

Original Article

Antimicrobial Sensitivity Pattern of *Helicobacter pylori* Isolates among Subgroup of Bangladeshi Patients

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Abstract :

This cross sectional study was carried out at Bangabandhu Sheikh Mujib Medical University (BSMMU) and International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from July 2008 to September 2009. Aim of the study was to find out the antimicrobial susceptibility profile of *Helicobacter pylori* isolates from dyspeptic patients. Total 224 dyspeptic patients from Out Patient Department (OPD) of BSMMU were initially enrolled after informed written consent. After upper GI endoscopy 157 patients were finally included who had erosions, ulcers or atrophic changes in the stomach or duodenum. Two biopsy samples were taken from each of them. Samples were incubated at 37°C in a double gas incubator with 5%O₂, 10%CO₂ and 85%N₂. Total 82 (52.23%) samples were found positive for *H. pylori*. Isolated organisms were then tested for sensitivity to Amoxicillin, Clarithromycin, Tetracycline, Levofloxacin and Metronidazole by Agar dilution method. Among 82 patients 51(62.2%) were male and 31(37.8) were female with a male:female ratio 1.6:1. Patients were categorized into two groups one having gastric or duodenal ulcer (30.5%) and other having no ulcer (69.5%). Among these isolates 92.7% were sensitive to Amoxicillin, 89% to Clarithromycin, 81.7% to Tetracycline, 80.5% to Levofloxacin and only 26.8% to Metronidazole. Beside these, 81.7% isolates were sensitive to both Amoxicillin and Clarithromycin, 74.4% to Amoxicillin and Tetracycline, 73.2% to Amoxicillin and Levofloxacin, 72% to Clarithromycin and Tetracycline, 59% to Clarithromycin and Levofloxacin and 51% to Tetracycline and Levofloxacin.

Key words: *Helicobacter pylori*, Culture and sensitivity, Antimicrobials, Ulcer, Erosions.

Introduction :

Helicobacter pylori is a slow growing, micro-aerophilic, highly motile, Gram negative, spiral bacterial organism

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that chronically infects the stomach of more than half of the human population¹. In most patients, *H. pylori* infection remains asymptomatic and only 10-20% of infected patients develop various gastro-duodenal pathologies including chronic gastritis, peptic ulcer disease, primary B-cell gastric lymphoma, and adenocarcinoma of the stomach^{2,3}. Prevalence of *H. pylori* is very high in Bangladesh, about 92% of adult have been found to be sero-positive for *H. pylori*⁴. In Bangladesh, prevalence of duodenal and gastric ulcer is 11.98% and 3.58% respectively among population aged 15 years and above⁵. Eradication of *H. pylori* allows ulcers to heal and greatly reduces the chance of recurrence⁶. Combined antibiotic therapy with a proton pump inhibitor is recommended to eradicate *H. pylori* infection but antibiotic resistance is a major problem^{7,8}. There is wide variability of antibiotic resistant's pattern and eradication rate with geographical region^{9,10}. It Most of the studies conducted in Bangladesh shown that the eradication rate was between 30-64%¹¹⁻¹³. This is much lower than those obtained in Western countries and the recurrence rate is also higher in our country^{14,15}. This low eradication rate in our country may be due to different antimicrobial sensitivity pattern of *H. pylori*. So, isolation of organism from dyspeptic patient and the antibiotic susceptibility pattern will guide to formulate the appropriate regimen for eradication of *H. pylori*. This study was done to find out the antibiotic susceptibility pattern of *H. pylori* isolates from dyspeptic patients in Bangladeshi population for Amoxicillin, Tetracycline, Clarithromycin, Metronidazole and Levofloxacin.

Materials and methods :

This cross sectional study was carried out in the Department of Gastroenterology, BSMMU and *H. pylori* laboratory of ICDDR, B from July 2008 to September 2009. Dyspeptic patients aged between 15 to 60 years attended to OPD of BSMMU who had not taken Proton Pump Inhibitor (PPI), H₂ receptor blocker or antibiotics in the preceding 2 weeks and had no contraindication for endoscopy were initially enrolled for the study. Every ethical issue was discussed with the patients regarding the study and informed written consents were obtained. Detailed clinical history was taken and thorough physical examination was done. After that, patients were underwent upper GI endoscopy. Patients found to have ulcers or erosions anywhere in the stomach and duodenum at endoscopy were included in the study. Endoscopic diagnosis was categorized into ulcer group and non-ulcer group. Two biopsies were taken, one from antrum within 2 cm of the pylorus and another from the body of stomach within 8 cm of the cardia along the greater curvature^{16,17}. Samples were stored in 1ml Brain Heart Infusion Agar (BHIA) containing 25% glycerol and transported to the laboratory for *H. pylori* culture. In the laboratory 200µl suspension of biopsy specimen was mixed with 100µl of 0.008M urea and 0.006N HCl to minimize contamination. Mixture was streaked on antibiotic supplemented blood-BHIA plates. Plates were incubated for 3 to 6 days at 37°C in a double gas incubator with 5%O₂, 10%CO₂, and 85%N₂. The incubated plates were periodically examined from 2 days for the growth of *H. pylori*. Presumptive *H. pylori* colonies were propagated on new plate for confirmation and stock for storage at -86°C. Isolated organisms were tested for sensitivity to Amoxicillin, Metronidazole, Tetracycline, Levofloxacin and Clarithromycin by Agar dilution method. The antimicrobial susceptibility was judged according to breakpoint criteria defined by National Committee for Clinical Laboratory Standards. All data were recorded in a printed data sheet, statistical analysis were done by commercially available SPSS software.

Results :

A total 224 dyspeptic patients were underwent upper GI endoscopy. Of them 157 had positive endoscopic findings namely- ulcers or erosions in the stomach or duodenum. Biopsy taken from them for culture and 82 patients (52.23%) were found to be positive for *H. pylori*. Among them 51 (62.2%) were male and 31 (37.8) were female with a male to female ratio 1.6:1. Patients were categorized into two groups one having gastric or duodenal ulcer (30.5%) and other having no ulcer (69.5%) (Figure: 1).

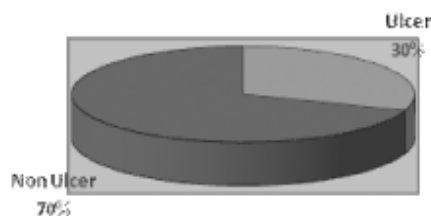


Figure 1 : Distribution of Patients According to Endoscopic Diagnosis

Out of 82 isolates, 76 isolates (92.7%) were sensitive to Amoxicillin, 73 (89%) to Clarithromycin, 67 (81.7) to Tetracycline, 66 (80.5%) to Levofloxacin and only 22 isolates (26.8%) were sensitive to Metronidazole. Nine (10.98%) isolates were sensitive to all tested antibiotics, 42 (51.22%) isolates showed resistant to single antibiotic, 31 (37.8%) isolates were resistant to more than one antibiotic and no isolates were found to be resistant to all five antibiotics (Table I).

Table I : Distribution of patients according to antimicrobial susceptibility

Antimicrobial agent	No. (%) sensitive isolates
Amoxicillin	76 (92.7)
Clarithromycin	73 (89)
Tetracycline	67 (81.7)
Levofloxacin	66 (80.55)
Metronidazole	22 (26.8)

Combined sensitivity of two antibiotics to same isolates was also calculated. Majority of isolates were sensitive to both Amoxicillin and Clarithromycin followed by Amoxicillin and Tetracycline. It was found that 67 (81.7%) isolates were sensitive to both Amoxicillin and Clarithromycin, 61 (74.4%), to both Amoxicillin and Tetracycline, 60 (73.25%) to both Amoxicillin and Levofloxacin, 59 (72%) to Clarithromycin and Tetracycline, 59 (72%) to Clarithromycin and Levofloxacin and 51 (62.2%) to Tetracycline and Levofloxacin.

Sensitivity of isolates was compared between ulcer group and non ulcer group. Here significant difference was only found among Metronidazole resistance, among 25 isolates of *H. pylori* from ulcer group only 1 isolate was sensitive to this antibiotic and 24 isolates were resistant to Metronidazole but among 57 isolates from non-ulcer group 21 were sensitive to Metronidazole. This difference is statistically significant (P=0.002). Regarding other antibiotics tested, no significant difference was observed among ulcer and non-ulcer groups (Table II).

Table II: Comparison of antibiotic susceptibility with endoscopic findings

Antimicrobials	Ulcer Group		Non Ulcer Group		P Value
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	
Amoxycillin	23 (92)	2 (8)	53 (93)	4(7)	0.875
Clarithromycin	20 (80)	5 (20)	53 (93)	4 (7)	0.083
Tetracycline	21 (84)	4 (16)	46 (80.7)	11 (19.3)	0.722
Levofloxacin	22 (88)	3 (12)	44 (77.2)	13 (22.8)	0.256
Metronidazole	1 (4)	24 (96)	21 (36.8)	36 (63.2)	0.002

NB: Statistical test was done by chi square test

Discussion :

There is wide variability of antibiotic sensitivity pattern of *H. pylori* in different country over the world. Metronidazole resistance was the most common type documented worldwide. In Bangladesh, a study conducted by Nahar S et al¹⁸ showed that Metronidazole, Tetracycline, Clarithromycin, and Amoxicillin resistance was 77.5, 15, 10, and 6.6% respectively. An Indian study at Chennai shown that overall *H. pylori* resistance rate was 77.9% to Metronidazole, 44.7% to Clarithromycin and 32.8% to amoxicillin¹⁹. Another Indian study at Kolkata showed that 85% isolates were resistant to Metronidazole and 7.5% to tetracycline²⁰. In present study we found 73.2% isolates were resistant to Metronidazole which is consistent with most of the study. High prevalence of Metronidazole resistance in our country might be due to frequent use of Metronidazole for other intestinal and gynecological problems.

In a study by Chang Wei-Lunet al²¹ shown that resistance rates for Clarithromycin was 10.6%. Huang L Pet al²² in China shown that resistant rate to clarithromycin was 8.3%. Kulsuntiwong Pet al²³ in Thailand shown that resistance to Clarithromycin was 5%. Rafeey Met al²⁴ in Iran found that resistance rate was 16%. Hooton Cet al²⁵ in Ireland found resistance to clarithromycin was 8.9%. Nahar S et al¹⁸ in Bangladesh shown that 10% of isolates were resistant to Clarithromycin. In this study it was found that 11.1% of *H. pylori* isolates were resistant to Clarithromycin, which is in agreement with previous majority study.

Thyagarajan SP et al²⁶ found that Ciprofloxacin and tetracycline resistance was from 1.0 to 4%. Rafeey M et al²⁴ in Iran found 5% isolates were resistant to tetracycline. In Thailand, study by Tangmankongworakoon N et al²⁷

showed 5.1% isolates were resistant to Tetracycline. In Bangladesh it was previously documented that, 15% of isolates were resistant to Tetracycline¹⁸. In present study 18.3% of isolates found to be resistant to tetracycline. These findings are also consistent with previous Bangladeshi study but a bit higher than that of most of the other studies. Tetracycline is also widely used in Bangladesh for diarrhoeal disease, for this reason resistance rate may be a bit higher.

Regarding amoxicillin resistance, in India it was 32.8%²⁶ and in Bangladesh it was 6.6%¹⁸. In this study 7.3% of isolates were resistant to amoxicillin. These findings are also comparable to previous study.

Ciprofloxacin sensitivity against *H. pylori* was extensively studied but limited study was conducted to see Levofloxacin sensitivity. Study conducted in Taiwan²¹, Hong kong²⁸, Japan²⁹ and Italy³⁰ shown that Levofloxacin resistant rate 9.4%, 11.5%, 15% and 14.6% respectively. In this study Levofloxacin resistance was found among 19.5% of *H. pylori* isolates and is almost consistent with previous study.

Amoxicillin & Clarithromycin were shown better combination regarding sensitivity profile, which were recommended worldwide as a 1st line therapy for *H. pylori* eradication.

Among ulcer group majority isolates 24/25 were resistant to Metronidazole in comparison to non-ulcer group 36/57, difference is statistically significant. There are no clear explanations of this finding but may be due to that, ulcer patient had more symptoms, for which they sought more medical consultation and took eradication treatment incompletely and subsequently might develops Metronidazole resistance. But if so, other antibiotics may also be resistant in ulcer group. Other explanation may be that Metronidazole resistance strains of *H. pylori* might be more ulcerogenic than sensitive strains. There are no published data regarding this issue, so it may be a more incidental finding.

Conclusion :

Helicobacter pylori are associated with peptic ulcer disease and are proven risk factors for carcinoma stomach, gastric MALTOMA and other variety of gastrointestinal and non-gastrointestinal disorder. Eradication of *H. pylori* reduces cancer risk and allow ulcer to heal. Combined antibiotic and PPI are recommended for *H. pylori* eradication but antibiotic resistance is a great problem worldwide and there is

also geographical variation of antimicrobial sensitivity pattern. So identification of regional or local antibiotic sensitivity profile will help to formulate appropriate treatment schedule for *H. pylori* eradication. In this study, it was found that majority of isolates were sensitive to Amoxicillin (92.7%), followed by 89% to Clarithromycin, 81.7% to Tetracycline, 80.5% to Levofloxacin and only 26.8% isolates were sensitive to Metronidazole. Regarding sensitivity to two antibiotics simultaneously 81.7% isolates were sensitive to both Amoxicillin and Clarithromycin, followed by 74.4% to Amoxicillin and Tetracycline. Most of the isolates from ulcer patient were Metronidazole resistant.

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