PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 2000 February; 6(1):119-121
World Journal of Gastroenterology
 ISSN 1007-9327

Brief Reports

Prevalence of primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in Singapore

Hua JS, Bow H, Zheng PY and **Khay-Guan Y**

Subject headings *Helicobacter pylori*; triple therapy; metronidazole; clarithromycin

INTRODUCTION

Eradication of *Helicobacter pylori*, a bacterium residing in stomach and causing peptic ulcer disease, can be achieved by using combination therapies consisting of one or two antibiotics with a proton pump inhibitor (PPI). The major antibiotics widely used in the regimens to eradicate *H. pylori* are metronidazole and clarithromycin[1-3]. However, resistance to these antibiotics by *H. pylori* affects the effectiveness of treatment. Treatment failure is often associated with resistance to metronidazole and clarithromycin[4-7]. In the United States, the frequency of resistance to metronidazole is about 25% with range from about 20% to more than 50% [8]. In the Netherlands and Germany, the incidence of metronidazole resistance is 17%[9] and 32%[10], respectively. In contrast, the prevalence of metronidazole resistance in developing countries was reported to be as high as 70%-90%[4].

Compared to metronidazole resistance, clarithromycin resistance is low with a range of 7%-14% in the United States[8], 1% in the Netherlands[9] and 3% in Germany[10]. Data on clarithromycin resistance in developing countries are rare.

The rise in antibiotic resistance emphasizes the need for surveillance of *H. pylori* sensitivity to antibiotics as in other infectious diseases. These data will allow clinicians to choose suitable therapy for their patients. The present study provides recent data on the prevalence of primary metronidazole and clarithromycin resistance of *H. pylori* in Singapore.

MATERIALS AND METHODS

H. pylori strains isolated from 282 consecutive of *H. pylori* positive patients (108 females and 174 males) undergoing routine endoscopy with informed consent for dyspepsia at the Singapore National University Hospital were included in this study. None of the 282 patients had been previously treated for *H. pylori* or had known exposure to antibiotics, bismuth compound or proton pump inhibitor in the past four weeks. Methods for isolation and culture of *H. pylori* were described previously[11,12]. Briefly, 2 gastric biopsies were obtained from the gastric antrum within 2cm of the pylorus from each patient. The biopsies were transported in 0.85% sterile saline to the laboratory for processing within 24h. The two biopsies were smeared onto a chocolate blood agar plate (blood agar base No.2 supplemented with 5% horse blood) without antibiotics followed by smearing onto a chocolate blood agar plate supplemented with antibiotics (vancomycin 3mg/L, colistin methane sulphate 7.5mg/L, nystatin 12500U/L and trimethoprim 5mg/L). The plates were incubated at 37¡æ in a humidified incubator (Forma Scientific) with 5% CO_2. Identification of *H. pylori* isolates was based on the results of Gram staining, cell morphology and positive reaction for catalase, oxidase and urease activity. These isolates were further confirmed by API ZYM Kit (BioMériux)[13], a semiquantitative micromethod for the rapid detection of the presence of 19 preformed enzymes.

The disk diffusion test was used for the testing of bacterial sensitivity to antimicrobial agents. An inoculum of 0.2 mL of *H. pylori* suspension equivalent to McFarland 3 turbidity standard was spread onto the chocolate blood agar plate. The plates were dried completely for 5 min - 10 min before a metronidazole disk (5μg, Oxoid) or clarithromycin disk (15 μg, Oxoid) was placed on the surface of each dried agar plate. These plates were incubated at 37℃ in 5% CO_2 atmosphere for

Jie Song Hua[1], Ho Bow[1], Peng Yuan Zheng[1] and Yeoh Khay-Guan[2]
Department of Microbiology[1] and Medicine[2], Faculty of Medicine, National University of Singapore, 5, Science Drive 2, Singapore 117597, Republic of Singapore
Dr. Jie Song Hua, male born on 1961-11-16 in Shanghai City, China, graduated from Shanghai Medical University, now a research fellow of microbiology, having 30 papers published.
This study is supported by the National University of Singapore Grant GR6431.
Correspondence to: Dr. Jie Song Hua, Department of Microbiology, National University of Singapore, 5, Science Drive 2, Singapore 117597, Republic of Singapore
Tel. +65-8743285, Fax. +65-7766872
Email. michuajs@nus.edu.sg
Received 1999-09-22 **Accepted** 1999-11-15

2-4 days. The inhibitory zone around each antibiotic disk was recorded accordingly. Inhibitory zones of less than 15mm for metronidazole[14,15] and 30mm for clarithromycin[16] were considered resistance.

Fisher's exact test was used for statistical analysis. *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

In a total of 282 *H. pylori* isolates, resistance to metronidazole was found in 130 isolates (46%; 95% confidence interval, 40.3% - 51.9%) and clarithromycin in 18 isolates (6%; 95% confidence interval, 3.2%-8.8%), respectively. Eight of 18 isolates (3 %; 95 % confidence interval, 1.92 %-3.68%) resistant to clarithromycin were also resistant to metronidazole.

Of the 20 isolates obtained in 1995, 4 (20%; 95% confidence interval, 2.5% - 37.5%) isolates were resistant to metronidazole. Of the 36 isolates obtained in 1996, 19(53%; 95% confidence interval, 36.7%-69.3%) isolates were resistant to metronidazole. In 1997 and 1998, 40/83 (48%; 95% confidence interval, 37.8%-58.7%) and 67/143 (47%;95% confidence interval, 39.9%-54.1%) isolates were found to be resistant to metronidazole, respectively (Table 1).

Of 282 isolates, 50/108(47%; 95% confidence interval, 37.5%-56.4%) and 80/174 (46%; 95% confidence interval, 38.6%-53.4%) isolates from females and males, respectively, were found to be resistant to metronidazole. No statistical difference was found between two genders ($P>0.05$).

Table 1 Prevalence of metronidazole resistance to *H. pylori*

Year	No.	Resistant isolate	Resistance %
1995	20	4	20
1996	36	19	53
1997	83	40	48
1998	143	67	47
Total	282	130	46

DISCUSSION

This study showed that during the 4 years period of investigation the metronidazole resistant rate increased from 20% in 1995 to 47% in 1998 with an average of 46% in 282 *H. pylori* isolates from Singapore. Our previous investigation in 1994 revealed 13% metronidazole resistant rate in 43 isolates[17]. It is, therefore, believed that resistance to metronidazole in Singapore rose to reach a platform of about 50%. On the other hand, clarithromycin resistance was 6% in the total of 282 isolates from Singapore in this study.

Since metronidazole attains high concentration in the stomach and is not influenced by pH, it is among the most antibiotics to be used to eradicate *H. pylori*. However, the effectiveness of treatment was compromised by emergence of metronidazole resistance[4-7]. Thus, it is of great importance to monitor the resistance. The prevalence of metronidazole resistance varies widely from country to country. A study of multicentre in Europe showed that metronidazole resistance is 28% with large variation from 7% in Spain to 49% in Greece[18]. Recent studies from Germany[10] and the Netherlands[9] reported the prevalences of metronidazole resistance are 32% and 17%, respectively. The finding of 46% of metronidazole resistance in Singapore in this study is relatively higher than those of developed countries, but the 6% of clarithromycin resistance in Singapore is similar to those in developed countries, such as 10% in France[19], 5% in Ireland[16], 1% the Netherlands[9], 3% in German[10] and 7%-14% in the United States[8]. This may be due to the fact that the history of metronidazole application in treating infectious diseases other than *H. pylori* in Singapore is much longer than that of clarithromycin. However, if the use of clarithromycin increases, the clarithromycin resistance could pose a serious problem in eradication of *H. pylori* in the future.

In 8 of 18 clarithromycin resistant isolates, metronidazole resistance was also found. The combination of resistance to 2 antibiotics has been reported elsewhere[9,10,18]. The data remind gastroenterologists to be cautious in the use of triple therapy comprising both clarithromycin and metronidazole when they treat patients with peptic ulcer disease. Such therapy if administered to patients infected with *H. pylori* which is resistant to both clarithromycin and metronidazole may result in treatment failure. On the other hand, the treatment may overcome the problem if the isolate is resistant only to one of these two antibiotics.

It is interesting to note that there is no discrimination of metronidazole resistance in terms of gender distribution. This is in contrast to the reports from Europe which state that women are more likely to harbour resistance metronidazole than men[8-10]. This difference possibly reflects equal exposure of metronidazole to males and females in Singapore.

In conclusion, in Singapore the prevalence of resistance to metronidazole is high (46%) as compared to developed countries. However, the prevalence of clarithromycin resistance is comparatively low (6%). The surveillance of *H. pylori* susceptibility to antibiotics is critical in guiding the clinicians on the effectiveness of

treatment regimens. If antibiotic susceptibility testing of *H. pylori* is not available, it is suggested that the clarithromycin-contained triple regimen be preferred to metronidazole-contained triple regimen in local population.

REFERENCES

1 Pohle T, Stoll R, Kirchner T, Heep M, Lehn N, Bock H, Domschlve H. Eradication of *Helicobacter pylori* with lansoprazole, roxithromycin and metronidazole—an open pilot study.*Aliment Pharmacol Ther*, 1998;12:1273-1278

2 Unge P. Eradication therapy of Helicobacter pylori. A review. Report from a workshop organized by the Swedish and Norwegian Medical Products Agencies, September 1995.*J Gastroenterol*, 1998;33(Suppl 10):57-61

3 Pohle T, Stoll R, Kirchner T, Heep M, Lehn N, Bock H, Domschke W. Eradication of *Helicobacter pylori* with lansoprazole, roxithromycin and metronidazole—an open pilot study.*Aliment Pharmacol Ther*,1998;12:1273-1278

4 Glupczynski Y. Antimicrobial resistance in Helicobacter pylori: a global overview.*Acta Gastroenterol Belg*,1998;61:357-366

5 Deltenre M, Ntounda R, Jonas C, De Koster E. Eradication of Helicobacter pylori: why does it fail Ital *J Gastroenterol Hepatol*, 1998;30(Suppl 3):S326-S328

6 Moayyedi P, Ragunathan PL, Mapstone N, Axon AT, Tompkins DS. Relevance of antibiotic sensitivities in predicting failure of omeprazole, clarithromycin, and tinidazole to eradicate Helicobacter pylori. *J Gastroenterol*,1998; 33(Suppl10):62-65

7 Mégraud F, Doermann HP. Clinical relevance of resistant strains of Helicobacter pylori: a review of current data.*Gut*, 1998;43 (Suppl 1):S61-S65

8 Graham DY. Antibiotic resistance in Helicobacter pylori: implications for therapy.*Gastroenterology*, 1998;115:1272-1277

9 van Zwet AA, de Boer WA, Schneeberger PM, Weel J, Jansz AR, Thijs JC. Prevalence of primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in The Netherlands.*Eur J Clin MicrobiolInfect Dis*,1996;15:861-864

10 Wolle K, Nilius M, Leodolter A, Muller WA, Malfertheiner P, Konig W. Prevalence of *Helicobacter pylori* resistance to several antimicrobial agents in a region of Germany.*Eur J Clin Microbiol Infect Dis*, 1998;17:519-521

11 Hua J, Yeoh KG, Ng HC, Zheng PY, Lim SG, Ho B. Improving the success of culturing *Helicobacter pylori* from gastric biopsies. *Microbios*, 1998;96:95-101

12 Hua J, Zheng PY, Teo KF, Khim MM, Ho B. *Helicobacter pylori* acquisition of metronidazole resistance by natural transformation *in vitro*. *WJG*, 1998;4:385-387

13 Kung JS, Ho B, Chan SH. Biotyping of Campylobacter pylori. *J Med Microbiol*, 1989;29:203-206

14 DeCross AJ, Marshall BJ, McCallum RW, Hoffman SR, Barrett LJ, Guerrant RL. Metronidazole susceptibility testing for -Helicobacter pylori: comparison of disk, broth, and agar dilution methods and their clinical relevance. *J Clin Microbiol*,1993;31:1971-1974

15 Hua J, Ng HC, Yeoh KG, Ho KY, Ho B. Characterization of clinical isolates of *Helicobacter pylori* in Singapore. *Microbios*, 1998;94:71-81

16 Xia HX, Buckley M, Keane CT, O'Morain CA. Clarithromycin resistance in Helicobacter pylori: prevalence in untreated dyspeptic patients and stability *in vitro*. *J Antimicrob Chemother*,1996; 37:473-481

17 Vijayakumari S, Khin MM, Jiang B, Ho B. The pathogenic role of the coccoid form of *Helicobacter pylori*. *Cytobios*, 1995;82:251-260

18 Glupczynski Y, Langenber W, Dankert J, Noach L, Rauws E, Mentis A. Results of a multicentre European survey in 1991 of metronidazole resistance in Helicobacter pylori. *Eur J Clin Microbiol Infect Dis*, 1992;11:777-781

19 Cayla R, Lamouilliatte H, Mégraud F, Quinton A. Primary resistance of *Helicobacter pylori* strains to metronidazole and to clarithromycin in France in 1993. Gastroenterology,1994;106 (Suppl S):A61

Edited by Wu XN