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Helicobacter pylori eradication in Western Australia using novel quadruple therapy combinations

C. Y. Tay, H. M. Windsor, F. Thirriot, W. Lu, C. Conway, T. T. Perkins & B. J. Marshall

School of Pathology and Laboratory Medicine M502, University of Western Australia, Nedlands, WA, Australia.

Correspondence to:
Dr C. Y. Tay, School of Pathology and Laboratory Medicine M502, University of Western Australia, Nedlands 6009, WA, Australia.
E-mail: alfred.tay@uwa.edu.au

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SUMMARY

Background
Helicobacter pylori eradication rates with standard triple therapy are declining worldwide. The optimal management of *H. pylori* is evolving and new treatment combinations for antibiotic resistant *H. pylori* strains are required, especially for patients with penicillin allergy.

Aim
To review the effectiveness of alternative antibiotic combinations and necessity of pre-antibiotic sensitivity testing.

Methods
A total of 310 consecutive patients who had failed at least one course of standard 7-day triple therapy initially prescribed by their physicians were included in this study between year 2007 and 2011. Antibiotics were prescribed based on pre-antibiotic sensitivity tests and, if any, patient's allergy to penicillin.

Results
In 98.7% of the patients' samples, *H. pylori* was successfully cultured. The proportion resistant to clarithromycin and metronidazole was 94.1% and 67.6% respectively, with 65% resistant to both. For the in-house primary quadruple therapy, with Proton pump inhibitor, Amoxicillin, Rifabutin and Ciprofloxacin (PARC), *H. pylori* was successfully eradicated in 95.2% of patients. For patients allergic to amoxicillin, an alternative quadruple therapy using Proton pump inhibitor, Bismuth subcitrate, Rifabutin and Ciprofloxacin (PBRC) gave an eradication rate of 94.2%. Patients needing alternative salvage therapy were given novel personalised combinations consisting of bismuth, rifabutin, tetracycline or furazolidone; the eradication rate was 73.8%.

Conclusions
Patients who present with antibiotic resistant *H. pylori* can be confidently treated with PARC, PBRC or other personalised salvage therapies. These regimens can be used when treatment options are limited by penicillin allergy. Pre-treatment *H. pylori* antibiotic sensitivity tests contributed to the high eradication rate in this study.

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INTRODUCTION

Antibiotic treatment and eradication of the gastric pathogen, *Helicobacter pylori*, is complex, involving multiple antibiotics that are combined with proton pump inhibitors (PPI) to increase the pH of the stomach for the duration of the course of the antibiotics to ensure high eradication rates. Treatment failure is generally attributed to lack of compliance with the drug regimen or infection by antibiotic resistant strains.[1] *H. pylori* infection is currently known to be associated with peptic ulcer disease, non-ulcer dyspepsia, premalignant lesions, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. Although the incidence of gastric cancer has declined in developed countries, it remains one of the most common types of cancer worldwide.[2–4] The prevalence of *H. pylori* infection varies significantly between developed (10–30%) and developing countries (70–90%).[5] In general, *H. pylori* prevalence is higher in lower socioeconomic groups, institutionalised individuals and those who have migrated from the developing world.[6, 7] In Australia, the prevalence of *H. pylori* was reported to be between 25% and 35%[8, 9] and the rate of failure to eradicate *H. pylori* has recently gradually increased.[10–12] In addition, there has been a significant increase in the number of migrants coming to Australia from the neighbouring developing countries.[13] It is therefore reasonable to expect an increase in the number of *H. pylori* cases in Australia.

The current first line triple therapy, recommended by the first Maastricht consensus report,[14] consists of a PPI, amoxicillin (AMX) and clarithromycin (CLR) or metronidazole (MTZ) and has generally been adopted worldwide. The current efficacy of this treatment is almost 80%; however, due to the increase in prevalence of CLR resistance, the failure rate of this treatment can be as high as 30%.[15] Prevalence of bacterial resistance varies in different geographical areas, and it has been shown to correlate with the consumption of antibiotics in the general population.[11, 16] For example, careful use of macrolide antibiotics in Northern European countries resulted in a reduced rate of *H. pylori* CLR resistance as compared with Central and Southern European countries, where CLR is widely prescribed.[10, 12, 17, 18] During the last two decades, widespread use of antibiotics, such as CLR for respiratory infections, MTZ for anaerobic bacterial infections and levofloxacin for urinary tract infections, has increased the occurrence of primary *H. pylori* resistance.[10, 11, 16] Therefore, the optimal management of *H. pylori* after failed eradication therapy is evolving and new treatments for antibiotic resistant *H. pylori* strains are required. There is also the need for a successful therapy for patients who are allergic to AMX and have failed first–line treatment. This study reports the antibiotic resistance of *H. pylori* in referred patients, reviews the necessity of pre-antibiotic sensitivity testing and ascertains the effectiveness of alternative antibiotics such as rifabutin (RFB), ciprofloxacin (CIP), bismuth subcitrate (BIS), furazolidone (FZD) and tetracycline (TET), in patients who have failed the standard triple therapy with PPI plus AMX, CLR and/or MTZ.

MATERIALS AND METHODS

Patients

A total of 310 consecutive patients attended the *H. pylori* Research Laboratory at Sir Charles Gairdner Hospital, Perth, Australia from 2007 to 2011. Median age for the patients was 53 years (range: 16–85) and the gender ratio was 91 male to 219 female. Patients were referred to the *H. pylori* Research Laboratory after failing the 7-day *H. pylori* standard triple therapy (PPI + AMX + CLR) and usually also failed a second 7-day or 14-day therapy of the same treatment or one with CLR replaced by MTZ. Penicillin-allergic patients had usually failed a PPI + CLR + MTZ therapy.

Helicobacter pylori infection was proven by positive bacterial culture of *H. pylori* or histology. At least 4 weeks after completion of treatment, the *H. pylori* status of the patients was determined by a C^{14}-urea breath test (UBT). Alternative quadruple therapy was provided when the initial in-house quadruple treatment failed. Analysis of *H. pylori* eradication efficacy was considered on an intention-to-treat basis including all eligible patients enrolled in the study regardless of compliance with the study protocol.

Isolation of *Helicobacter pylori*

At upper gastrointestinal endoscopy, an antral and a corpus biopsy were each transported in 0.2 mL saline to the *H. pylori* Research Laboratory at the University of Western Australia for culture. The biopsies were diced into small fragments with a sterile scalpel and then inoculated onto two agar plates, one nonselective and the other, selective. The nonselective plates used were Columbia blood agar plates (CBA) (Columbia agar base; Oxoid, Adelaide, Australia) with 5% horse blood and the selective plates were Pylori plates (bioMérieux, Marcy. L'Étoile, France) or CBA plates with 'Dent' supplement (Oxoid). After 72–96 h of incubation at 37°C and 10% CO_2, six to eight *H. pylori* colonies were subcultured separately onto a fresh nonselective CBA and incubated for additional 72 h. The growth of the subcultured colonies was examined by Gram

stain and tested for catalase, oxidase and urease activity before being pooled together for antibiotic sensitivity testing. A cohort of 23 patients did not have an endoscopy, but had *H. pylori* isolated using the Entero-Test Hp string test (HDC Corporation, Mountain View, CA, USA).[19] In the laboratory, the bacteria adhering to the string were washed off with brain heart infusion broth (BHIB) and plated onto selective media plates. The further isolation of *H. pylori* continued as described above.

Antibiotic sensitivity test

Antibiotic sensitivity testing was performed using e-Test (bioMérieux, Murarrie, Australia) for AMX, CLR, MTZ, TET, Rifampicin (RIF) and CIP. Resistance was defined according to National Committee for Clinical Laboratory Standards (NCCLS)[20]: AMX, minimum inhibition concentration (MIC) ≥ 2 μg/mL; CLR, MIC ≥ 1 μg/mL; MTZ, MIC ≥ 8 μg/mL; TET, MIC ≥ 1 μg/mL; RIF, MIC ≥ 4 μg/mL; and CIP, MIC ≥ 1 μg/mL.

Quadruple therapies

The in-house standard primary quadruple therapies, PBRC and PARC (Table S1), were designed for patients with and without penicillin allergy respectively. The PARC consists of Rabeprazole (20 mg, 3 times daily for 10 days), AMX (1000 mg, 3 times daily for 10 days), RFB (150 mg, starting from day 6, 2 times daily for 5 days) and CIP (500 mg, starting from day 6, 2 times daily for 5 days), whereas, PBRC consists of Rabeprazole (20 mg, 3 times daily for 10 days), Bismuth subcitrate (BIS) (240 mg, 4 times daily for 10 days), RFB (150 mg, 2 times daily for 10 days) and CIP (500 mg, 2 times daily for 10 days). In cases where patients were not successfully treated by PARC or PBRC treatment or were carrying *H. pylori* proven resistant to CIP and/or RIF, a more personalised therapy was prescribed. The personalised therapy was principally dependent on the pre-antibiotic sensitivity result. Patients were mainly given FZD (100 mg, 3 times daily for 10 days) in combination with PPI, BIS and one other antibiotic. The choice of other antibiotic included AMX, CIP, MTZ, RFB and TET (500 mg, 4 times daily for 10 days).

RESULTS

In the period 2007–2011, *H. pylori* was successfully cultured from 306 patients (98.7%) of the 310 patients attending this research clinic. Most of the patients were aged between 36 and 65 years (Figure 1). The number of female patients ($n = 219$) was more than double the number of male patients ($n = 91$) and this difference is significant according to the Western Australian population statistic (average for the age groups with the greatest numbers).[21] Analysis of the nature of antibiotic resistance of CLR and MTZ did not show any correlation with the gender.

A high resistance rate to CLR and MTZ was observed among these patients as all of them had completed at least one course (usually two) of the standard 7-day triple therapy containing PPI and combinations of AMX, CLR or MTZ before they were referred to this research clinic. The proportion resistant to CLR and MTZ was 94.1% ($n = 288$) and 67.6% ($n = 207$) respectively, with 65% ($n = 199$) resistant to both. Comparing the resistance rates between 2007 and 2011 (Table 1), MTZ was shown to be stable at 66.7–71.4% and CLR was shown stable at 95.4–98.6% except for year 2011 where the resistance rate was observed at 71.0%. Antibiotic resistance rates to CIP, RIF, AMX and TET were recorded as 5.6%, 2.0%, 0% and 0% respectively over the time period (Table 1).

The PARC quadruple treatment was primarily prescribed to patients who were proven not allergic to penicillin and the *H. pylori* isolated was shown sensitive to RIF and CIP by pre-treatment antibiotic sensitivity testing. It was the most frequently prescribed treatment to the patients in this research clinic. A total of 210 (or 67.7%) patients (female = 138; male = 72) received the PARC treatment during the period 2007–2011. All patients received a post-treatment C^{14}-UBT to determine the eradication rate. The PARC treatment demonstrated a high cure rate of 95.2% (or $n = 200/210$) (Table 2). Five of the ten patients who failed this treatment wished to receive alternative personalised quadruple treatment without further endoscopy.

The PBRC treatment was primarily prescribed for patients who had a history of penicillin allergy. A total

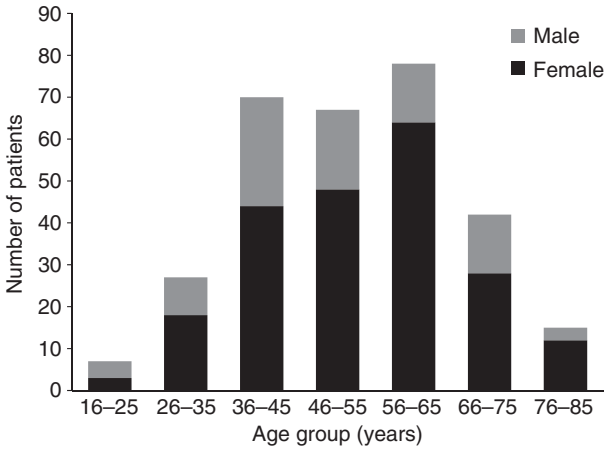

Figure 1 | Number of *Helicobacter pylori* patients classified by gender and age.

of 69 (or 22.2%) patients (female = 60; male = 9) received this treatment. PBRC demonstrated a cure rate of 94.2% by post-treatment UBT (Table 2). Two of these four patients who were not successfully cured were further treated with the alternative personalised treatment.

The alternative personalised treatments, which mainly consisted of FZD, when prescribed as primary salvage treatment, delivered a cure rate of 77.4% (n = 31); and when the 11 patients who were not successfully treated with PARC or PBRC were included, the cure rate was 73.8% (n = 42). The different combinations of antibiotics and the cure rates for patients who received these regimens are listed in Table 3. Only 10% of the patients included in this study received these treatment combinations. They were given these treatment combinations as antibiotic sensitivity testing revealed that their *H. pylori* isolates were resistant to CIP and RIF. Four of eight of these patients who failed eradication with one combination therapy were further treated with an alternative treatment and 50% (n = 4) of them were cured.

DISCUSSION

This study reviews the antibiotic resistance profiles and treatment plans for patients at the *H. pylori* Research Laboratory over the 5-year period between 2007 and 2011. These patients were not randomly selected and the resistance rates shown in this study do not reflect the entire pre-treatment Australian *H. pylori* population. This study was not controlled or blinded as it included a large cohort of consecutive patients referred to this research clinic by their general practitioners after (usually two) treatment failures. According to the antibiotic resistance profile collected in this study, the failure of the standard triple therapy, in agreement with many others,[11, 22, 23] is mainly due to the emergence of antibiotic-resistant strains (especially for CLR and MTZ). CLR and MTZ are two of the most commonly prescribed antibiotics for respiratory tract and anaerobic infections respectively. Frequent use of these antibiotics, not necessarily for *H. pylori* infections, contributes to the resistance of *H. pylori* to these two antibiotics.[24] Moreover, *de novo* antibiotic resistance can be easily introduced by a simple mutation in the *H. pylori* chromosome e.g. mutations in the 23S rRNA, *gyrA*, *rpoB* and *rdxA* genes are associated with CLR, CIP, RIF and MTZ resistance respectively.[25–30] Nevertheless, the authors believe that the CLR/MTZ-based triple therapy is still effective as a first-line treatment, at least within Australia.

Female patients are more likely to carry *H. pylori* strains resistant to both CLR and MTZ than male

Table 1 | *Helicobacter pylori* antibiotic resistance rates (percentage) from 2007 to 2011

	n	CLR (%)	MTZ (%)	CIP (%)	RIF (%)	AMX (%)	TET (%)
2007	54	96.3	66.7	3.7	0.0	0.0	0.0
2008	70	98.6	71.4	7.1	0.0	0.0	0.0
2009	86	96.5	67.4	7.0	3.5	0.0	0.0
2010	65	95.4	63.1	3.1	0.0	0.0	0.0
2011	31	71.0	71.0	6.5	9.7	0.0	0.0
2007–2011*	306	94.1	67.6	5.6	2.0	0.0	0.0
Female	217	93.5	70.5	6.0	1.8	0.0	0.0
Male	89	95.5	60.7	4.5	2.2	0.0	0.0

AMX, amoxicillin; CIP, ciprofloxacin; CLR, clarithromycin; MTZ, metronidazole; RIF, rifampicin; TET, tetracycline.

* *H. pylori* was isolated from 306 of 310 patients (98.7%).

Table 2 | Eradication rate of the therapy prescribed

Treatment	*n*	*H. pylori* eradicated	Success rate	PPI	AMX	BIS	RIF	CIP	MTZ	TET	FZD
PARC	210	200	95.2%	●	●		●	●			
PBRC	69	65	94.2%	●		●	●	●			
Personalised Therapy*	(31, 11)	(26, 5)	(77.4%, 45.4%)	●	◐	◐	◐	◐	◐	◐	◐
Total	310										

Symbol '●', prescribed drug; symbol '◐', optional drug; AMX, amoxicillin; BIS, bismuth subcitrate; CIP, ciprofloxacin; FZD, furazolidone; MTZ, metronidazole; PPI, rabeprazole; RIF, rifabutin; TET, tetracycline.

* Used as first-line treatment, used as second-line treatment.

Table 3 | The different combination of antibiotics used in personalised treatment

Personalised treatment	*n*	Cured	Rate (%)
PBAF	15	11	73.3
PBRF	4	4	100.0
PMA	3	2	66.7
PBAT	2	0	0.0
PBTF	2	2	100.0
PBTM	2	2	100.0
PBAC	1	1	100.0
PBR	1	1	100.0
PBRMF	1	1	100.0
	31	24	77.4

A, AMX; B, BIS; C, CIP; F, FZD; M, MTZ; P, PPI; R, RFB; T, TET.

patients due to the higher incidence of previous treatment of gynaecological conditions with antibiotics.[11, 31–34] Although the resistance rate between genders was not proved statistically significant in this study (P-value$_{MTZ}$ = 0.11; P-value$_{CLR}$ = 0.77), the higher ratio of female-to-male patients, especially among the older women, suggests that mature women are more likely to carry antibiotic-resistant strains, which then lead to a poorer eradication rate in standard MTZ/CLR-based treatment.

The in-house primary PARC treatment plan contained a triple dose of PPI (Rabeprazole), a high-dose AMX (3 g/day), a restricted antibiotic (RFB) and a quinolone (CIP). Similar to other RFB-based triple therapy studies,[34, 35] this treatment was shown to deliver a high cure rate of 95.2% (n = 210). The high-dose AMX (3 g/day) 10 days' treatment was adopted from the 'old' dual therapy which was reported with a high cure rate (>90%) in the early years of *H. pylori* treatment.[36, 37] However, the dual therapy only achieved a 67% cure rate in one of our earlier trials.[38] As a result, RFB and a quinolone, a method adopted from Wong WM *et al.*,[34] were combined with the dual therapy to improve the cure rate. CIP was chosen as the quinolone because of its affordability and easy access. Interestingly, although the efficacy of CIP had been reported to underperform in acidic conditions and is currently discouraged by the Maastricht consensus report IV,[1] some studies have shown that the efficacy can be improved by having high dosage of PPI.[39, 40] On the other hand, RFB combinations with newer alternative quinolones such as norfloxacin, levofloxacin, moxifloxacin and gatifloxacin have been frequently reported with successful rates >90% in recent years. The consistent success of PARC/PBRC therapy shown in this study suggests that it should be continued as the appropriate combination, at least in Australia and other countries with low quinolone resistance. The only concern from this high-dose antibiotic therapy was the possibility of other complications such as *Clostridium difficile* colitis.[41, 42] However, not a single case of such a complication was reported from the treatment group. Other common adverse side-effects such as diarrhoea, loose stools, nausea, headache, joint pain and rashes were reported from 11% of our patients taking the PARC treatment. As for patients who were allergic to penicillin (n = 69), this study showed that PBRC therapy (AMX substituted by BIS) was able to achieve a cure rate of 94.2%. Bismuth subcitrate is a mineral that has been widely used for treating ulcers. It is known not only for its topical bactericidal effects and the synergistic effects with other antibiotics, but most of all, *H. pylori* has never been known to develop resistance to it.

Personalised treatments (mainly PBAF) were designed for patients who were shown to carry *H. pylori* resistant to RFB or CIP and for those who were not cured with PARC/PBRC. This study has demonstrated a cure rate of 77.4% (n = 31); and when we included the 11 patients who failed PARC/PBRC, the cure rate became 73.8% (n = 42). The combinations of BIS, TET and FZD had been previously reported with high efficacy,[43–45] but they were not commonly prescribed due to their adverse side-effects and consequent poor compliance. In addition, FZD is well known to be associated with many adverse side-effects and it is currently restricted in many European countries and the United States.[45] Indeed, FZD is reported as a mutagenic, genotoxic and potentially carcinogenetic.[46, 47] However, MTZ is also listed as a potential carcinogen by FDA and yet it is commonly used worldwide.[45] The sensitivity of *H. pylori* to furazolidone seems to vary from one geographical region to another. For example, 1.3% was reported in Bulgaria,[48] 2% in Spain,[49] 4.5% in Iran,[50] 8.7% in China, Zhengjiang province[51] and 13% in Brazil.[52] Nevertheless, the low cost and high efficacy made it a good alternative antibiotic.[50, 53, 54] High-dose FZD has been reported to increase the cure rate, but also significantly increases the incidence of severe side-effects.[45] About 50% of the patients who received this treatment reported at least one of the following side-effects: diarrhoea, loose stool, dark urine, headache or nausea. As a result, for these reasons, unless proven *H. pylori* eradication failed in all other possible treatments, FZD should be avoided.

Rifampicin resistance in *H. pylori* was rare, but was observed in 3 cases in both 2009 and 2011. Investigation revealed that all of these patients had previous exposure to

RFB treatment for either mycobacterial or meningococcal infections. In addition, one patient had prematurely ceased an RFB-based anti-*H. pylori* treatment. Pre-exposure to RFB plays an important role in its resistance in *H. pylori* infection.[55, 56] In Australia, because of tight restrictions imposed on prescribing RFB, resistance was not commonly expected. Despite its useful synergism with other antibiotics, in rare cases, it is known to associate with serious adverse effects (mainly myelotoxicity). Frequent use of RFB in populations with a high prevalence of tuberculosis might lead to the development of resistant *M. tuberculosi.*[57, 58] As a result, in agreement with the Maastricht IV Consensus Report,[1] it should remain restricted only to patients who had failed multiple previous eradication regimens.

A mean CIP resistance rate of 6% was detected in this study. This rate was considered low when compared to 15.6% in Nigeria,[59] 20.9% in Portugal,[18] 14.3% in Spain[60] and 15.7% in Korea.[61] In many other countries, due to the overall increase in the consumption of fluoroquinolones for various other infections (such as urinary and respiratory tract infections), there has been an increase in fluoroquinolone resistance. For example, Portugal had 0% resistance rate in 1990–1993, but 20.9% in 1998–1999[18]; Taiwan had 2.8% resistance rate in 1998–2003, but 11.8% in 2004–2007[62]; and South Korea had 0% resistance rate in 1987, but 33.3% in 2003.[63] Then again, although the resistance rate to CIP was shown low in this study of refractory *H. pylori* infection in Western Australia, due to the ease of acquiring resistance, care should always be taken when prescribing fluoroquinolones. Of interest, very few studies had used CIP in combination treatments. Hence, this study is reassuring to note that this less expensive quinolone, together with other antibiotics, is apparently just as effective as the newer ones i.e. norfloxacin, levofloxacin, moxifloxacin and gatifloxacin.

Pre-treatment antibiotic sensitivity testing has contributed to the high eradication rate demonstrated in this study. The importance of pre-treatment antibiotic sensitivity testing has been long debated. Such efforts have been claimed inefficient, especially in a community where RFB resistance is low.[64–66] In this study, over 90% of patients who carried MTZ- and CLR-resistant *H. pylori* strains were successfully treated with the in-house PARC therapy. Although testing the antibiotic sensitivity required additional cost and effort, the patients ultimately benefited by avoiding 'guess work' or experimental treatment plans. Repeated failure in *H. pylori* eradication not only increases the risk of developing antibiotic resistance in both *H. pylori* and other gastrointestinal micro-organisms, but patients are more likely to become pessimistic and less compliant.

CONCLUSION

Current *H. pylori* triple therapy is effective, but clinicians are still challenged by patients with persistent infection after standard CLR- and MTZ-based treatment as well as those with penicillin allergy. Antibiotic resistance remains the main reason for treatment failure. For these patients, current guidelines suggest a case-by-case approach at the specialist care level. Most patients who have no eradication after two courses of standard triple therapy are likely to be resistant to both MTZ and CLR. This study shows that these *H. pylori* strains are usually sensitive to CIP and RFB, which means that the PARC salvage therapy could be reasonably prescribed without the expense of antibiotic sensitivity testing, especially among countries with low quinolone resistance. However, pre-treatment antibiotic sensitivity testing is very useful when therapy with novel antibiotic combination is contemplated. We believe that the 73.8% eradication rate from these novel antibiotic combinations guided by pre-antibiotic sensitivity testing has greatly benefitted these patients, especially those with penicillin allergy.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow-chart displaying the treatment strategy.

Table S1. The in-house treatment plans.

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