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Leading article

Antimicrobial resistance and *Helicobacter pylori*

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Twelve years since its initial isolation and culture by Marshall and Goodwin, *Helicobacter pylori* is now firmly established as the principle cause of chronic gastritis and peptic ulcer, with eradication therapy indicated in all patients with an active or previous *H. pylori*-associated peptic ulcer (NIH Consensus Development Panel, 1994). Indeed, the impact of *H. pylori* on the management of dyspepsia is such that eradication therapy is often used empirically in dyspeptic patients in primary care without documenting the presence of either infection or an ulcer (Milne *et al.*, 1995), even though studies have failed to show a role for *H. pylori* in non-ulcer dyspepsia (NIH Consensus Development Panel, 1994). However, on the basis of seroepidemiological data, the World Health Organisation has recently classified *H. pylori* as a Type-I carcinogen (International Agency for Research on Cancer, 1994), further highlighting the importance of this common pathogen.

Initially, treatments against *H. pylori* involved complex multiple drug regimens containing bismuth and two antibiotics (metronidazole and either amoxycillin or tetracycline). However, treatment failure was common due to poor patient compliance and metronidazole resistance in *H. pylori* (Glupczynski & Burette, 1990). As a consequence, eradication of infection was only undertaken in patients with severe or chronic disease. Simpler treatment regimens of either amoxycillin or clarithromycin combined with proton pump inhibitors (PPIs) (e.g. omeprazole or lansoprazole) as potent inhibitors of acid secretion, were tried but were compromised by inconsistent efficacy, side-effects and cost (Penston, 1994). Lately, 1-week, low-dose combinations of two from three antibiotics (clarithromycin, amoxycillin and a nitroimidazole) with a PPI have consistently achieved eradication rates >90% (Goddard & Logan, 1995). As the efficacy of anti-*H. pylori* therapy improves, however, the mechanisms behind treatment failure become more difficult to study. Numerous factors have been implicated but the most important are poor patient compliance, inadequate drug-delivery and antimicrobial resistance (Malfertheiner, 1993).

Several problems surround antimicrobial susceptibility testing of *H. pylori* and have led many clinicians to underestimate the importance of such testing. The inherent difficulties of sample collection by endoscopic biopsy in part mean that considerable care (including use of fresh plates and humid conditions) is required for routine isolation to be successful (Goodwin *et al.*, 1985), as well as the need for rapid transport and use of appropriate transport media before culture.

At present, there is no standardized method for susceptibility testing of this organism. Even under optimal conditions the organism can show variable growth making classical susceptibility tests difficult to interpret or the results from different studies difficult to compare (DeCross *et al.*, 1993). In addition, the relative merits and clinical significance of tests based on MIC_{50}, MIC_{90}, and MBC remain unclear and the testing parameters of inoculum size, growth phase of organism, culture medium, atmosphere and duration of incubation all need to be evaluated and validated. Disc-diffusion techniques may be simple and quick (Xia *et al.*, 1994), but are less suitable for slow growing bacteria such as *H. pylori*. Agar dilution methods are more reliable and reproducible but are time consuming and expensive. The recently developed E-test, (a semi-quantitative variant of the disc diffusion test using a non-porous plastic with an exponential concentration gradient of antimicrobial agent), has been validated for sensitivity testing of *H. pylori* (Glupczynski *et al.*, 1991) and may prove to be the ideal method for assessing antimicrobial resistance of *H. pylori* for routine clinical practice. However, the correlation between in-vitro sensitivity and in-vivo effectiveness may not be complete (i.e. pharmacological resistance). In addition, there is some uncertainty as to whether resistance occurs through selection of resistant strains within a

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mixed population in an individual (i.e. primary resistance) or development of resistance by previously sensitive strains (i.e. secondary resistance).

The prevalence of metronidazole resistant (MR) *H. pylori* varies between different populations and between different groups within these populations (European Study Group, 1992). As a general rule, prevalence is lower in developed countries compared to developing countries, though certain populations within developed countries may also have high rates of matronidazole resistance due to early acquisition of resistant strains in their country of origin. For example, the overall prevalence of MR *H. pylori* in the UK is 26%, but this increases to 90% in inner-city populations (Karim & Logan, 1995). In a study of Bangladeshi inhabitants of the East End of London (Banatvala *et al.*, 1994), the prevalence of MR *H. pylori* was far higher in those patients born in Bangladesh (possibly secondary to use of metronidazole for diarrhoeal disease) than in those patients born in the UK. This study, consistent with others, also showed MR *H. pylori* were more frequently isolated from women compared to men: this may be related to greater use of nitroimidazoles by women. Significantly, two reports suggest that the prevalence of resistance in developed countries is increasing, though this may reflect previous sampling errors unmasked by increased surveillance in some cases.

Several studies have documented the relationship between nitroimidazole resistance within individuals and failure of standard bismuth-based triple therapy for *H. pylori* (Table). Whether such an effect occurs with low-dose 1-week therapy is less clear. Buckley *et al.* (1995) found eradication rates of 90% in patients with metronidazole sensitive *H. pylori*, but only 47% in those with MR *H. pylori*. Moayyedi *et al.* (1995), however, found no such difference and although this group used tinidazole rather than metronidazole, concordance of *H. pylori* susceptibility to both drugs seems complete. The difference may relate to methodological variation, emphasising the importance of standardisation for in-vitro testing. The development of MR *H. pylori* following unsuccessful bismuth-based triple therapy is also recognised and may explain why treatment is less likely to succeed in those whom have received previous treatment compared with those being treated *de novo*.

In vitro, co-administration of bismuth with nitromidazoles limits the emergence of MR *H. pylori* (Goodwin *et al.*, 1988). Ranitidine bismuth citrate (the bismuth salt of ranitidine recently launched in the UK by Glaxo-Wellcome) has also been shown to limit the development of resistance to several antimicrobial agents *in vitro*, although the clinical relevance of this observation remains untested.

The mechanisms behind nitroimidazole resistance are complex and little work has been done in this area. Several mechanisms have been proposed including interference with drug penetration across the cell wall, decreased nitro-reduction within the organism or enhanced DNA-repair mechanisms. Smith & Edwards (1995) showed that the mechanism of action of the nitroimidazoles is related to their redox potential and that anaerobiosis abolishes resistance to metronidazole, therefore suggesting that the mechanism is probably mediated through the activation of anaerobic metabolic pathways which function less or not

Table. The effect of metronidazole resistance by *H. pylori* on the efficacy of bismuth-based triple therapy

Study	Regimen	Duration (days)	No. of patients	Eradication rate susceptible	Eradication rate resistant
Burette, Glupczynski & De Prez (1992)	CBS, amoxycillin, metronidazole	14	57	97%	63%
Bell *et al.* (1992)	CBS, tetracycline, metronidazole	14	40	91%	32%
Rautelin, Kosunen & Seppälä (1992)	CBS, amoxycillin, metronidazole	14		91%	63%
Lian *et al.* (1993)	CBS, tetracycline, metronidazole	14	71	86%	43%

CBS, Colloidal bismuth subcitrate.

at all under microaerophilic conditions. A recent study by Hoffman *et al.* (1995) has shown that the pyruvate:oxidoreductase activity (thought to be necessary for the reduction of metronidazole to its toxic form) of MR *H. pylori* is lower than that of sensitive strains and also that the acquisition of this mechanism is unlikely to be plasmid-borne. Metronidazole-resistance appears to be stable and reversion to susceptible strains has not been observed.

Clarithromycin, unlike older macrolides such as erythromycin, is very effective against *H. pylori in vivo* and is widely used in combination with a PPI with or without a nitroimidazole. However, clarithromycin-resistant *H. pylori* have already been isolated and although the prevalence elsewhere is generally low (<5%), in France and Belgium, two countries where consumption of macrolides is high, the prevalence is about 10%. From the limited data available, the prevalence of clarithromycin resistant *H. pylori* following treatment failure is between 20–60% (Cayla *et al.*, 1995), and both primary and secondary clarithromycin resistance appear to predict treatment failure. The long term clinical significance of this, however, is unclear since one in-vitro study has shown clarithromycin resistance may not be stable.

The underlying processes behind acquisition of macrolide resistance have recently been elucidated by Versavolic *et al.* (1995). In this study, post-transcriptional adenylation and point mutations within the 23S ribosomal RNA (i.e. the site of action of macrolides) were shown to decrease the affinity of clarithromycin for the 23S ribosome component and thus interfere with antimicrobial activity.

Antimicrobial resistance has also been seen with other groups of drugs. For ciprofloxacin, once used to treat *H. pylori* but now avoided due to rapid emergence of resistance following failed treatment, resistance has been shown to be associated with single point mutations in the bacterial genome (Moore *et al.*, 1995). More significantly, a national UK surveillance study of antimicrobial resistance has found *H. pylori* isolates resistant to tetracycline, a key component of bismuth-based triple therapy (Karim & Logan, 1995). This finding, though, needs to be confirmed by other groups before its true significance is understood. Fortunately, so far, *H. pylori* has not been shown to develop resistance to any of the penicillins. However, despite good in-vitro activity against *H. pylori*, these antibiotics are of variable benefit *in vivo*.

What, then, are the implications of antimicrobial resistance for the treatment of *H. pylori* infection? For patients infected with sensitive strains the current regimens are highly effective making resistance of minor importance. However, in patients with *H. pylori* resistant to nitroimidazoles, bismuth-based triple therapy is likely to fail and subsequent successful treatment will be more difficult still. Susceptibility to nitromidazoles and clarithromycin may also be important for low-dose 1-week triple therapies. Identification of patients infected with resistant strains, either by knowledge of the prevalence of resistance in a particular population or by the direct assessment in individuals, will allow the use of alternative treatments. Such assessments can only be made if the facilities are available, but as yet the routine testing of *H. pylori* sensitivities is unavailable in most centres in Europe.

On the evidence available, we suggest that where the prevalence of metronidazole and/or clarithromycin resistance by *H. pylori* is low, routine pre-treatment susceptibility testing is not required, but the prevalence of resistant strains should be monitored at a regional or national level. Such monitoring would obviate the need for all departments to perform routine susceptibility testing but would require an agreed standardized protocol.

If the prevalence of resistance is high, and the recommended eradication regimens are known to be less effective in patients infected with resistant strains, we would advise routine pre-treatment testing in all patients being considered for *H. pylori* treatment. The definition of high prevalence is somewhat arbitrary, although greater than of 30% and 5% for metronidazole and clarithromycin, respectively, would not be unreasonable in light of the limited data available on the effect of antimicrobial resistance on the efficacy of treatment. Of course, in developing countries where MR *H. pylori* is endemic, it may be assumed that everyone has MR *H. pylori* and only macrolide resistance need be monitored. Together with the high rate of re-infection in these populations this emphasises the need for the development of simple effective regimens for patients infected with resistant *H. pylori*.

In our opinion, whilst the clinical significance of any extra-gastric consequences of *H. pylori* infection remain unclear, the high efficacy and tolerability of newer treatment regimens make it likely that reluctance to use eradication therapy will fade, resulting in more patients being given such treatment

Moreover, if global eradication is deemed a realistic and desirable goal, the development of multi-drug resistant organisms will hinder this. It is therefore important for gastroenterologists and microbiologists to take steps to curb such developments by actively discouraging the use of ineffective and resistance-promoting treatments as well as monitoring changes in the pattern of antimicrobial resistance. It is also vital that susceptibility testing methods are standardized and validated to allow comparisons to be made between different studies, as without such manoeuvres it may be impossible to monitor the clinical impact of antimicrobial resistance on a world-wide scale.

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ANDREW F. GODDARD[a]
ROBERT P. H. LOGAN[b]
[a]*Division of Gastroenterology*, and [b]*Institute of Infections and Immunity, University Hospital, Nottingham NG7 2UH, UK*
Tel: +44-115-970-9918; Fax. +44-115-942-2232.

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