H PYLORI ANTIBIOTIC RESISTANCE: PREVALENCE, IMPORTANCE, AND ADVANCES IN TESTING

F Mégraud

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The discovery that *Helicobacter pylori* infection is the main cause of most gastroduodenal diseases has been a major breakthrough in gastroenterology. It has dramatically changed the management of these diseases which are now considered as infectious diseases and are treated with antibiotics.

Triple therapy, including two antibiotics, amoxicillin and clarithromycin, and a proton pump inhibitor given for a week has been recommended as the treatment of choice at several consensus conferences.[1–6] However, this treatment may fail for several reasons, as reported elsewhere.[7] In fact, the main reason for failure was found to be *H pylori* resistance to one of the antibiotics used (that is, clarithromycin). Other treatments have also been proposed, including metronidazole, a drug for which resistance is also a problem although to a lesser extent, as well as tetracycline, fluoroquinolones, and rifamycins for which resistance has become an emerging issue.[8 9]

Our aim was to review the prevalence of *H pylori* resistance to these various antibiotics, their clinical importance, and methods of testing, especially in light of the resistance mechanism which allows application of molecular methods.

PREVALENCE OF *H PYLORI* RESISTANCE TO ANTIBIOTICS

Numerous studies have been performed to determine the prevalence of *H pylori* resistance to antibiotics. However, many of them have drawbacks, in particular concerning the number and representativeness of the strains tested.

Most of the studies were performed in specialised centres, with recruitment of special cases which are not always representative of patients as a whole and, because these studies are monocentric, the number of patients may be low, leading to wide confidence intervals of the prevalence rates obtained.

Ideal studies involving patients who are representative of a given region are few. An alternative has been to analyse prevalence data obtained from clinical trials aiming to evaluate new regimens. As prevalence is in essence an evolving phenomenon, only the most recent articles have been taken into account in this review. However, given the delay in publication, only data from the end of the last decade are essentially available.

Prevalence of *H pylori* resistance to clarithromycin

Only primary resistance has been reviewed, and adults and children were considered separately. An important difference is noted between the Northern and Southern parts of Europe (table 1). For adults in Northern Europe, the global prevalence was less that 5% while in Southern Europe it was as high as up to 20% or more. For children from all European countries a high prevalence has also been reported, ranging from 12.4% to 23.5% (table 2).

A prospective multicentre survey was also carried out in Europe in 1998. Investigators from 22 different centres located in 17 countries were involved and used a similar protocol, with Etest as the testing method. Overall, the minimum inhibitory concentrations (MICs) of 1274 isolates were determined with a mean of 64 isolates per centre, ranging from 21 to 115. The global primary resistance rate for clarithromycin was 9.9% (95% confidence interval (CI) 8.3–11.7). Interestingly, when the results were broken down according to the different regions in Europe, significant differences were observed which are in line with the data indicated above (that is, the prevalence of clarithromycin resistance in Northern Europe was low (4.2% (95% CI 0–10.8), it was higher in Central/Eastern Europe (9.3% (95% CI 0–22), and was the highest in Southern Europe (18% (95% CI 2.1–34.8)).[44]

A similar survey involving children in Europe was carried out more recently. Investigators from 16 paediatric centres from 14 countries collected their data over a four year period (1999–2002). A primary resistance rate of 24% was obtained (Koletzko, personal communication). A retrospective survey was also performed in Eastern Europe by Boyanova and colleagues.[45] Results on antibiotic resistance from 18 centres in Eastern European countries were gathered from 1998 to 2000 (1337 isolates). The prevalence of clarithromycin resistance was 9.5% (95% CI 7.9–11.1) with no

Correspondence to:
F Mégraud, Laboratoire de Bactériologie, Hôpital Pellegrin, Place Amélie Raba-Léon, 33076 Bordeaux Cedex, France;
francis.megraud@chu-bordeaux.fr

Table 1 Primary resistance of *Helicobacter pylori* to clarithromycin, metronidazole, tetracycline, and amoxicillin in adults in different parts of the world

Country	Years	Type of study	Method of testing	No of strains tested	Clari^R global prevalence (%)	95% CI	Metro^R global prevalence (%)	95% CI	Tetra^R global prevalence (%)	Amoxi^R global prevalence (%)	Reference
Europe											
Bulgaria	96–98	TriC	DD	103*	8.7	4.1–15.9	ND		ND	ND	Boyanova[10]
Croatia	2001	MonoC	Etest	196	8	4.7–12.9	33	26.6–4.02	ND	0	Bago[11]
France	96–99	MultiC	AD	659	15	12.4–18.0	31.5	28.0–35.3	ND	0	Mégraud[12]
Germany	95–00	MonoC	Etest	1644	2.2	1.5–3.0	26.2	24.1–28.4	0	0	Wolle[13]
Germany	95–96	MultiC	Etest	188	4	1.9–8.2	32	25.3–39.1	ND	ND	Dammann[14]
Italy (Central)	98–02	MonoC	AD	406	23.4	19.4–27.8	36.7	32.0–41.6	ND	0.2	Toracchio[15]
Italy (North)	99	MultiC	Etest	167	1.8	0.4–5.2	14.9	9.9–21.3	ND	0	Pilotto[16]
Netherlands	97–98	MultiC	Etest	231	1.7	0–3.4	21.2	16.1–27.1	0	0	Debets-Ossenkopp[17]
Portugal	90–99	TriC	Etest	132	22	15.2–30.0	34.1	26.1–42.8	0	0	Cabrita[18]
Spain	95–98	MonoC	Etest	235	12.9	8.7–17.7	23.5	18.1–29.3	0.7	0	Cuchi Burgos[19]
Sweden	97–98	MultiC	AD	203	2.9	1.1–6.3	26.1	20.2–32.6	ND	0	Mégraud[20]
UK	94–99	MonoC	DD	1064	4.4‡	3.3–5.8	40.3	37.4–43.3	0.5	0	Parsons[21]
UK	95–98	MonoC		843	3.9	2.7–5.5	36	32.7–39.3	ND	0.4	Teare[22]
North America											
Mexico	95–97	MonoC	Etest	144	25	18.2–32.9	76.3	68.6–83.1	ND	0†	Torres[23]
USA	93–99	MultiC	AD	3439	10.6	9.6–11.7	21.6	20.2–23.0	ND	0.08	Osato[24]
USA	98–99	MultiC	AD	422	12	9.1–15.6	ND		ND	0	Laine[25]
USA	00–01	MultiC	AD	106	12.2	6.7–20.0	33.9	25.0–43.8	ND	ND	Laine[26]
South America											
Brazil	96–00		AD	203	9.8	6.1–14.8	53	46.1–60.2	ND	ND	Prazeres-Magalhaes[27]
Middle East											
Iran	02	BiC	DD	120	17	10.5–24.6	ND		ND	ND	Mohammadi[28]
Israel	00–01	MonoC	Etest	110	8.2	3.8–15.0	38.2	29.1–47.9	0	0.9	Samra[29]
Far East											
Hong Kong	97–01	MonoC	DD	991	4.5	3.3–6.0	29	26.3–32.0	0.5	0.3	Ling[30]
Japan	95–00	MonoC	AD	593	11	8.6–13.8	9	6.8–11.5	ND	0.3	Perez Aldana[31]
Japan	96–99	BiC	AD	388	12.9	9.7–16.6	12.4	9.3–16.1	ND	0	Kato[32]
Korea	94–99	BiC	AD	456	5.9	3.9–8.5	40.6	36.0–45.2	5.3	0	Kim[33]
Korea	96–00	MonoC	BD	224	5.4	2.8–9.2	41.9	35.4–48.7	ND	ND	Eun[34]
Singapore	93–96	MonoC		459	ND		62.7	58.1–67.2	ND	ND	Teo[35]
Singapore	02	MonoC	AD	120	ND		31.7	23.5–40.8	ND	ND	Lui[36]
New Zealand	93–98	MonoC	DD	225	6.8	3.8–10.8	32	26.0–38.5	ND	ND	Fraser[37]

Data from studies including more than 100 strains published during the last five years.
*Includes 42 children.
†Transient resistance was observed in 19% of the strains.
‡Clarithromycin susceptibility performed on 812 strains only.
DD, disk diffusion method; AD, agar dilution method; BD, broth dilution method; ND, not determined; 95% CI, 95% confidence interval; C, centre.
Clari^R, clarithromycin resistant; Metro^R, metronidazole resistant; Tetra^R, tetracycline resistant; Amoxi^R, amoxicillin resistant.

significant difference between the different countries. Outside Europe, the prevalence of clarithromycin resistance tends to be lower. A systematic review of the studies performed in Canada before the year 2000 estimated resistance to be less than 4%.[46] However, it has already reached 10–15% in the USA based on data from clinical trials and regardless of the region. In the Middle East, a prevalence of 5.4% in Israel[29] and 17% in Iran[28] have been reported. In the Far East, the prevalence is higher in Japan (11–12%) than in Hong Kong (4.5%) and Korea (5–6%) (table 1).

Most of the studies provide data over several years and therefore it is possible to see the evolution of resistance. However, the number of strains per year is usually low, so only a trend can be considered, and this trend is towards an increased prevalence over time.

The essential risk factor for clarithromycin resistance is previous consumption of macrolides,[12 18] and if resistance is higher in children it is because there was increased prescription of these drugs, notably in children during the last decade essentially for respiratory tract infections. A study in Japanese families showed that despite the fact that the children's strains were usually identical to one parent strain by molecular fingerprinting, the children's strains often became clarithromycin resistant after clarithromycin

Table 2 Primary resistance of *Helicobacter pylori* to clarithromycin, metronidazole, and amoxicillin in children in Europe

Country	Years	Type of study	Method of testing	No of strains	Clari^R prevalence (%)	95% CI	Metro^R prevalence (%)	95% CI	Amoxi^R prevalence (%)	Reference
Austria	1997–2000	BiC	Etest	117	20.3	13.6–29	16	10.1–24.2	0	Crone[38]
Belgium	1989–2000	MonoC	DD	555	16	13.1–19.4	18	14.9–21.5	0	Bontems[39]
Bulgaria	2000–2001	MonoC	AD	115	12.4	6.8–19.6	15.8	9.5–23.6	0	Boyanova[40]
France	1994–1999	MonoC	Etest	150	21	15.1–28.8	43	35.3–51.7	0	Kalach[41]
Poland	1998–2000	MonoC	AD	98	23.5	15.5–33.1	ND		ND	Dzierzanowska-Fangrat[42]
Spain	1991–1999	MonoC	AD	246	21.1	16.2–26.8	23	18.0–29.0	0	Lopez Brea[43]

Data from studies including more than 100 patients published during the last five years.
Clari^R, clarithromycin resistant; Metro^R, metronidazole resistant; Amoxi^R, amoxicillin resistant.
DD, disk diffusion method; AD, agar dilution method; ND, not determined; 95% CI, 95% confidence interval; C, centre.

treatment.[47] No association with age was noted in adults in most studies or significant regional differences in a large country such as the USA. Interestingly, some studies compared macrolide consumption and the ensuing resistance in the corresponding countries over the years. For example, emergence of resistance to clarithromycin in Estonia in 1998 followed introduction of this drug in 1997.[48] In Japan, clarithromycin consumption multiplied by four between 1993 and 2000, and this led to a fourfold increase in clarithromycin resistance.[31] However, this was not case in the Netherlands; despite a threefold increase in clarithromycin prescriptions between 1993 and 1997, there was no significant increase in macrolide resistance.[17] This fact could be explained by the prudent use of antibiotics in this country. Indeed, when a comparison of antibiotic consumption was made in countries in the European Community, the Dutch had the lowest consumption.[49] Considering macrolide consumption evaluated in this last study in specific European regions, a perfect correlation could be made with the prevalence of clarithromycin resistance observed in the multicentre study of Glupczynski and colleagues.[44]

Although it is recognised that there is in vitro cross resistance between all macrolides, it is not clear whether all macrolides have the same potential to select resistant strains in vivo, depending on their diffusion in the gastric mucosa. Theoretically, based on in vitro data, when clarithromycin is present in the gastric mucosa at subinhibitory concentrations, it may select resistant strains. However, for other macrolides which may not even reach such a subinhibitory concentration, there is often little impact on the resistance selection process. With clarithromycin, in the case of treatment failure, emergence of resistance occurs in at least two thirds of strains.[50] This is not the case with azithromycin. In a French study, the rate of secondary resistance was only 23% after azithromycin therapy despite a high failure rate (62%).[51] The impact of erythromycin on selection of resistant strains may be more important. In Iran, the clarithromycin resistance rate is already 17% even though this drug has not been introduced in this country but erythromycin is used.[28] A similar situation was observed in France in 1993 (that is, an *H pylori* clarithromycin resistance rate of 8% was already noted probably due to the intense use of macrolides (erythromycin, josamycin, etc.) during the previous decade.[52]

Most studies did not mention any difference in prevalence according to the patient's disease status, with the exception of two French studies and one in Germany. Firstly, in our prospective study on antimicrobial resistance, while the risk of harbouring a resistance strain for patients with non-ulcer dyspepsia (NUD) or other diseases was 1, it was only 0.08 (95% CI 0.011–0.66) for those with peptic ulcer disease.[12] Similar results were observed when risk factors for failure of *H pylori* therapy in all of the French clinical trials were studied.[53] In the subsample on which clarithromycin susceptibility was performed, strains from 5.6% of peptic ulcer patients were resistant compared with 16.7% of strains from NUD patients ($p = 0.0005$). The same observation was made in Germany.[54] This could be related to the fact that virtually all of the peptic ulcer disease patients were infected with *cag* positive strains versus only half of the NUD patients, and these strains may be easier to eradicate,[55] possibly because their generation time is shorter or because they may be in closer contact with cells and therefore more accessible to the antibiotic. Another possibility is that NUD patients are simply greater consumers of antibiotics; in a Croatian study, for example, after multiple treatments of these two groups of patients, the prevalence of resistance was significantly higher in NUD patients.[56]

Another controversial point is the stability of the point mutation leading to resistance. Indeed, there are only a few positions, especially in domain V of the 23S rDNA, where mutations are found and where they change the spatial configuration of the ribosome, while mutations at other spots probably lead to non-viable organisms.[57] It was nevertheless possible to obtain some other mutants in vitro.[58] The key question lies in the impact of the mutations on the fitness of the bacterium. It is clear that if the mutation has a biological cost, it will not be maintained when selection pressure stops. In two studies, resistant mutants were followed after subculture in vitro as well as at different time points in vivo but resistance remained.[59 60] However, others claim that clarithromycin resistance is not stable over the long term.[61]

The point mutation involved in resistance is interesting to consider. Its determination was made by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or sequencing in 12 studies (table 3). The most frequent mutation was A2143G (69.8%) but its prevalence varied from 53% to 95%, followed by A2142G (11.7%) and A2142C (2.6%). This last mutation is probably underestimated because the relevant restriction enzyme to detect it was not used in most of the studies. However, one study reported

Table 3 Prevalence of the different mutations associated with clarithromycin resistance in recent studies

Country	No of strains tested	A2143G (No (%))	A2142G (No (%))	A2142C (No (%))	T2717C (No (%))	No detection (%)	Reference
Germany (adults)	16	9 (52.7)	3 (16)			4 (11)	Wolle[13]
Italy	12				7 (58.3)	5 (41.6)	Fontana[62]
Poland (children)	23	16 (69.5)	4 (17.3)	2 (8.6)		1 (4.3)	Dzierzanowska-Fangrat[42]
Spain (children)	28	23 (82.1)	2 (7.1)			3 (10.7)	Alarcon[63]
European multicentre study	10	7 (70)	2 (20)			1 (10)	Mégraud[19]
Brazil	19	14 (73.6)*	3 (18.7)			2 (10.5)	Prazeres-Magalhaes[27]
Hong Kong	23	22 (95.6)	1 (4.3)				Ling[30]
Iran	20	15 (75)	1 (5)	3 (15)		1 (5)	Mohammadi[42]
Japan	12	11 (92)				1 (8)	Kato[32]
Japan	9	2 (22.2)	6 (66.6)			1 (11.1)	Umegaki[64]
Korea	12	8 (66.6)				4 (33.3)**	Kim[65]
Taiwan	12	10 (83.3)	1 (8.3)			1 (8.3)	Yang[66]
Total	196	137 (69.8%)	23 (11.7%)	5 (2.6%)	7 (3.6%)	24 (12.2%)	

*Two strains also harboured the A2142G mutation.
**These four strains had a T2182C mutation.

completely different results (that is, seven cases of a new mutation T2717C associated with a low MIC (1 mg/l) and five cases where no mutation was found). Such results warrant confirmation.

While there are differences in MICs between strains harbouring these different mutations, A2142G being the highest, the clinical relevance appears to be minimal.

Prevalence of *H pylori* resistance to amoxicillin

In all of the surveys reported in table 1, resistance to amoxicillin is either null or less than 1%, indicating that it is not yet a problem. Fortunately, plasmid borne beta lactamase resistance has never been encountered. In contrast, a strain has been found with an MIC of 8 mg/l due to a mutation in the *pbp*-1A gene[67], probably selected following multiple cures of amoxicillin.[68] By culturing an amoxicillin susceptible strain with increasingly higher concentrations of amoxicillin, it was also possible to obtain strains with MICs of 4–8 mg/l which exhibited a decreased affinity for this drug.[69] Strains with high MICs obtained in vivo[67 70] or in vitro[71] have been used to transform susceptible strains. In all cases the authors obtained transformants with increased MICs but not higher than 0.5–2 mg/l. However, sequencing of *pbp*-1A in these different experiments[67 70 71] showed different mutations associated with resistance.

Other strains with decreased susceptibility (MIC 0.5 mg/l instead of 0.05 mg/l) are sometimes encountered. They have not been explored with regard to the mechanism involved but they may be the result of transformation. Their impact on *H pylori* eradication is also unknown. The existence of tolerant strains was raised in the past, and at that time a lack of a fourth PBP, PBP-D, was proposed as the mechanism.[72] Four of these strains have been studied recently. Transformants reached an MIC of 8 mg/l, which is still lower than that of clinical isolates. The suggestion was made that resistance was due to a mosaic block of PBP-1A which contained 10 amino acid changes.[73] Decreased susceptibility also observed for other antibiotics could not be clearly related to an efflux mechanism.

Very high resistance rates to amoxicillin have been reported in some prevalence studies.[74 75] However, these results must be interpreted with caution until the strains have been explored in depth.

Prevalence of *H pylori* resistance to tetracycline

Resistance to tetracycline is also very low, or even absent, in most countries. Cases have been reported in Spain (0.7%),[19] the UK (0.5%),[21] and Hong Kong (0.5%)[30] but also at a higher rate (5.3%) in Korea[33] (table 1).

With tetracycline, as well as with other antibiotics, resistance increases with the use of these drugs due to selection pressure. The resistance mechanism has been described as a change in three contiguous nucleotides in the 16S rRNA gene (AGA 926–928→TTC).[76 77]

In vitro experiments have shown that when mutations occur in only one or two of these nucleotides, resistance is at a low level (4 mg/l), which is clinically insignificant; only the triple mutation leads to stable and high resistance levels.[78 79] The need for a triple mutation may explain the rarity of tetracycline resistance.

Prevalence of *H pylori* resistance to metronidazole

The prevalence of *H pylori* resistance to metronidazole varies from 20% to 40% in Europe and the USA, with one exception in Northern Italy.[16] It is well known that the prevalence is much higher in developing countries (50–80%), for example Mexico (76.3%)[23] (table 1). In contrast, the prevalence rate is quite low in Japan (9–12%).[31 32]

In the European multicentre study previously mentioned,[44] the global resistance rate to metronidazole was 33.1% (95% CI 7.5–58.9) with no significant difference between the North (33% (95% CI 7.1–69.2)) and South (40.8% (95% CI 27.3–54.3)) but a significantly lower prevalence in Central and Eastern parts (29.2% (95% CI 17.9–41.5)) ($p<0.01$). The global resistance rate shows a slight increase in comparison with that of a previous study carried out seven years earlier (1991) in Europe but where the centres were not exactly the same (27.5% (122/443) (95% CI 23.4–32.0)).[80]

The study by Boyanova and colleagues[45] in Eastern Europe reported a slightly higher prevalence using Etest (34.7% (95% CI 29.9–39.5)). The prevalence in Canada in the systematic review by Fallone was 18–22%.[46]

Emphasis should be made concerning the poor correlation between the different methods used to test *H pylori* resistance to metronidazole, with up to 10–20% discrepancies. Furthermore, reproducibility using a given method is also not good.[20 24 81] Nevertheless, although the exact prevalence rate obtained must be interpreted with caution, the trends of high, medium, or low resistance observed seem real.

In contrast with resistance mechanisms for other antibiotics, the resistance mechanism to metronidazole is not as straightforward.[82 83] Clearly, alterations of the *rdx*A gene are of prime importance but it has not been possible to identify a clear panel of point mutations which could explain the phenomenon. Moreover, other genes such as *frx*A also seem to be involved.[84]

When risk factors are studied, past use of metronidazole, which is common in tropical countries for parasitic diseases, is once more involved. In developed countries, most studies have reported a higher resistance rate in women than in men, probably due to the use of nitroimidazole drugs to treat gynaecological infections.[44] The use of nitroimidazole for dental infections may also add to selection pressure. In the USA there were no marked regional differences.

Interestingly, metronidazole resistant strains were found significantly more frequently among NUD patients from different ethnic backgrounds than in peptic ulcer patients (56.4% *v* 19.8%, respectively; $p<0.001$) in a recent study from Singapore while it was not mentioned in previous publications.[36]

Prevalence of *H pylori* resistance to fluoroquinolones

As in other bacteria, resistance of *H pylori* to fluoroquinolones is due to point mutations in the quinolone resistance determining regions of *gyr*A.[85] The prevalence of this resistance has been determined in only a limited number of studies. Only one country, Portugal, has reported a high resistance rate: 20.9% in strains isolated from 110 adult patients.[18] In the Netherlands, the rate was 4.7% (231 strains tested) with a drug, trovafloxacin, not yet introduced on the market, confirming cross resistance between the different molecules from this antibiotic group.[17] In France, a rate of 3.8% was reported between 1993 and 1999 on 655 strains[52] and was recently confirmed in another study (3.3%).[86] In five Eastern European countries the rate was also 3.9%.[45]

Again, resistance to fluoroquinolones mirrors the use of these drugs. Despite a high rate of resistance in adults in

Table 4 *Helicobacter pylori* eradication with triple therapy (proton pump inhibitor-amoxicillin-clarithromycin) according to clarithromycin susceptibility or resistance

Reference	Type of study	Treatment	Eradication rate		
			Overall	Clarithromycin susceptible	Clarithromycin resistant
Bago[10]	Randomised	OAC 1 week	84% (37/44)	89.7% (35/39)	40% (2/5)
Bochenek[94]	Randomised	PAC 1 week	66.5% (137/206)	77.3% (130/182	29.1% (7/24)
Ducons[95]	Successive	LAC 1 week	79% (79/100)	85.5% (77/90)	20% (2/10)
Hoshiya[96]	Not randomised	OAC, LAC 1 week	77.5% (83/107)	83.1% (79/95)	33.3% (4/12)
Kalach[97]†	Successive	OAC, LAC 1 week	81.9% (50/61)	100% (50/50)	0% (0/11)
Katelaris[98]	Randomised	PAC 1 week	80.4% (37/46)	86% (36/42)	25% (1/4)
Kawabata[99]	Randomised	LAC, RAC 1 week	78% (135/173)	85.5% (130/152)	23.8% (5/21)
Kihira[100]	Randomised	RAC	92.6% (79/85)	96.3% (79/82)	0% (0/3)
Laine[25]	Randomised	OAC	85.8% (91/106)	94.6% (88/93)	23% (3/13)
Lamouliatte[101]	Randomised	PAC 1 week	81.1% (82/101)	86% (82/95)	0% (0/6)
Lamouliatte[102]*	Randomised	OAC 1 or 2 weeks	48.3% (57/118)	80% (48/60)	15.5% (9/58)
Lehman[103]	Randomised	LAC 2 weeks	72% (23/32)	87% (23/29)	0% (0/3)
Lind[104]	Randomised	OAC 1 week	95.7% (113/118)	97% (111/116)	100% (2/2)
McMahon[105]	Not randomised	LAC 2 weeks	71.6% (38/53)	87.5% (35/40)	23% (3/13)
Miki[106]	Randomised	OAC, R AC 1 week	87% (120/138)	97.5% (119/122)	6.3% (1/16)
Murakami[107]	Randomised	RAC, LAC 1 week	85.3% (227/266)	94% (226/241)	4% (1/25)
Peitz[108]*	Randomised	OAC 1 week	48.5% (17/35)	83% (10/12)	30% (7/23)
Pilotto[109]	Randomised	PAC 1 week	85% (34/40)	87% (33/38)	50% (1/2)
Poon[110]	Randomised	LAC 1 week	84% (37/44)	95% 37/39)	0% (0/5)
Tankovic[111]	Successive	OAC 1 week	67.6% (69/102)	79% (67/85)	12% (2/17)
Total			78.2% (1545/1975)	87.8% (1495/1702)	18.3% (50/273)

*Second line treatment.
†Children.
O, omeprazole; L, lanzoprazole; P, pantoprazole; C, clarithromycin; M, metronidazole; R, ranitidine; A, amoxicillin; PPI, proton pump inhibitor.

Portugal, children who were not treated with quinolones did not harbour any resistant strains.[18]

This also indicates the high risk of selection of resistant strains which may jeopardise the new promising fluoroquinolone based rescue therapies (proton pump inhibitor-amoxicillin-levofloxacin or moxifloxacin).

Prevalence of *H pylori* resistance to rifabutin

The prevalence of *H pylori* resistance to this group of antibiotics is not known but is probably extremely low as these drugs, until recently, were used only in a limited number of patients to treat mycobacterial infections. For example, Heep *et al* did not find a single resistant strain from 81 tested in 1999[87] in Germany and nor did Fujimura *et al* among 52 strains in Japan.[88] However, the use of these drugs could also lead to the emergence of resistant strains, as was observed in one case of treatment failure.[89] Again, resistance is due to point mutations in the *rpo*B gene, as for other bacteria, and concerns all rifamycin drugs.[90 91]

Double resistant strains

As clarithromycin and metronidazole are the antibiotics most frequently used with amoxicillin, it was interesting to see if both resistances were evenly distributed. Most of the studies presented in tables 1 and 2 give an indication of the prevalence of these double resistant strains. It was found that they were twice as frequent as expected. However, given their proportion among the total number of strains, their prevalence is still low in Europe (0.8–9.1%) and in Asia (2–3%), and much higher in developing countries such as Mexico (18%). The situation is different after failure of a therapy using both clarithromycin and metronidazole, and up to 50% of strains may then harbour double resistance.[50]

Table 5 *Helicobacter pylori* eradication with triple therapy (proton pump inhibitor-clarithromycin-metronidazole) according to susceptibility or resistance to both antibiotics

Reference	Type of study	Type of treatment	Eradication rate				
			Overall	Metronidazole susceptible		Metronidazole resistant	
				Clarithromycin resistant	Clarithromycin susceptible	Clarithromycin resistant	Clarithromycin susceptible
Bazzoli[112]	Randomised	OCT	91.3 % (21/23)	0	18/18	0/1	3/4
Damman[14]	Randomised	PCM	85.1% (160/188)	0/3	123/125	0/1	37/59
Ellenrieder[113]	Successive	PCM	92.8% (78/84)	2/3	63/67	0	13/14
Hurenkamp[114]	Randomised	OCM	100% (74/74)	1/1	57/57	0	16/16
Lind[25]	Randomised	OCM	89.4% (102/114)	4/6	73/75	0	25/33
Pilotto[109]	Randomised	PCM	82.8% (29/35)	2/3	24/25	0	3/7
Poon[110]	Randomised	LCM	73.5% (25/34)	0/2	15/16	0/3	10/13
Savarino[115]	Randomised	OCM	73.1% (30/41)	2/4	18/20	0/2	10/15
Total				50.0% (11/22)	97.0% (391/403)	0/7	72.6% (117/161)

O, omeprazole; L, lanzoprazole; P, pantoprazole; C, clarithromycin; M, metronidazole; T, tinidazole.

Table 6 *Helicobacter pylori* eradication with triple therapy (ranitidine bismuth citrate-clarithromycin-metronidazole) according to susceptibility or resistance to both antibiotics

				Eradication rate			
				Metronidazole susceptible		Metronidazole resistant	
Reference	Type of study	Type of treatment	Overall	Clarithromycin resistant	Clarithromycin susceptible	Clarithromycin resistant	Clarithromycin susceptible
Bardhan[116]	Randomised	RBC-CM	97.6% (41/42)	0	100% (32/32)	0/1	100% (9/9)
Savarino[115]	Randomised	RBC-CM	97.2% (35/36)	2/2	100% (17/17)	66% (2/3)	100% (14/14)
Sung[117]	Randomised	RBC-CM	92.8% (52/56)	1/1	91.3% (21/23)	0/1	96.7% 30/31
Total				100% (3/3)	97.2% (70/72)	40% (2/5)	98.1% (53/54)

RBC, ranitidine bismuth citrate; C, clarithromycin; M, metronidazole.

CONSEQUENCES OF *HELICOBACTER PYLORI* RESISTANCE TO ANTIBIOTICS

Antibiotic resistance is important if it leads to treatment failure. We reviewed the clinical relevance in 1998 at a time when data on susceptibility testing were scarce.[92] One year later a systematic review was published based on data from 16 of 172 arms where proton pump inhibitor-clarithromycin-amoxicillin therapy was given, and for which susceptibility testing was performed.[93] Table 4 is not a systematic review but presents data from 20 recent studies (1999–2003) and 1975 patients receiving the same treatment where susceptibility testing was performed. Unfortunately, these studies still represent a low proportion of the studies carried out (for example, 436 of 2751 patients among the French studies[53]) and sometimes the data are not presented in an adequate manner. A major difference in eradication rates was found: 87.8% when strains were clarithromycin susceptible compared with 18.3% when strains were clarithromycin resistant. The Mantel-Haenszel pooled odds ratio (OR) was highly significant (OR 24.5 (95% CI 17.2–35.0), $p<0.001$). It was 22.5 using the fixed effect model and 28.7 with the random effect model.

This 70% decrease in clinical success is higher than the 53% decrease reported in the meta-analysis by Houben and colleagues[93] and confirms the high clinical relevance of clarithromycin resistance. Fortunately, the prevalence of clarithromycin resistance is still low in many places, as shown in table 1, but deserves monitoring at least in some centres.

Conversely, these results indicate the high success rate of this treatment (87.8%) when strains are susceptible, regardless of the proton pump inhibitor, dosage of the different drugs, and duration of the treatment.

Data from eight studies where a nitroimidazole compound was administered with clarithromycin instead of amoxicillin were also reviewed (table 5). When considering nitroimidazole resistance alone, there was a 25% decrease in the success rate compared with nitroimidazole susceptible strains (72.6% *v* 97%), which is identical to that observed by Houben and colleagues[93] indicating that nitroimidazole resistance is less clinically relevant than clarithromycin resistance. The Mantel-Haenszel pooled OR was 11.3 (95% CI 5.7–22.3; $p<0.001$). It was 10.4 using the fixed effect model and 9.8 with the random effect model.

Furthermore, in the context of this treatment, clarithromycin resistance seems to lead less often to treatment failure (50% *v* 18.3%). A small number of strains were resistant to both antibiotics and none could be eradicated, reinforcing the fear of using this combination as a first line treatment because resistance to both drugs may be selected.

Few studies have used ranitidine bismuth citrate (RBC) instead of a proton pump inhibitor but data available indicate better efficacy of this combination, especially on metronidazole resistant strains (table 6). This result may be due to synergism between RBC and antibiotics.[118] Such a synergy may also exist when a bismuth based quadruple therapy is used.[26 119]

The combination of proton pump inhibitor-amoxicillin-metronidazole has also been used in six trials (table 7). For metronidazole susceptible strains, the eradication rate was similar to the association of amoxicillin-clarithromycin (susceptible strains: 89.4% *v* 87.8%) which is inferior to the combination with clarithromycin (when strains are also susceptible: 89.4% *v* 97%; ($p<0.001$). A 25% drop in efficacy was also observed when strains were metronidazole resistant.

Interestingly, some authors compared a susceptibility testing strategy with an empirical treatment (table 8).

Table 7 *Helicobacter pylori* eradication with triple therapy (proton pump inhibitor-amoxicillin-metronidazole) according to susceptibility or resistance to metronidazole

		Eradication rate		
Reference	Type of treatment	Overall	Metronidazole susceptible	Metronidazole resistant
Bardhan[120]	OAM 1 week	81.5% (274/336)	87% (233/266)	57% (41/70)
Bayerdorffer[121]	OAM 1 week	88% (96/109)	91% (81/89)	75% (15/20)
Isomoto[122]*	RAM 2 weeks	91.6% (33/36)	93% (27/29)	86% (6/7)
Lamouliatte[102]*	OAM 2 weeks	69.8% (67/96)	81% (38/47)	59% (29/49)
Murakami[123]*	RAM 1 week	92.9% (79/85)	96.8% (61/63)	81.8% (18/22)
Pilotto[109]	PAM 1 week	85.2% (29/34)	96% (24/25)	56% (5/9)
Total		83% (578/696)	89.4% (464/519)	64.4% (114/177)

*Second line *H pylori* eradication treatment.
O, omeprazole; P, pantoprazole; R, rabeprazole; M, metronidazole; A, amoxicillin.

Table 8 Comparison of an empirical treatment with a susceptibility testing strategy to eradicate *Helicobacter pylori*

		Empirical treatment group			Susceptibility testing group		
			% eradication			% eradication	
Reference	Treatment	n	ITT	PP	n	ITT	PP
Lamouliatte[102]†	OAM 2 weeks	57	63.2	72.1	113	74.3	78.3
Miwa[124]	LAM	39	92.4	94.7	38	81.6	83.3
Neri[125]	OCA RBC-CT	116	67		116	76*	
Romano[126]	OMC	39	79.5	77.5	40	95	97.4
Toracchio[127]	OMC	56	75	81	53	91	98

*Eradication rate was 64% using OCA and 87% using RBC-CT.
†Second line treatment.
O, omeprazole; A, amoxicillin; L, lanzoprazole; M, metronidazole; C, clarithromycin; T, tinidazole; RBC, ranitidine bismuth citrate.
ITT, intention to treat; PP, per protocol.

There was a marked advantage in using the susceptibility testing strategy in two studies. Indeed, the strategy depends very much on the treatment used. For Neri *et al*, an empirical treatment using RBC was the most efficient.[125]

ADVANCES IN TESTING

Overwhelming evidence indicates that antibiotic resistance in *H pylori* is essentially due to chromosomal mutations. An important consequence of this finding is that transmission of resistance essentially occurs vertically from the organism in which the mutation appears to its descendants, and therefore a progressive increase in resistance is observed according to selection pressure rather than a rapid outbreak-like spread when resistance in plasmid borne. However, genetic exchanges seem to be numerous between different strains of *H pylori* and therefore the possibility of transmission of resistance does exist. This opportunity is probably on the decrease in our Western societies given that gastric infections are now essentially due to a unique strain. In vitro studies have shown, for example, that metronidazole resistance could be transmitted by transformation.[128]

The genes involved in mutations have been readily identified and presented earlier in this article. They are summarised in table 9. In most cases there is a limited number of point mutations which allows the development of molecular methods to be used for their detection.

The usual methods of phenotypic detection of resistance are still widely used. The recent European standardisation[81] has led to recommendation of a similar protocol to that of the US NCCLS. Given the important difference observed in MICs between clarithromycin susceptible and resistance strains, the simple and cheap disk diffusion method has been validated. The best results were obtained using an erythromycin disk.[129] New methods have been developed. These methods are essentially molecular methods given that point mutations are the unique mechanism of resistance in *H pylori*, and PCR based methods are most often used. These methods have been extensively reviewed in 2002 in this journal.[130] However, for metronidazole the complexity of the phenomenon does not allow this approach and a new phenotypic method has been proposed.

Table 9 Genes involved in point mutations or other genetic events leading to antibiotic resistance in *Helicobacter pylori*

Antibiotic group	Gene involved
Macrolides	23S *rrn*
Metronidazole	*rdxA, frxA*
Quinolones	*gyrA*
Rifamycins	*rpoB*
Amoxicillin	*pbp*-1A
Tetracycline	16S *rrn*

Detection of clarithromycin resistance

Among the numerous methods described (table 10), the most promising for the future is application of real time PCR. The first apparatus, the Lightcycler, was designed to perform quantitative PCR[146] but now has a major application in detecting point mutations. A technology named fluorescence resonance energy transfer (FRET) can be applied. In the first article by Gibson and colleagues[140] in 1999, a DNA double strand specific fluorophor SYBR Green 1 and a second fluor dye Cy5 on a probe were used to test *H pylori* strains. This method was then applied to gastric biopsies.[141] Later, another approach was used: in addition to the specific primers targeting 23S rDNA, two probes were designed: (1) a sensor probe 5′ labelled with LC-Red 640 and 3′ phosphorylated, which hybridises with the region containing the mutation site and (2) an anchor probe 3′ labelled with fluorescein which hybridises three bases upstream from the former. When the anchor probe is excited, an energy transfer occurs to the sensor probe and a signal is emitted. After amplification, a melting step is performed which leads to different melting points for the wild-type and mutants.[142–144] This method allows detection of *H pylori* and determination of its clarithromycin susceptibility directly from biopsy specimens

Table 10 Molecular methods for *Helicobacter pylori* testing of clarithromycin resistance

Based on amplification of 23S rRNA gene
- Sequencing
- RFLP (restriction fragment length polymorphism)[131 132]
- OLA (oligonucleotide ligation assay)[133]
- DEIA (DNA enzyme immunoassay)[134 135]
- PHFA (preferential homoduplex formation assay)[136]
- INNO-LiPA (line probe assay)[137]
- Mismatch PCR[138]
- DG-DGGE (double gradient-denaturing gradient gel electrophoresis)[139]
- FRET (fluorescence resonance energy transfer)[140–144]

Based on hybridisation
- FISH assay (fluorescence in situ hybridisation assay)[145]

in one reaction which takes two hours and limits the possibility of contamination with PCR products as the entire reaction occurs in the same tube. It has proven to be highly sensitive and specific. However, because there are not many 23S rRNA sequences from other helicobacters available in data banks, and because other helicobacters are seldom found, specificity among uncommon *Helicobacter* species has not been extensively tested.

A recent development is the application of this technology, not to gastric biopsy samples, but to stool samples, which offers a non-invasive method of performing susceptibility testing. Preliminary results have been presented[147] and look promising.

Additional progress for those using the more traditional PCR-RFLP method was the description of a new restriction enzyme allowing detection of the A2142C mutation.[148]

The possibility of detecting clarithromycin resistance without performing PCR also exists, by fluorescence in situ hybridisation (FISH). This method has been applied to the detection of *H pylori* and its clarithromycin resistance by Trebesius and colleagues.[145] It consists of an rRNA based whole cell in situ hybridisation using a set of fluorescent labelled oligonucleotide probes. Labelling of intact single bacteria is monitored by fluorescence microscopy. This method allows detection of *H pylori* with a 16S rRNA probe labelled with the fluorochrome Cy3 (red) and of resistant mutants with a 23S rRNA probe labelled with fluorescein (green) simultaneously. This method proved to be sensitive and specific compared with standard methods of culture and susceptibility testing. No discrepant result was noted for 45 cases.[149] In another study, there were 11 discrepant results among 69 cases. These cases corresponded to mixed infections with susceptible and resistant strains.[150] This method has the advantage of being independent of nucleic acid preparations, it is not prone to inhibition such as PCR, and is quick. It also allows visualisation of the bacteria, including coccoidal forms, and can be performed on paraffin embedded biopsy samples.[151] A limitation could be the observer dependent result, and sometimes difficulty in reading.

All of the techniques described highlight the fact that mixed infections between clarithromycin susceptible and resistant bacteria are frequent. In a study performed in our laboratory, multiple genotypes were found in 21.1% of cases.[143] Usually the essential part of the bacterial population corresponds to the susceptible genotype, indicating that resistance occurs spontaneously, remains at a low concentration, and is subsequently selected by administration of the drug.

Detection of metronidazole resistance

An interesting approach based on the detection of the RdxA protein by immunoblotting with specific rabbit anti-RdxA antibodies has been proposed.[152] A 24 kDa immunoreactive band corresponding to the RdxA protein is observed in all metronidazole susceptible isolates while it is not found in most metronidazole resistant isolates. However, this band was present in approximately 10% of metronidazole resistant isolates in the two studies performed. Complementary experiments proved that these isolates contained mutations associated with inactivation of the *rdx*A gene.[153]

Despite this limit, this new approach looks promising, given the high discrepancies observed when using different phenotypic methods and even the lack of reproducibility with a given method.

Detection of other resistances

Although the primary material for developing molecular tests for other antibiotics (tetracycline, quinolones, rifamycin) do exist, these tests have not yet been developed, probably because of the low frequency of resistant strains. Better knowledge of the *pbp*1 gene domain where mutations occur[154] should help in developing a molecular method for the rare amoxicillin resistant *H pylori*.

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