PATTERNS OF *HELICOBACTER PYLORI* ISOLATE RESISTANCE TO FLUOROQUINOLONES, AMOXICILLIN, CLARITHROMYCIN AND METRONIDAZOLES

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Abstract. *Helicobacter pylori* eradication using the three antibiotic regimen of amoxicillin, clarithromycin and metronidazole often fails, making it imperative to find substitutes. The following study made use of 72 *H. pylori* isolates derived from pyloric antrum mucosa biopsies of gastritis and chronic dyspepsia patients treated at the Cipto Mangunkusumo National General Hospital and three private hospitals in Jakarta. Testing for *H. pylori* sensitivity to various antimicrobials was conducted using the disk diffusion method (Kirby Bauer) and procedures determined by the Clinical and Laboratory Standards Intitute (CLSI)/NCCLS. The resistance rates of the isolates were 100% for metronodazole, 27.8% for clarithromycin, 19.4% for amoxicillin, 6.9% for ciprofloxacin, norfloxacin and ofloxacin, 2.8% for sparfloxacin and gatifloxacin, and 1.4% for levofloxacin and moxifloxacin. Fluoroquinolons have the lowest resistance compared to amoxicillin, clarithromycin and metronidazole.

INTRODUCTION

Warren and Marshall discovered in 1983 that *Helicobacter pylori* plays a major role in active chronic gastritis, stomach ulcers, duodenum ulcers and gastric carcinoma (Blaser *et al*, 1995; Lee, 1996; Marshall, 1996; Kupier, 1999). Prevalence of *H. pylori* infection is considerably higher in developing countries, such as Indonesia (Ketut, 1995). Infection is associated with geographical, socioeconomic and environmental sanitation factors (Rourke *et al*, 2003). *H. pylori* infection is prevalent in nearly 95% of those with duodenal ulcers and in 80% and 90% of those with gastric ulcers (Mitchell, 1999).

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During the initial stage of infection, the disease generally proceeds without symptoms. Patients come to know they are infected with *H. pylori* when the infection is chronic, producing ulcers or cancer. Treatment of *H. pylori* is important in the prevention of ulcers and gastric carcinoma. To date there have been frequent relapses. Resistance to amoxicillin, clarithromycin and metronidazole, three antibiotics currently employed to eradicate the bacteria, has been on the increase in a number of countries (Megraud, 2004). There is an urgent need to find other antibiotics, such as the fluoroquinolone group, to combat *H. pylori*.

MATERIALS AND METHODS

Study samples were gastric antrum biopsies. Biopsies was taken by endoscopy in 126 patients suffering from chronic gastritis and dyspepsia. There were 67 males, age 25

to 65 years (average 30), and 59 females age 22 to 65 years (average 28) treated as out patients at Cipto Mangunkusumo National General Hospital and three private hospitals in Jakarta. Biopsies were taken after informed consent was given.

The gastric biopsy samples were placed in Stuart transport medium, stored in a cold thermos flask (using ice), then immediately transported to the microbiology laboratory, Faculty of Medicine, Trisakti University.

Isolation and identification

Isolation for *H. pylori* was conducted at the Microbiology Department, Faculty of Medicine, Trisakti University, by placing the finely cut biopsy tissues in Brucella blood agar (Brucella agar containing 5% sheep blood and 0.2% vitamin K) and Dent selective medium (Brucella agar with 5% sheep blood and Dent supplement from Oxoid). They were incubated at 37ºC for 3 to 5 days in a humid place with 5% oxygen and 10% carbon dioxide. Incubation was done using an anaerobic jar containing *Campylobacter* BR 56 and BR 46 catalyst from Oxoid. By 3 to 5 days, if fine, translucent colonies were seen in the Brucella agar medium and greyish colonies in the Dent medium, then these colonies were assumed to be *H. pylori.*

The colonies were then microscopically identified and biochemically tested. Bacteria samples were subjected to Gram staining. Gram-negative bacteria and in spirals with two S or U curves, are most probably *H. pylori*. Biochemical testing was made with a rapid urease test using motility indole urease (MIU) (Kumala *et al*, 2001), catalase, and oxydase. All tested isolates were put in tryptic soy broth with 10% glycerol as stock before being stored at -70ºC. The isolates were stored at this temperature until antibiotic sensitivity was testing.

Antibiotic sensitivity testing

The disk diffusion method was performed according to Kirby Bauer method *H. pylori* colonies were suspended in Brain Heart Infusion broth to MacFarland 2 turbidity standard. The solution (0.1 ml) was then spread evenly on Brucella blood agar. Antibiotic disks for ciprofloxacin, gatifloxsacin, moxifloxasin, levofloxasin, norflosxacin, ofloxacin, amoxicillin, clarithromycin and metronidazole were placed on the Brucella blood agar. Each agar culture plate had three kinds of antibiotics tested and was processed *duplo*-wise. *H. pylori* ATCC 43504 served as a quality control unit. The plate was subsequently incubated similar to the *H. pylori* isolation method. The inhibition zone diameter was measured in millimeters. Sensitivity of *H. pylori* to an antibiotic was in accordance with the Clinical and Laboratory Standards Institute (CLSI/NCCLS, 2005).

RESULTS

Of the 126 samples, 2 were excluded due to contamination with *Candida* sp. Seventy-two (56.1%) were isolated and tested for antibiotic sensitivities (Table 1). The total *H. pylori*

Table 1
Resistance pattern of *H. pylori* to fluoroquinolones, amoxicillin, clarithromycin and metronidazole.

Antibiotics		Total of resistant isolates n (%)
Metronidazole	(5 µg)	72 (100)
Clarithromycin	(15 µg)	20 (27.8)
Amoxicillin	(20 µg)	14 (19.4)
Ciprofloxacin	(5 µg)	5 (6.9)
Norfloxacin	(10 µg)	5 (6.9)
Ofloxacin	(5 µg)	5 (6.9)
Sparfloxacin	(5 µg)	2 (2.8)
Gatifloxacin	(5 µg)	2 (2.8)
Moxifloxacin	(5 µg)	1 (1.4)
Levofloxacin	(5 µg)	1 (1.4)

Total number of isolates = 72; n = number of total of resistant isolates

isolates resistant to metronidazole were 72 (100%), clarithromycin 20 (27.8%), and amoxicillin 14 (19,4%). The resistance to other antibiotics was as follows: fluoroquinolone ciprofloxacin, norfloxacin and ofloxacin 5 (6.9%), and sparfloxacin and gatifloxacin 2 (2.8%). Only one *H. pylori* isolate (1.4%) was resistant to levofloxacin and moxifloxacin.

DISCUSSION

H. pylori was totally resistant to metronidazole. The data are consistent with earlier research conducted in several areas of Indonesia, such as Malang (Achmad, 1996) and Bali (Suate and Suyasa, 1995). Central Africa has reported 70-90% resistance (Walt, 1996) and Costa Rica 95.1% (Quintana-Guzman *et al*, 1998). This may be due to frequent use of metronidazole in the treatment of diarrhea caused by ameba, trichomoniasis in women and other anaerobic bacterial infections when incorrect observing dosing and duration of medication are used (Jenks *et al* 1999). Another cause is gene mutation. Mutation of the *rdxA* gene accelerates *H. pylori* resistance to metronidazole (Kwon *et al*, 2000; Paul *et al*, 2001). Other studies regarding amoxicillin and clarithromycin have also revealed resistance. *H. pylori* resistance to various antibiotics has been found in a number of countries (Wu *et al*, 2000; Torres *et al*, 2001; Megraud, 2004).

Resistance to clarithromycin was found in 27.8%. This is because clarithromycin is frequently used in the treatment of upper and lower respiratory tract infections in children and adults (Loivukene *et al,* 2002; Perez *et al*, 2002). Another factor contributing to resistance is mutation of the *H. pylori* 235 rRNA gene, and A2143G (Matsumura *et al*, 2001; Francesco *et al*, 2006).

H. pylori sensitivity to amoxicillin is still acceptable, although 19.4% resistance cannot be ignored. Frequent use of this antibiotic for the treatment of respiratory and skin infections in our country may contribute to bacterial resistance. A study by Achmad in 1996 found *H. pylori* resistance to amoxicillin to be present in more than 90%. The difference in results from this study may be attributed to different bacterial strains in different regions (Achmad, 1996; Thyagarajan *et al*, 2003), the density of different inoculums (Berger *et al,* 1993), medium and incubation time (Hartzen *et al*, 1997). Paul *et al* in 2001 proved that *H. pylori pbpI* gene mutation makes it resistant to amoxicillin.

Resistance to ciprofloxacin in a current study (6.9%) is consistent with the literature which found resistance to ciprofloxacin is still low at 5-15% (Zeiler and Grohe, 1984). Similar results have been seen in studies carried out in France and Eastern European countries where resistance rates were 3.8% and 3.9%, respectively (Birac *et al*, 1999; Boyanova *et al*, 2002). Our study found the ciprofloxacin resistance rate was higher than that for sparfloxacin, gatifloxacin, levofloxacin and moxifloxacin. A reason for this high resistance may be that ciprofloxacin is often used in the treatment of urinary tract and respiratory infections (Stamm and Stapleton, 1998).

In this study, only one *H. pylori* isolate was resistant either to levofloxacin or moxifloxacin. Levofloxacin has better tissue penetration and fewer side effects than ciprofloxacin (Croom and Goa, 2003). Moxifloxacin has good penetration and accumulates in the gastric mucosa (Wirtz *et al*, 2004).

Based on this study, most *H. pylori* isolates in Jakarta are beginning to be resistant to antibiotics, notably those often used in standard treatment, such as clarithromycin, metronidazole and amoxicillin. Metronidazole, due to 100% resistance, is no longer effective in the eradication of *H. pylori*. Instead, the fluoroquinolone group may be more effective in eradicating *H. pylori*.

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