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High *Helicobacter pylori* Resistance to Metronidazole but Zero or Low Resistance to Clarithromycin, Levofloxacin, and Other Antibiotics in Malaysia

Khean-Lee Goh* and Parasakthi Navaratnam†

*Division of Gastroenterology and Hepatology, University of Malaya, Kuala Lumpur, Malaysia, †Department of Medical Microbiology, School of Medicine, Monash University Malaysia, Petaling Jaya, Malaysia

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Reprint requests to: Khean-Lee Goh, Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail: klgoh56@gmail.com

Abstract

Objective: Bacterial resistance to antibiotics is the single most important determinant of treatment success. The objective of this study was to determine the prevalence of *Helicobacter pylori* resistance to clarithromycin, amoxicillin, metronidazole, tetracycline, levofloxacin, rifabutin, and furazolidone in our local bacterial strains.

Methods: Samples from consecutive ninety patients were obtained for culture and sensitivity testing. Resistance to individual antibiotics were tested using the E-test and MIC_{90} read from the strips. Resistance to rifampicin and nitrofurantoin were used as a surrogate for rifabutin and furazolidine.

Results: There was a high prevalence of resistance to metronidazole 68/90 (75.5%). No male (34/45 (75.5%) versus female (35/45 (77.7%) difference in frequency of metronidazole resistance was noted ($p = 1.000$). There was zero resistance $_0$ to clarithromycin, levofloxacin, amoxicillin, and nitrofurantoin/furazolidone. Resistance to rifampicin/rifabutin was for breakpoints of 1 and 4 μg/mL of 14.4 and 2.2% respectively.

Conclusions: Although there was high bacterial resistance to metronidazole, the absence of resistance particularly to the key antibiotics used in *H. pylori* eradication therapy: clarithromycin and levofloxacin is reassuring to note. Continued monitoring of antibiotic resistance should be carried out.

The use of multiple antibiotics in the treatment of *Helicobacter pylori* has given rise to the emergence of bacterial resistance to antibiotics. The pattern of bacterial resistance to antibiotics however varies from region to region. For example, high rates of clarithromycin have been reported in Europe [1,2] and in Japan and China [3–7] but lower rates in other parts of Asia [8,9], while rates of resistance to metronidazole is high in many countries in Asia but lower in Western countries [10].

We have tracked the prevalence of resistance to metronidazole and clarithromycin over several years [11–16] in our center. Consistently, resistance to clarithromycin has been zero or very low [12,14–16]. However, resistance to metronidazole has steadily increased to almost 80% [15]. With the increasing use of newer or different antibiotics for the treatment of *H. pylori* in Malaysia, there is concern about the background primary *H. pylori* resistance to these antibiotics.

The objective of this study was to determine the prevalence of resistance to six antibiotics: clarithromycin, amoxicillin, metronidazole, levofloxacin, rifabutin, and furazolidone which are used in the treatment of *H. pylori* in our local setting.

Methods

Consecutive patients with previously untreated *H. pylori* infection were prospectively recruited for the study from January to August 2009.

Culture of *Helicobacter pylori*

Four gastric biopsies of patients (two from antrum and two from the body of the stomach) were obtained from *H. pylori*-positive patients. The biopsies were directly plated on Columbia sheep agar plates (Bio Med

Laboratories, Malacca, Malaysia) and placed into an anaerobic jar in the endoscopy unit. The jars were then brought to the microbiology laboratory within 2 hours of biopsy and incubated at 37 °C under microaerophilic conditions for up to 7 days. Colonies of *H. pylori* were provisionally identified by their colonial morphology, characteristic Gram-stained appearance and positive oxidase, catalase, and urease reaction.

Antibiotic Sensitivity Testing

Cultures were tested with epsilometer (E-test) (AB BioMerieux, Solna, Sweden) test for bacterial resistance to antibiotics. Pure cultures of *H. pylori* from blood agar plates were inoculated into 5 mL of sterile saline to obtain a turbidity of equivalent to McFarland's opacity standard no.4. Using a cotton-tipped swab, sheep agar plates were inoculated to obtain a confluent growth of the organism. A single E-test strip is placed after the inoculum had dried. The plates were incubated at 37 °C under microaerophilic conditions for 72 hours after which the Minimum inhibitory concentration (MIC) was read.

Breakpoints for resistance were used according to CLSI/EUCAST guidelines – metronidazole ≥8.0 μg/mL, clarithromycin ≥1.0 μg/mL, amoxicillin ≥1.0 μg/mL, and levofloxacin ≥1.0 μg/mL. Testing for rifampicin was used for rifabutin and nitrofurantoin for furazolidine. Breakpoints used for nitrofurantoin/furazolidine used was ≥4 μg/mL. No breakpoints exist for rifampicin/rifabutin but arbitrarily based on previous reports, breakpoints of 1, 4, and 16 μg/mL were chosen [17–19]. The study was approved by the ethical committee of the University of Malaya Medical Centre and performed in accordance with GCP-ICH guidelines.

Statistical Analysis

Data was put into Statistical Package for the Social Sciences (SPSS version 11.5, Chicago, Illinois, USA) database and analysis carried out using the same program.

Results

Samples from 90 patients were obtained for culture and sensitivity testing. The mean age was 50.5 ± 14.4 years. The male-to-female ratio was 1 : 1.

The overall resistance to antibiotics is as shown in Table 1. There was a high prevalence of resistance to metronidazole 68/90 (75.5%). In 59 patients (65.5%), the MIC_{90} was ≥256 μg/mL. Three patients had MIC of 64.0, 1, 48.0, 2, 24.0, 2, 16, and 1, 8 μg/mL. The remaining cultures (22/90–24.4%) had very good sensitivity

Table 1 MIC_{90} of *Helicobacter pylori* strains

Antibiotic	Median MIC_{90} (μg/mL)	Range of MIC (μg/mL)	Resistant strains N = 90 (%)
Metronidazole	256	≤0.016–≥256	68 (75.5)
Clarithromycin	≤0.016	–	0
Amoxicillin	≤0.016	–	0
Levofloxacin	0.094	≤0.002–0.50	0
Rifampicin	0.25	≤0.016–4.0	*
Nitrofurantoin	0.032	≤0.032–0.50	0

See Table 2.

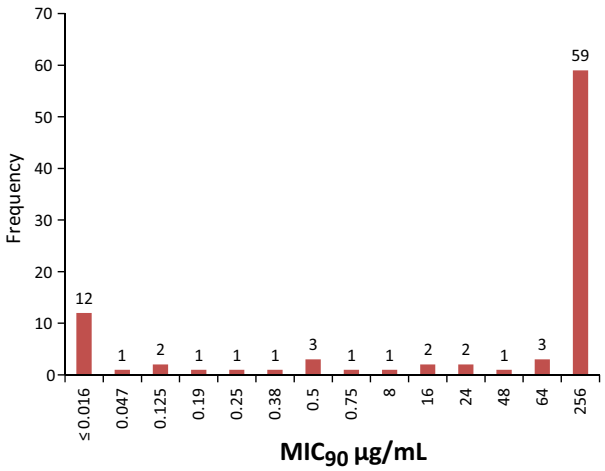

Figure 1 Distribution of MIC_{90} to metronidazole.

with a MIC of 0.75 – ≤0.016 μg/mL. No male (34/45 (75.5%)) versus female (35/45 (77.7%)) difference in frequency of metronidazole resistance was noted (p = 1.000). The spread of MIC_{90} is as shown in Fig. 1.

The MIC_{90} to clarithromycin and amoxicillin were ≤0.016 μg/mL for all 90 samples giving a resistance rate of zero for both these antibiotics. The MIC_{90} to levofloxacin for all 90 strains were below the breakpoint of 1.0 μg/mL. The values ranged from ≤0.002 to 0.75 μg/mL with a median of 0.094 μg/mL (25–75% IQR: 0.064–0.190 μg/mL).

The MIC_{90} to rifampicin ranged from ≤0.016 to 4.0 μg/mL with a median of 0.25 μg/mL (25–95% IQR: 0.125–0.50 μg/mL). Using a high breakpoint of 16 μg/mL, no resistant strains were detected. However, using breakpoints of 1 and 4 μg/mL, 14.4 and 2.2% resistant strains were detected respectively (Table 2).

The MIC_{90} to nitrofurantoin for all 90 strains were below the breakpoint of 4.0 μg/mL. Seventy-eight of 90 (86.6%) had a MIC_{90} of 0.032 μg/mL. The values ranged from ≤0.032 to 0.094 μg/mL with a median of 0.032 μg/mL (25–95% IQR: 0.032–0.032 μg/mL).

Table 2 Resistance to rifampicin based on different breakpoints

Breakpoint (μg/mL)	No of resistant strains (%)
≥1	13 (14.4)
≥4	2 (2.2)
≥16	0

Table 3 Antibiotic resistance over time in the University of Malaya Medical Centre, Malaysia

Year	n	% Resistance to MTZ	% Resistance to clarithromycin
1992 [11]	20	10.8	–
1995 [12]	20	–	0
1997 [13]	75	44.0	–
1997 [14]	63	54.0	0
2000 [15]	74	77.0	0
2006 [16]	107	–	2.9
2009 (present study)	90	75.5	0

Discussion

Resistance to antibiotics is a major problem in the effective treatment of *H. pylori*. Resistance to clarithromycin in particular has been shown to impact adversely on eradication success. For example, Ducon et al. [20] showed a 20% eradication success in clarithromycin-resistant strains compared with 83% in sensitive strains and in a Korean study, treatment success was absolute in all sensitive strains but zero in patients harboring resistant strains [21]. In a review of the published data, Megraud and Doermann recorded a drop of 70% in eradication rates in resistant versus sensitive strains [22] in a pooled analysis of several studies. In a systematic analysis of published studies, Houben et al. [23] computed a decline in success rate of 56% with 1 or 2 week clarithromycin containing triple therapy. A more recent meta-analysis by Fischbach et al. [24] recorded a decrease in eradication rate of 66% with Proton-pump inhibitor PPI–clarithromycin–amoxicillin triple therapy. Clarithromycin is a key antibiotic, and the emergence of resistance will negate against its usefulness in *H. pylori* eradication therapy.

High resistance rates to clarithromycin of up to 40% have been reported in several studies. This is especially worrisome with the findings of high resistance rates in children [2,5,25]. The reason for this increase is undoubtedly the widespread use of macrolides for treatment of various other bacterial infections and especially for respiratory tract infections [26]. This has been well shown in studies where the increase in prescription of clarithromycin was accompanied by an increase in resistance to the antibiotic [27,28].

Our finding of zero or low resistance among our isolates is therefore gratifying to note. A low rate of resistance to this antibiotic has also been noted from another recent published study from Malaysia where a prevalence of 2.1% was recorded [29]. Several reports over time from our own centre have consistently recorded a very low or zero clarithromycin resistance rates [12,14,15] (Table 3). A recent study using molecular methods of detection reaffirms this finding by demonstrating a low prevalence (2.9%) of mutations in the 23S rRNA gene [16].

An ''in-clinical practice'' study from our centre showed a satisfactory eradication rate (per protocol analysis) of 84.4% with a PPI–clarithromycin–amoxicillin 1 week triple therapy [30]. Our findings are in keeping with a more recent study from Hong Kong, China which also showed a high eradication rate with a PPI–clarithromycin-containing triple therapy of 92.7% [31]. Hung et al. also noted a relatively low clarithromycin resistance rates among their patients.

Conversely, metronidazole resistance rates have risen steadily from 10.8% in our initial report to over 70% in latter studies (Table 3). In our present study, we have again shown a high metronidazole resistance rate. The recommended treatment for *H. pylori* in Malaysia utilizes amoxicillin instead of metronidazole except in cases of penicillin allergy. The high rate of metronidazole resistance has been widely reported in tropical countries where the antibiotic is often used for parasitic diseases or even empirically for any ''infectious'' diarrheal disease. In western countries, metronidazole is used for vaginal infections and a female predominance of metronidazole resistance has been reported. No gender difference was observed with metronidazole resistance in our study however, indicating a wider use for treatment of diarrheal diseases in our locality. Detection of resistance to metronidazole tends to vary with different methods used. However, a consistently high prevalence reported over several years reliably points to a high background primary resistance in our local population. A recent meta-analysis showed a decrease in efficacy of 18% in resistant strains [24].The high rate of metronidazole resistance has discouraged the use of metronidazole in most eradication therapies used in Malaysia.

The usual dose of clarithromycin used is 1 g daily and metronidazole 800 mg per person per day given in twice daily dose for 1 week. This has been recommended by our Malaysian Working Party for *H. pylori* and has not changed over the past 12 years [32].

It is also important to note the zero resistance to amoxicillin, which is a key antibiotic used in our local

setting. Isolated reports of amoxicillin resistance have been reported from various locations [33], but universally this has been distinctly rare [26]. Levofloxacin is a newly introduced antibiotic in Malaysia. Again it was reassuring to note that resistance was zero to this antibiotic. In countries where prescription of this antibiotic is widespread, an exponential increase in resistance has been observed. For example, in Taiwan levofloxacin resistance has increased 2.8% in 2003 to 11.8% in 2007 [34], in France from 3.3% 1999 to 17.2% in 2005 [35], and in Korea from 0% in 1999 to 21.5% in 2006 [36,37]. In Malaysia, although increasingly used for urinary, biliary tract and respiratory infections, levofloxacin has been reserved for use as a second-line therapy for *H. pylori* [38].

Furazolidone and rifabutin have both been used for second-line rescue therapies. They have generally not been popular locally. With increasing emergence of resistance to the more commonly used antibiotics, these drugs may however have to be considered for second-line rescue therapy.

The resistance pattern for rifampicin/rifabutin is important to note. Using a high breakpoint of 16 μg/mL, no resistant strains were detected. However, using lower and more realistic breakpoints of 1 and 4 μg/mL, 14.4 and 2.2% of the strains respectively were detected to be resistant. This is not unexpected as tuberculosis is still common in Malaysia [39] and rifampicin is frequently used in anti-tuberculosis treatment regimens.

There has been a resurgence in the use of bismuth-containing compounds such as colloidal bismuth subcitrate to overcome the declining eradication rates in several countries. International authorities have now recommended bismuth-containing quadruple and sequential therapies as first-line treatment for *H. pylori* [40]. Bismuth compounds are not readily available in Malaysia in recent years, but the use of these drugs should certainly be considered as well. Continued monitoring of resistance to antibiotics remains important to determine whether the favorable antibiotic profile that we have in our local setting remains the same in the future.

Conflict of Interest

No external source of funding was obtained for this study. The authors have no conflict of interest in carrying out this study.

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