Original Article

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

MMSU Islam1, S Nahar2, MN Sarker3, ASM Salimullah4, MA Rahman5, DS Ahmed6, MQ Hasan7, ASMA Raihan8, MR Bhuiyan9, SA Sarker10

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 that chronically infects the stomach of more than half of the human population. 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. Prevalence of H. pylori is very high in Bangladesh, about 92% of adult population aged 15 years and above have been found to be sero-positive for H. pylori. In Bangladesh, prevalence of duodenal and gastric ulcer is 11.98% and 3.58% respectively among population aged 15 years and above. Eradication of H. pylori allows ulcers to heal and greatly reduces the chance of recurrence. Combined antibiotic therapy with a proton pump inhibitor is recommended to eradicate H. pylori infection but antibiotic resistance is a major problem. There is wide variability of antibiotic resistant's pattern and eradication rate with geographical region. Most of the studies conducted in Bangladesh shown that the eradication rate was between 30-64%. This is much lower than those obtained in Western countries and the recurrence rate is also higher in our country. 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.
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).

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, 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

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. (%) sensitive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>76 (92.7)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>73 (89)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>67 (81.7)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>66 (80.55)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>22 (26.8)</td>
</tr>
</tbody>
</table>

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).
Metronidazole resistance was the most common type documented worldwide. In Bangladesh, a study conducted by Nahar S et al\(^{18}\) 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 amoxicillin\(^{19}\). Another Indian study at Kolkata showed that 85\% isolates were resistant to Metronidazole and 7.5\% to tetracycline\(^{20}\). In present study we found 73.2\% isolates were resistant to Metronidazole for other intestinal and gynecological problems.

In a study by Chang Wei-Lun et al\(^{21}\) shown that resistance rates for Clarithromycin was 10.6\%. Huang L Pet\(^{22}\) in China shown that resistant rate to clarithromycin was 8.3\%. Kulsuntiwong Pet\(^{23}\) in Thailand shown that resistance to Clarithromycin was 5\%. Rafeey M et al\(^{24}\) in Iran found that resistance rate was 16\%. Hooton Cet al\(^{25}\) in Ireland found resistance to clarithromycin was 8.9\%. Nahar S et al\(^{18}\) 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 al\(^{26}\) found that Ciprofloxacin and tetracycline resistance was from 1.0 to 4\%. Rafeey M et al\(^{24}\) in Iran found 5\% isolates were resistant to tetracycline. In Thailand, study by Tangmankongworakoon N et al\(^{27}\) showed 5.1\% isolates were resistant to Tetracycline. In Bangladesh it was previously documented that, 15\% of isolates were resistant to Tetracycline\(^{28}\). 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 Taiwan\(^{29}\), Hong kong\(^{30}\), Japan\(^{31}\) and Italy\(^{32}\) 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 :**

\textit{Helicobacter pylori} are associated with peptic ulcer disease and are proven risk factors for carcinoma stomach, gastric MALOMA 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

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**Table II: Comparison of antibiotic susceptibility with endoscopic findings**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Ulcer Group</th>
<th>Non Ulcer Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (%)</td>
<td>Resistant (%)</td>
<td>Sensitive (%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>23 (92)</td>
<td>2 (8)</td>
<td>53 (93)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>20 (80)</td>
<td>5 (20)</td>
<td>53 (93)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>21 (84)</td>
<td>4 (16)</td>
<td>46 (80.7)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>22 (88)</td>
<td>3 (12)</td>
<td>44 (77.2)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>1 (4)</td>
<td>24 (96)</td>
<td>21 (36.8)</td>
</tr>
</tbody>
</table>

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 al\(^{18}\) 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 amoxicillin\(^{19}\). Another Indian study at Kolkata showed that 85\% isolates were resistant to Metronidazole and 7.5\% to tetracycline\(^{20}\). 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.
also geographical variation of antimicrobial sensitivity pattern. So identification of regional or local antibiotic sensitivity profile will help to formulate appropriate treatment schedule for \textit{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.

References: