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Antimicrobial Susceptibility of *Helicobacter pylori* Strains Isolated in Bangladesh

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Antimicrobial susceptibility of 120 *Helicobacter pylori* isolates to metronidazole, tetracycline, clarithromycin, and amoxicillin was determined, and 77.5, 15, 10, and 6.6% of the isolates, respectively, were resistant. Only *rdxA* inactivation and both *rdxA* and *frxA* inactivation were responsible for metronidazole resistance in 66% (8 of 12) and 33% (4 of 12) of the isolates, respectively.

Eradication of *Helicobacter pylori* infection by treatment with two antimicrobial agents (clarithromycin and amoxicillin or metronidazole) and a proton pump inhibitor is recommended by various consensus groups (10, 16, 20). Antimicrobial resistance in *H. pylori* is a growing problem as it is the most important factor in determining treatment outcome. The prevalence of antimicrobial resistance varies with geographical regions (3, 25). Metronidazole resistance in *H. pylori* has been shown to be due to mutation in *rdxA*; mutation in *frxA* has also been shown to be associated with metronidazole resistance (11, 12, 23). In Bangladesh, the prevalences of *H. pylori* infection among infants, children, and adults are 61, 84, and 92%, respectively (1, 21, 22); however, information on antimicrobial susceptibility to commonly used drugs in *H. pylori* treatment is lacking. This study was conducted to evaluate (i) the prevalence of primary antibiotic resistance to commonly used antimicrobial agents and (ii) the genetic basis for metronidazole resistance in *H. pylori* isolates from Bangladesh.

Consecutive patients attending the Gastroenterology Department of Dhaka Medical College Hospital for upper gastrointestinal endoscopy were enrolled during 1999 to 2001. Diagnosis of peptic ulcer (PU) and non-ulcer dyspepsia (NUD) or gastritis was based on endoscopic examination of the stomach and duodenum. Biopsy samples were taken from each patient for culture.

Bacteria were grown in brain heart infusion agar with 7% sheep blood and incubated at 37°C in 5% O_2, 10% CO_2, and 85% N_2 for 3 to 6 days. The MICs of amoxicillin, clarithromycin, metronidazole, and tetracycline for the isolates were determined by the agar dilution method as described elsewhere (18, 19). All tests were repeated twice, and *H. pylori* 26695 was used as a control. β-Lactamase production was tested by the chromogenic cephalosporin method (6). The molecular mechanism of susceptibility and resistance to metronidazole was studied in 12 isolates. Metronidazole-susceptible (Mtz^s) isolates were further studied (by inactivation of *rdxA* alone or *rdxA* and *frxA* for conversion into an Mtz^r phenotype) by transformation of Mtz^s isolates with plasmids pBS-*rdxA*-cam (*rdxA*::*cat*) and pBS-*frxA*-kan (*frxA*::*kan*) as described earlier (11, 12).

A total of 278 consecutive patients between 15 and 78 years of age were enrolled, and among them, 72.7% (202 patients) were male and 27.3% (76 patients) were female. Among the patients, 162 had PU and 116 had NUD and 62.6% (174 of 278) were culture positive for *H. pylori*. Among the culture-positive patients, 121 (69.5%) were male and 53 (30.4%) were female and 112 (64.3%) had PU and 62 (35.6%) had NUD. Of the 174 isolates, a total of 120 were available for antimicrobial susceptibility testing and 73.3% (88 of 120) and 26.6% (32 of 120) were from PU and NUD patients, respectively. Among the isolates, 77.5% (93 of 120), 15% (18 of 120), 10% (12 of 120), and 6.6% (8 of 120) were resistant to metronidazole, tetracycline, clarithromycin, and amoxicillin, respectively (Table 1). The range and distribution of MICs for the isolates are shown in Fig. 1. All amoxicillin-resistant isolates were β-lactamase negative. Antimicrobial susceptibilities of the isolates collected from patients with PU and NUD and males and females were compared, and no significant difference ($P \leq 0.05$) in antimicrobial resistance was observed among these groups (Table 1).

Inactivation of only *rdxA* was sufficient to confer the Mtz^r phenotype in 66% (8 of 12) of isolates, 33% (4 of 12) of isolates were Mtz^s, and inactivation of only *frxA* had little effect on Mtz^s of all 12 isolates. Subsequent *frxA* inactivation of all *rdxA*-deficient strains increased the MIC of metronidazole from 16 μg/ml to 32 μg/ml for the eight strains which became resistant after only *rdxA* inactivation, and four strains which were sensitive after only *rdxA* inactivation reverted to the Mtz^r phenotype (MIC, 32 μg/ml).

Resistance to metronidazole was the most common type of

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TABLE 1. Antibiotic susceptibility of 120 *H. pylori* strains isolated during 1999 to 2001 from Dhaka, Bangladesh

Antimicrobial agent	No. (%) of resistant isolates in patients:				
	All patients (n = 120)	PU (n = 88)	NUD (n = 32)	Male (n = 88)	Female (n = 32)
Amoxicillin	8 (6.6)	7 (8.0)	1 (3.1)	4 (4.5)	4 (12.5)
Clarithromycin	12 (10)	8 (9.1)	4 (12.5)	7 (8.0)	5 (15.6)
Metronidazole	93 (77.5)	67 (76.1)	26 (81.2)	68 (77.3)	25 (78.1)
Tetracycline	18 (15)	12 (13.6)	6 (18.7)	14 (16.0)	4 (12.5)

resistance, with worldwide rates of 10 to 90% (3, 25). The high prevalence (77%) of metronidazole resistance in Bangladesh might be due to frequent use of metronidazole for other intestinal and gynecological problems. Previous use of metronidazole has been shown to be associated with *H. pylori* resistance to this antimicrobial agent (17). Two types of Mtz^s *H. pylori* were isolated in the present study: type I, requiring only inactivation of *rdxA* to become resistant; and type II, requiring inactivation of both *rdxA* and *frxA* to become resistant. Only *frxA* inactivation did not have any role in metronidazole resistance, as only subsequent inactivation of *frxA* in *rdxA*-inactivated isolates reverted from the Mtz^s phenotype to the Mtz^r phenotype and increased the MIC for the Mtz^r phenotype. This is in contrast to the findings of Kwon et al., who interpreted that the resistant phenotype can obtained by inactivation either of *frxA* or *rdxA* (13). Thus, resistance to metronidazole in *H. pylori* is mainly due to mutation in the *rdxA* gene and results from de novo mutation in the resident *rdxA* gene, rather than lateral transfer of a mutant *rdxA* gene.

The reported prevalence of primary resistance to clarithromycin ranges between 0 and 15% in most countries (3, 25). Around 10% of the isolates in the present study were clarithromycin resistant. In Bangladesh, clarithromycin was introduced in the late 1990s, and it has been widely used for eradication of *H. pylori*. Previous use of macrolides has been shown to be associated with *H. pylori* resistance to clarithromycin (17).

Amoxicillin resistance was not considered important until recently identified in the United States, Canada, and Italy (7, 8). Amoxicillin is one of the most commonly used antimicrobial agents in Bangladesh in recent years. Although 6.6% of the isolates were resistant, none was positive for β-lactamase. Amoxicillin resistance develops due to structural alterations in one of the penicillin-binding proteins (4, 5, 9) or changes in other proteins involved in cell wall synthesis (2, 15, 26), and the resistant phenotype may be lost due to freezing or storage. All isolates tested in the present study were frozen at least once, and the low prevalence of the resistance phenotype may be due to loss during storage. Primary resistance to tetracycline ranges between 5 and 59% in Asian countries (14, 24, 27). Around 15% of the isolates in the present study were tetracycline

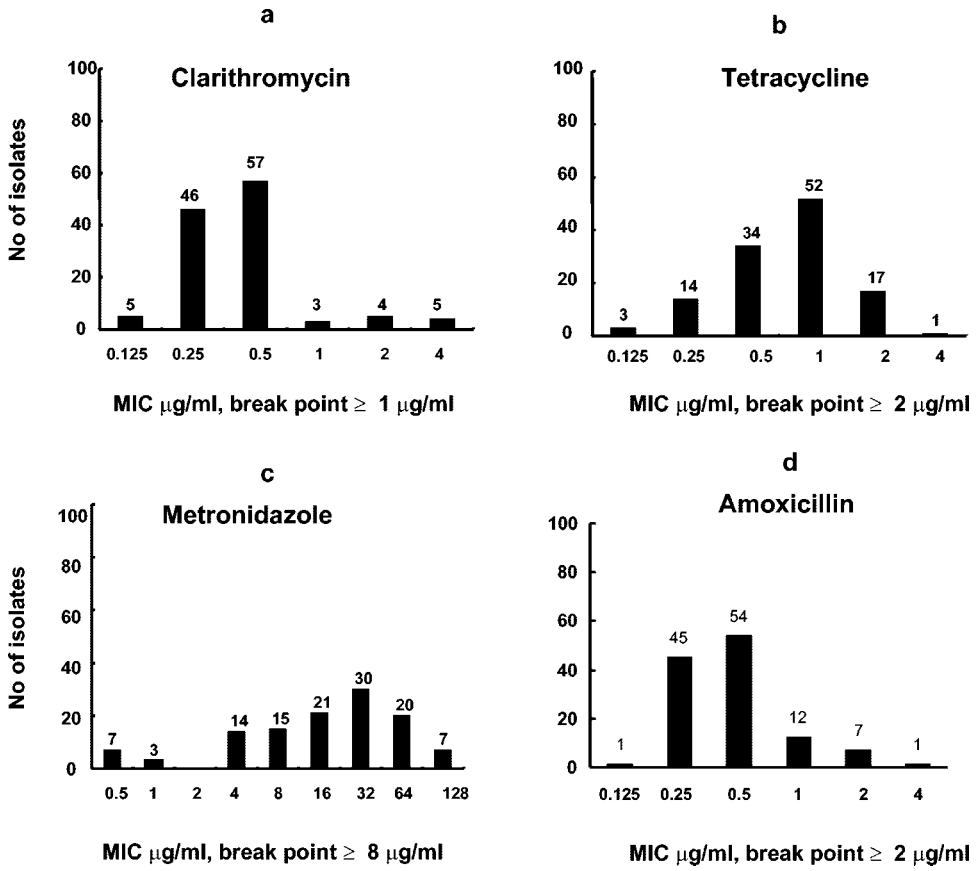

FIG. 1. Distribution of MICs of clarithromycin (a), tetracycline (b), metronidazole (c), and amoxicillin (d) for 120 *H. pylori* isolates. The numbers above the bars represent the numbers of the isolates for which the particular MIC applies.

resistant, which is in agreement with an earlier finding from this region.

Therefore, it is reasonable to conclude that in our geographical area, antibiotic resistance is an emerging problem for the treatment of *H. pylori*-infected patients. The present study also demonstrates the need for continuous monitoring of the antimicrobial susceptibility in *H. pylori* for determination of optimal treatment regimens.

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