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Determination of Antibiotic Sensitivity Pattern Along With Isolation of Helicobacter Pylori from Gastric Mucosa in North West Region of Rajasthan

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Original Research Article

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Abstract: The Importance of *H. pylori* as an etiological agent in gastroduodenal 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, Amoxycillin and Clarithromycin were used In against 100 clinical isolates Antibiotic sus-ceptibility was determined by disk diffusion and agar dilution methods against The resistance pattern, amoxicillin and metronidazole (AMR^R MET^R) 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.

INTRODUCTION

Helicobacter pylori (H.pylori) is a small, curved, highly motile, gram-negative bacillus colonizes the stomach of about 50% of people around the world. Colonization with H. pylori is not a disease, but H. pylori are an etiologic agent of acute or chronic gastritis, and a predisposing condition to peptic ulcer disease, gastric lymphoma and gastric carcinoma [1-3]. Many studies believe that H. pylori eradication leads to curing gastritis and peptic ulcer disease, and possibly as well has an important effect on regression of atrophic gastritis and prevention of gastric cancer[4-5]. It plays a role in adenocarcinoma of the distal stomach, mucosa-associated lymphoid tissue lymphoma and primary gastric non-Hodgkin's lymphoma, as well as in a number of extra gastric diseases [6].

The extensive use and limited option of the antibiotics have resulted in the raise of antibiotic resistance in H. pylori. Resistance to metronidazole is observed in 10 to 50% of the cases in developed countries, but can be as high as 90% in developing worlds (7)Infections with this bacterium cause considerable morbidity, and impose a major burden upon health care systems worldwide. Infection with one strain of the organism does not protect against subsequent co-infection with a different strain; hence a high rate of polyclonal infection results. This allows for exchange of DNA between different strains, which could promote the spread of genes encoding important virulence factors or resistance to antibiotics [7]

MATERIALS AND METHODS

Patient population

We evaluated a total of 110 adult patients [6 gastric cancer (GC), 47 peptic ulcer dyspepsia) PUD) and 57 nonulcer dyspepsia (NUD)] undergoing upper gastroduodenal endoscopy for diagnosis and treatment purposes in the gastroenterology departments of Sardar patel medical college and PBM Hospital, Bikaner, Rajasthan between January and July 2017.

Bacterial strains

Two antral and corpus biopsy specimens each were obtained from the patients at endoscopy. The biopsies were immediately placed in sterile bottles contain-ing 0.9% sterile physiological saline. Gastric biopsy specimens were ground with tissue homogenizer and then inoculated onto Brucella Agar (Merck, Germany) with 10% sheep blood and 10% fetal bovine

serum(GIPCO), and Campylobacter Selective Supplement (Merck, Germany), and incubated under microaerophilic (5% O2, 10% CO2, and 85% N2) conditions at 37º C for 3 to 5 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 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% sheep blood, and incubated under microaerophilic (5% O2, 10% CO2, and 85% N2) conditions for 3 days [9]. The bacterial suspension was adjusted to a final concentration of 3×108 CFU/ml in 1.0 ml sterile saline solution. The suspensions were spread on Mueller-Hinton agar plates (Merck, Germany) supplemented with 10% fetal bovine serum (GIPCO) by cotton swabs and then disks containing metronidazole (5 µg), amoxicillin (10 µg), clarithromycin (15 µg), and tetracycline (30 µg) (HiMedia Laboratories Co., India), were placed on the agar surface. The plates were incubated under microaerophilic conditions for 3 days at 37ºC. Then, the inhibition zone diameters were considered as resistant (R), intermediate (I) or susceptible (S). The E. coli strain ATCC 25922 was included as a quality control in all assays.Plates free of antibiotic was included as negative controls in every MIC determination. A inhibition zone size ≤16 mm was consider resistant for metronidazole, ≤25 mm for amoxicillin resistance, and ≤30 mm for clarithromycin, and tetracycline resistance. An inhibition zone larger than those sizes was determined to be[10].

RESULTS

A total of 110 subjects were enrolled in the study. Their mean age was 42.5 ± 14.7 years With 49 male and 61 female subjects, the overall male female ratio was 1:1.2. The antibiotic susceptibility results of the isolates are shown in Table 1. Of the 110 isolates, Overall resistance rates were: 26.5% (29/110) for clarithromycin and 63.8% (69/110) for metronidazole. Tetracycline resistance was identified in only three isolates (2.4%), and 8 (7.3%) isolates showed resistance to amoxicillin56.1% were susceptible to tetracycline and 55.3% to clarithromycin. Marked resistances were noted for amoxicillin (85.6%) and metronidazole (93.2%). Most strains (60%) were resistant (MtR/ ClaS) or intermediate (MtI/ClaS), whereas sixteen strains (19.3%) were resistant to both antibiotics (MtR/ClaR), and 28(25.3%) resistant to two different antibiotics (MtR/TetR, MtR/AmR, MtR/ClaR) and 3.6% to three antibiotics (MtR/ClaR/TetR, MtR/ClaR/AmR). Fifty nine percent of the clarithromycin resistant strains also showed resistance in metronidazole.Thirty (27.2%) showed multi-resistance to tetracycline, amoxicillin and metronidazole Zone diameter breakpoints for clarithromycin testing was <14 mm resistance (R) and ‡14 susceptible (S); for tetracycline and amoxicillin <16 mm (R) and ‡16 (S); and metronidazole testing was <10 mm (R) and ‡10 (S).

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

Serial number	Antibiotics	Number of strains Showing pattern (%)
1	Clarithromycin CLR	3(2.7)
2	Amoxicillin AMX	1(0.9)
3	Metronidazole MET	5(4.5)
4	CLR^R AMX^R	2(1.8)
5	CLR^R MET	3(2.7)
6	TET^R MET^R	4(3.6)
7	AMX^R MET^R	21(19.1)
8	CLR^R TET^R AMX^R	3(2.7}
9	CLR^R TET^R MET^R	1(0.9)
10	CLR^R AMX^R MET^R	23(20.9)
11	TET^R AMX^R MET^R	24(21.8)
12	CLR^R TET^R AMX^R MET^R	19(17.2)
Total		110(100)

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.

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