Abstract: The Importance of *H. pylori* as an etiological agent in gastro-duodenal disease had suggested antibiotic treatment as a main target for the elimination of infection and to determine the prevalence of *Helicobacter pylori* in patients with gastro-duodenal pathologies and the susceptibility patterns of isolates. Consecutive dyspeptic patients for endoscopy were recruited in the study. Gastric biopsies were collected from the patients and *H. pylori* isolated and identified. The present study a total six antimicrobial agents such as Ciprofloxacin, Metronidazole, Norfloxacin, Tetracyclin, Amoxicillin and Clarithromycin were used. In against 100 clinical isolates *Helicobacter pylori*, Antibiotic susceptibilities were determined by disk diffusion and agar dilution methods against Tetracycline, Amoxicillin and Metronidazole (AMR<sup>®</sup> MET®) was the most common (23.7%) amongst the isolates Ninety-two (83.6%) of the 110 patients (mean age 42.5 ± 15.7, range 14–70 years) were positive for *H. pylori*. The antibiotic susceptibility rates were 61% for tetracycline, 54.3% for clarithromycin, 16.4% for amoxicillin and 1.8% for metronidazole. Antimicrobial susceptibility results also revealed 12 antibiotypes based on resistance to the antimicrobial agents investigated. More than 60% of the isolates exhibited multi-drug resistance to three or four antibiotics. Studies attributed the high level of resistance to the frequent use of the drugs to treat various other infections, ineffective drug control policy and the current treatment regimen in Bikaner.

Keywords *H. pylori*, gastric biopsies, antibiogram.
A reference strain of H. pylori (NCTC 11638) was included as a control. Confirmed isolates were suspended in 20% glycerol and stored at 86 LC (Sanyo, Japan) for future experiments [8].

Antimicrobial susceptibility testing of isolates

For the disk diffusion assay, 110 strains of H. pylori were used to examine susceptibility to clarithromycin, tetracycline, amoxicillin and metronidazole. Antibiotic susceptibility testing: H. pylori isolates were grown on Brucella Agar (Merck, Germany) plates supplemented with 10% fetal bovine serum(GIPCO), and Campylobacter Selective Supplement (Merck, Germany), and incubated under microaerophilic (5% O2, 10% CO2, and 85% N2) conditions for 3 days. Organisms were identified as H. pylori on the basis of morphology on Gram stain examination and by oxidase, catalase, and urease tests (11). A reference strain of H. pylori (NCTC 11638) was included as a control.Confirmed isolates were suspended in 20% glycerol and stored at 86 LC (Sanyo, Japan) for future experiments [8].

Antimicrobial susceptibility results of isolates

Table 1: Antibiotic sensitivity results of H. pylori strains isolated from gastric biopsy specimens

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Antibiotics</th>
<th>Number of strains Showing pattern (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clarithromycin CLR</td>
<td>3(2.7)</td>
</tr>
<tr>
<td>2</td>
<td>Amoxicillin AMX</td>
<td>1(0.9)</td>
</tr>
<tr>
<td>3</td>
<td>Metronidazole MET</td>
<td>5(4.5)</td>
</tr>
<tr>
<td>4</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; AMX&lt;sup&gt;R&lt;/sup&gt;</td>
<td>2(1.8)</td>
</tr>
<tr>
<td>5</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; MET</td>
<td>3(2.7)</td>
</tr>
<tr>
<td>6</td>
<td>TET&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>4(3.6)</td>
</tr>
<tr>
<td>7</td>
<td>AMX&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>21(19.1)</td>
</tr>
<tr>
<td>8</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; TET&lt;sup&gt;R&lt;/sup&gt; AMX&lt;sup&gt;R&lt;/sup&gt;</td>
<td>3(2.7)</td>
</tr>
<tr>
<td>9</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; TET&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>1(0.9)</td>
</tr>
<tr>
<td>10</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; AMX&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>23(20.9)</td>
</tr>
<tr>
<td>11</td>
<td>TET&lt;sup&gt;R&lt;/sup&gt; AMX&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>24(21.8)</td>
</tr>
<tr>
<td>12</td>
<td>CLR&lt;sup&gt;R&lt;/sup&gt; TET&lt;sup&gt;R&lt;/sup&gt; AMX&lt;sup&gt;R&lt;/sup&gt; MET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>19(17.2)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>110(100)</td>
</tr>
</tbody>
</table>

DISCUSSION

H. pylori-associated disorders such as peptic ulcer disease generally treat completely after eradication of H. pylori with antibiotics. Antimicrobial resistance is an increasing difficulty in H. pylori treatment Barthel and Everett [11] dismissed culture as the gold standard for the diagnosis of H. pylori infection. We used culture to investigate the presence of H. pylori infection among dyspeptic patients and found a prevalence of 83.3 (92/110), higher than the 72% (67/93) reported by Palmer et al. [12]. The difference may be because of the detection method used. Moreover, culture enabled us to detect the susceptibility pattern of our isolates to guide empiric treatment. In a developing...
country such as India with limitations in expertise, culture remains an affordable technique in most laboratories. We evaluated 110 H. pylori isolates from patients. Of the 110 H. pylori isolates, 32 (28.9%) exhibited resistance to at least one of the four antimicrobial agents. Our results revealed antimicrobial susceptible rates of 56.1% for tetracycline, 55.3% for clarithromycin, 14.4% for amoxicillin and 6.8% for metronidazole. A similar study in Western Nigeria documented 100% resistance of H. pylori strains to amoxicillin, tetracycline and metronidazole [13]. We think this could be because of the differences in local antibiotic prescription practices and usage in the community.

The high prevalence of clarithromycin resistance (63.8%) observed in our study may be partly because of the use of other less expensive macrolides linked to cross-resistance with clarithromycin as suggested earlier, as clarithromycin is an expensive drug, and hence less abused by the public [14-15].

The resistance rate of 2.4% observed for tetracycline in our study is low compared with the 100% reported by Smith et al. 2001 [16]. Our study also revealed a very high resistance rate of isolates to amoxicillin (85.6%). This is similar to that reported by Smith et al. 2001. However, many studies have reported marked susceptibility (100%) to amoxicillin [17-19]. This may be because of the prescription practices in the different regions where these studies were conducted, as it has been reported that the prevalence of antimicrobial resistance varies with geographical region [20]. The possibility of bacterial strains acquiring resistance to amoxicillin is therefore strong. Colonisation of the stomach with b-lactam-resistant bacteria may lead to the transfer of amoxicillin resistance to H. pylori. The antimicrobial resistance of H. pylori isolates to two, and multiple antimicrobial agents was found in 25.3% and 3.6%, respectively. Multiple antibiotic resistances were observed in 8 of 27 (29.6%) resistant isolates (17).

The higher resistance observed with metronidazole in females may be because of the use of the drug in the treatment of trichomoniasis and bacterial vaginosis, which is especially common in our environment. For clarithromycin, it might have been provoked by the use of erythromycin in pregnancy or other macrolides for chlamydial or non-gonococcal urethritis / cervicitis with subsequent cross-transfer of resistance as suggested earlier [21].

CONCLUSION

In conclusion, the determination of H. pylori antibiotic resistance can help clinicians to select a valuable empiric treatment. Our study revealed low rates of susceptibility of our isolates to the currently recommended treatment regimen used in Bikaner. Despite the small number of patient and no follow-up, our results give an indication of the need to (1) establish baseline susceptibility data for empiric treatment of cases, and (2) conducting studies involving newer and broad spectrum antibiotics to address resistance.

REFERENCES

Helicobacter pylori resistance to clarithromycin, metronidazole, and amoxicillin—influence on treatment outcome. The American journal of gastroenterology, 93(3), 386.


