Primary Helicobacter Pylori Resistance to Clarithromycin and Metronidazole in Singapore

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ABSTRACT

The aim of this study was to investigate the effect of metronidazole resistance (MtzR) and clarithromycin resistance (ClaR) on the eradication rate for omeprazole, clarithromycin, and metronidazole triple-therapy regimen and on the development of posttherapy drug resistance in a region of high rates of MtzR. One hundred ninety-six Helicobacter pylori isolates were recovered from patients with duodenal ulcer, gastric ulcer, or nonulcer dyspepsia during upper endoscopy. The prevalences of MtzR, ClaR, and dual resistance were 37.8%, 13.8%, and 8.7%, respectively. The intention-to-treat eradication rates for metronidazole-susceptible (87.2% vs. 67.6%; P = .001) and clarithromycin-susceptible (86.4% vs. 40.7%; P < .001) strains were significantly higher than the rates for resistant strains. Multiple logistic regression analysis implicated younger age (<40 years old), MtzR, ClaR, and the diagnosis of nonulcer dyspepsia as independent factors that predicted treatment failure. The rates of posttreatment MtzR, ClaR, and dual resistance were 88%, 88%, and 75%, respectively. MtxR and ClaR significantly affected the success of eradication therapy. Posttreatment rates of resistance were high and were related to the presence of pretreatment antibiotic resistance

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 2 cm of the pylorus from each patient. The biopsies were transported in 0.85% sterile saline to the laboratory for processing within 24 h. 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 3 mg/L, colistin methane sulphate 7.5 mg/L, nystatin 12500 U/L and trimethoprim 5 mg/L). The plates were incubated at 37¡æ in a humidified incubator (Forma Scientific) with 5% CO2. 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 (5 µg, Oxoid) or clarithromycin disk (15 µg, Oxoid) was placed on the surface of each dried agar plate. These plates were incubated at 37 °C in 5% CO2 atmosphere for 2-4 days. The inhibitory zone around each antibiotic disk was recorded accordingly. Inhibitory zones of less than 15 mm for metronidazole[14,15] and 30 mm 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 (Table11).

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

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).

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.

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Footnotes

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