Antimicrobial susceptibility of Helicobacter pylori strains isolated in Bangladesh

Shamsun Nahar
*International Centre for Diarrhoeal Disease Research*

Asish K. Mukhopadhyay
*National Institute of Cholera and Enteric Disease, Calcutta, India*

Rasel Khan
*International Centre for Diarrhoeal Disease Research*

Mian Mashhud Ahmad
*Dhaka Medical College Hospital*

Simanti Datta
*National Institute of Cholera and Enteric Disease, Calcutta, India*

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

**Recommended Citation**
Nahar, Shamsun; Mukhopadhyay, Asish K.; Khan, Rasel; Ahmad, Mian Mashhud; Datta, Simanti; Chattopadhyay, Santanu; Dhar, Swapan Chandra; Sarker, Shafiqul Alam; Engstrand, Lars; Berg, Douglas E.; Nair, Balakrish; and Rahman, Motiur, "Antimicrobial susceptibility of Helicobacter pylori strains isolated in Bangladesh." *Journal of Clinical Microbiology*. 42,10. 4856-4858. (2004).
https://digitalcommons.wustl.edu/open_access_pubs/2589
Authors
Shamsun Nahar, Asish K. Mukhopadhyay, Rasel Khan, Mian Mashhud Ahmad, Simanti Datta, Santanu Chattopadhyay, Swapan Chandra Dhar, Shafiqul Alam Sarker, Lars Engstrand, Douglas E. Berg, Balakrish Nair, and Motiur Rahman
Antimicrobial Susceptibility of Helicobacter pylori Strains Isolated in Bangladesh

Shamsun Nahar, Asish K. Mukhopadhyay, Rasel Khan, Mian Mashhud Ahmad, Simanti Datta, Santanu Chattopadhyay, Swapan Chandra Dhar, Shafiquil Alam Sarker, Lars Engstrand, Douglas E. Berg, G. Balakrish Nair and Motiur Rahman


Updated information and services can be found at:
http://jcm.asm.org/content/42/10/4856

These include:

REFERENCES
This article cites 25 articles, 12 of which can be accessed free at:
http://jcm.asm.org/content/42/10/4856#ref-list-1

CONTENT ALERTS
Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Antimicrobial Susceptibility of *Helicobacter pylori* Strains Isolated in Bangladesh

Shamsun Nahar,¹ Asish K. Mukhopadhyay,² Rasel Khan,¹ Mian Mashhud Ahmad,³ Simanti Datta,² Santanu Chattopadhyay,² Swapan Chandra Dhar,³ Shafiqul Alam Sarker,¹ Lars Engstrand,⁴ Douglas E. Berg,⁵ G. Balakrish Nair,¹ and Motiur Rahman¹*

International Centre for Diarrhoeal Disease Research,¹ and Dhaka Medical College Hospital,³ Dhaka, Bangladesh; National Institute of Cholera and Enteric Disease, Calcutta, India;² SMI, Stockholm, Sweden;² and Departments of Molecular Microbiology and Genetics, Washington University School of Medicine, St. Louis, Missouri

Received 25 March 2004/Returned for modification 28 April 2004/Accepted 18 May 2004

Antimicrobial susceptibility of 120 *Helicobacter pylori* isolates from Bangladesh.

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). 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% O₂, 10% CO₂, and 85% N₂ 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 (Mtzs) isolates were further studied (by inactivation of *rdxA* alone or *rdxA* and *frxA* for conversion into an Mtzr phenotype) by transformation of Mtzs 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 ≤ 0.05) in antimicrobial resistance was observed among these groups (Table 1).

Inactivation of only *rdxA* was sufficient to confer the Mtzr phenotype in 66% (8 of 12) of isolates, 33% (4 of 12) of isolates were Mtzr, and inactivation of only *frxA* had little effect on Mtzr 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 Mtzr phenotype (MIC, 32 μg/ml).

Resistance to metronidazole was the most common type of
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 \textit{H. pylori} resistance to this antimicrobial agent (17). Two types of Mtz\textsuperscript{r} \textit{H. pylori} were isolated in the present study: type I, requiring only inactivation of \textit{rdxA} to become resistant; and type II, requiring inactivation of both \textit{rdxA} and \textit{frxA} to become resistant. Only \textit{frxA} inactivation did not have any role in metronidazole resistance, as only subsequent inactivation of \textit{frxA} in \textit{rdxA}-inactivated isolates reverted from the Mtz\textsuperscript{s} phenotype to the Mtz\textsuperscript{r} phenotype and increased the MIC for the Mtz\textsuperscript{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 \textit{frxA} or \textit{rdxA} (13). Thus, resistance to metronidazole in \textit{H. pylori} is mainly due to mutation in the \textit{rdxA} gene and results from de novo mutation in the resident \textit{rdxA} gene, rather than lateral transfer of a mutant \textit{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 \textit{H. pylori}. Previous use of macrolides has been shown to be associated with \textit{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 \(\beta\)-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-resistant.

![Figure 1](http://jcm.asm.org/)

**FIG. 1.** Distribution of MICs of clarithromycin (a), tetracycline (b), metronidazole (c), and amoxicillin (d) for 120 \textit{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 Helicobacter 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.

This study was conducted at the ICDDR, B: Centre for Health and Population Research with the support of SIDA agreement no 2001-3970, component 75000255/A/7500260.

ICDDR, B acknowledges with gratitude the commitment of SIDA to the Centre's research efforts.

REFERENCES


