

Clarithromycin resistance in *Helicobacter pylori* and its clinical relevance

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INTRODUCTION

The macrolide clarithromycin has emerged as the most important antibiotic in combined therapy for eradication of *H. pylori* infection^[1,2]. However, concerns about increasing clarithromycin resistance in *H. pylori* and its impact on the efficacy of eradication therapy have been raised since its widespread acceptance in *H. pylori* therapy^[3,4]. Here, we sought to review the geographic prevalence of clarithromycin resistance in *H. pylori* and its molecular mechanisms, and assess the clinical relevance of clarithromycin resistance.

Geographic prevalence of clarithromycin resistant *H. pylori*

The worldwide, prevalence of primary (pre-treatment) clarithromycin resistance to *H. pylori* ranges from 0.8% to 18% (Figure 1)^[5-29]. The reported prevalence in China is between 4.8% and 7.5%, while the rate in Australia ranges from 6.1% to 7.8%^[5,6,11,12].

Molecular mechanisms of clarithromycin resistance

Versalovic *et al* were the first to identify an A→G transition mutation within a conserved loop of 23S rRNA of *H. pylori*, and its association with clarithromycin-resistance^[30]. The mutation occurs commonly at two gene positions cognate with positions 2058 and 2059 of *Escherichia coli*-23S rRNA, which were re-named 2143 and 2144, and now revised as 2142 and 2143, respectively^[4,31]. Point mutations may occasionally occur at other positions, and can be a transition (A→G) or a transversion (A→C), but the transition is far more frequent^[4,32-35]. Moreover, Versalovic *et al*^[32] also observed that the A2142G mutation was associated with a high level of resistance (MIC>64 mg/L)

than the A2143G mutation^[32]. These observations are supported by others studies^[33,36].

It has been reported that macrolide-resistance was not stable in some strains of *H. pylori in vitro*^[17]. This phenomenon was also observed *in vivo*; i.e., strains developed resistance post-treatment and then reverted to being susceptible after a period of follow-up^[17,30]. Versalovic *et al* cultured five genotypically identical isolates subsequently from one patient before and after treatment with clarithromycin alone^[30]. They observed that the first two post-treatment isolates with a low-level clarithromycin resistance had an A2143G mutation, which was not present in the susceptible pretreatment isolate or in the last two post-treatment isolates with reverted susceptibility^[30]. This suggests that the mutation may be unstable^[35]. However, Hulten *et al* reported that clarithromycin resistance was stable after 50 subcultures *in vitro*^[35], which is consistent with other studies^[37].

Cross-resistance between macrolides in *H. pylori* has been observed^[12,17,30]. Generally, *H. pylori* strains resistant to clarithromycin are also resistant to erythromycin, azithromycin and roxithromycin or vice versa. These observations have been confirmed at the molecular level^[36].

Detection of clarithromycin resistance in *H. pylori*

The methods currently used for susceptibility testing of *H. pylori* to clarithromycin include agar dilution method, broth dilution method, disc diffusion test and the Epsilonometer test (E-test)^[17,38]. The agar dilution method determines the minimal inhibitory concentrations (MICs) of antibiotics against bacteria. This method is time consuming and not feasible for routine use. However, it is a reliable technique which is usually carried out as a reference method for other techniques^[17,38,39]. Broth dilution method is rarely used because of the difficulty in growing *H. pylori* in broth. The disc diffusion test is the easiest and cheapest way of testing susceptibility. However, this test requires strict standardization before it can be used^[39]. The E-test, developed in 1988, provides the MIC of a strain directly by using a diffusion-like method^[40]. A plastic-coated strip contains a preformed antimicrobial gradient on one side and a scale on the other. The reading is taken at the point where the

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ellipse of growth inhibition intersects the strip. Standardization and correlation with the agar dilution method are also required prior to application. This method is now widely used by many investigators^[12,13,15,16,18,22-28]. At present, no "gold standard" method has been proposed for testing *H. pylori* susceptibility to antibiotics including clarithromycin and metronidazole, as there is still a need for standardization regarding the appropriate medium, the supplementation, the size of the inoculum, the incubation atmosphere, the appropriate time to read the plates and the breakpoint differentiating resistance and susceptibility^[38]. Since cross-resistance exists between macrolides, erythromycin susceptibility testing may be useful in predicting (determining) clarithromycin resistant *H. pylori* strains^[12,17]. Erythromycin susceptibility testing is well established in many microbiological laboratories, and it is much cheaper than clarithromycin susceptibility testing at present.

The association between point mutations on the 23S rRNA gene and macrolide resistance in *H. pylori* potentially provides a new approach for diagnosing macrolide resistant *H. pylori* strains. Although cycle DNA sequencing of the 23S rRNA gene amplicons is regarded as the reference method, simpler techniques have been developed^[38]. These include polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP), an oligonucleotide ligation assay (PCR-OLA), a DNA enzyme immunoassay (PCR-DEIA), a reverse hybridisation line probe assay (PCR-LiPA), and a preferential homoduplex formation assay (PCR-PHFA)^[30,31,33,41-43]. The PCR-based molecular techniques are quicker than microbiological susceptibility testing, and more importantly, they can be performed directly on gastric biopsies and gastric juice^[10,44,45].

Clinical relevance of clarithromycin resistance in *H. pylori*

Studies have shown that clarithromycin resistance in *H. pylori* substantially affects the success rate of eradication regimens containing clarithromycin (Table 1). Generally, dual therapy with an antisecretory agent (e.g., H₂ antagonist or proton pump inhibitor) and clarithromycin achieves eradication rates of 60% to 80% for susceptible strains, but less than 40% for resistance strains (Table 1). Triple therapy with an antisecretory agent, clarithromycin and another antibiotic (i.e., amoxycillin or metronidazole) increases the eradication rates to 80%-95% for susceptible strains, but the rates remain under 40% for resistant ones (Table 1). A preliminary study reported that a combination of ranitidine bismuth citrate and

clarithromycin eradicated *H. pylori* at a rate of 98% and 92%, respectively, for both susceptible and resistant strains, but remains to be confirmed^[13].

Current anti *H. pylori* treatment regimens consisting of clarithromycin do not achieve an eradication rate of 100%. Emergence of clarithromycin-resistant strains during ineffective treatment has also been observed; the prevalence of clarithromycin-resistant strains cultured after treatment ranges between 40% and 100% (Table 1). This implies a likelihood of potential spread of clarithromycin-resistant strains in the population. Thus, the prevalence of clarithromycin resistance in *H. pylori* may exhibit a similar trend to the prevalence of metronidazole resistance in *H. pylori*. In Ireland, the prevalence of metronidazole resistant strains was 7% in 1989, 34% in 1992 and 38% in 1996^[17]. In Australia, the prevalence of metronidazole resistance was 17% in 1988, but increased to 40% in 1995 and over 60% in 1998^[11,47]. It is most likely that this increase is due to the use of metronidazole as a key agent in classic triple therapy (consisting of bismuth, metronidazole and tetracycline or amoxycillin), or increased use of this drug for other infections. Similarly, the current prevalence of clarithromycin-resistant strains of 6%-8% in Australia is much higher than the rate of 1.9% reported four years ago in this country^[11,12,48]. This increase in the prevalence of clarithromycin resistance has been also reported in Europe and the United States^[14,20,27,49]. It is assumed that prescriptions of macrolides, especially the new members such as spiramycin, roxithromycin, azithromycin and clarithromycin have been increased over the past years for the treatment of respiratory infection, sexually transmitted diseases and other infectious diseases. Thus, patients treated with any member of macrolides alone may select macrolide resistant *H. pylori* organisms (if infected), as cross-resistance exists between macrolides.

Overall, *H. pylori* resistance to clarithromycin is of less clinical relevance as compared with resistance to metronidazole, mainly because of the low prevalence and the possible reversibility of resistance in some strains. Susceptibility testing is not routinely required before treatment because of the low prevalence of clarithromycin resistance (Figure 1). However, *H. pylori* should be cultured and tested for clarithromycin susceptibility in patients who have failed the therapy containing clarithromycin (Table 1). Moreover, any previous use of macrolides not aimed at anti-*H. pylori* infection should be also taken into account when clarithromycin is chosen for eradication of *H. pylori*.

Table 1 Effect of primary clarithromycin resistance on the efficacy of eradication therapy for *Helicobacter pylori* infection

Authors	Treatment regimens	Eradication rate (%)		Prevalence of resistant strains post-treatment (%)*
		Susceptible strains	Resistant strains	
Liu <i>et al.</i> , 1996 ^[5]	LFC or BFC	98(45/46)	0(0/4)	100(5/5)
Suzuki <i>et al.</i> , 1998 ^[8]	LAC	94(66/70)	0(0/1)	40(2/5)
Miyaji <i>et al.</i> , 1997 ^[9]	OC or LC	64(9/14)	0(0/5)	80(20/25)
	OAC or LAC	85(57/67)	28(2/7)	(Overall)
	OCM or LCM	86(68/79)	38(3/8)	
Maeda <i>et al.</i> , 1998 ^[10]	LAC	85(29/34)	40(2/5)	63(5/8)
Megraud <i>et al.</i> , 1997 ^[13]	OC	70(33/47)	38(3/8)	81(17/21)
	RbCC	98(42/43)	92(11/12)	(Overall)
Debets-Ossenkopp <i>et al.</i> , 1996 ^[16]	RC	81(58/72)	0(0/1)	73(11/15)
Tompkins <i>et al.</i> , 1997 ^[18]	OC	80(101/127)	0(0/4)	74(14/19)
Moayyedi <i>et al.</i> , 1998 ^[19]	OCT	91(104/114)	40(2/5)	Nor reported
Schutze <i>et al.</i> , 1996 ^[24]	RC	75(21/28)	20(1/5)	91(10/11)
Laine <i>et al.</i> , 1998 ^[25]	AC	35(73/208)	7.7(2/2.6)	53(70/131)
	OAC	81(153/190)	27(4/15)	(Overall)
Yousfi <i>et al.</i> , 1996 ^[26]	RanMC	87(20/23)	25(1/4)	67(4/6)
Buckley <i>et al.</i> , 1997 ^[46]	OMC	85(71/84)	0(0/3)	58(7/12)

O, omeprazole; C, clarithromycin; A, amoxycillin; Ran, ranitidine; M, metronidazole; Rbc, ranitidine bismuth citrate; L, lansoprazole; Rox, Roxithromycin; B, colloidal bismuth subcitrate (CBS).

*The number of resistant strains post treatment was greater than the number of resistant strains before treatment in all the studies, suggesting acquisition of clarithromycin resistance during the unsuccessful treatment.

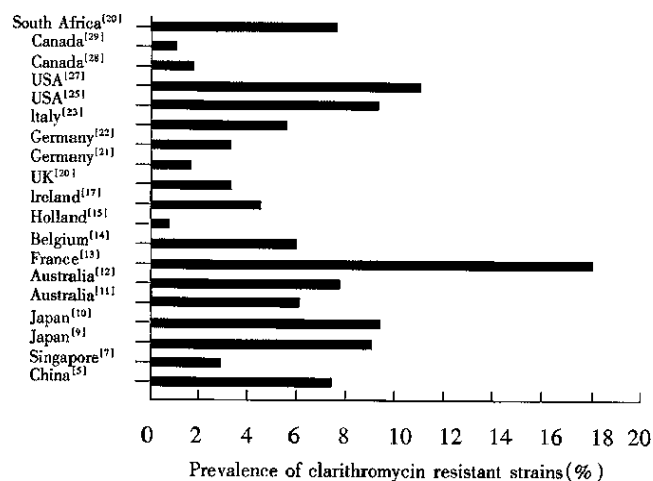


Figure 1 Worldwide prevalence of clarithromycin resistant *H. pylori* strains. Each of these studies included at least 50 strains. The number after each country is the reference number.

Conclusions

The prevalence of clarithromycin resistant *H. pylori* is low, but appears to be increasing. Point mutations in the 23S rRNA gene, mainly at the positions 2142 and 2143 with a transition of A→G, are responsible for the resistance. Although current triple therapies containing clarithromycin are able to eradicate up to 90% of susceptible strains, the eradication rates may be significantly reduced for resistant strains. Moreover, unsuccessful treatment with regimens containing clarithromycin can be associated with acquisition of resistance to the drug, which may explain the increasing rate of clarithromycin resistance.

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