

## 8 Studies on *Helicobacter pylori*

### 8. Studies on *Helicobacter pylori*

#### 8.1 Correlation of histology with genotypes of *Helicobacter pylori* isolated from cases of peptic ulcer, non ulcer dyspepsia, gastric carcinoma and lymphoma

Investigator:

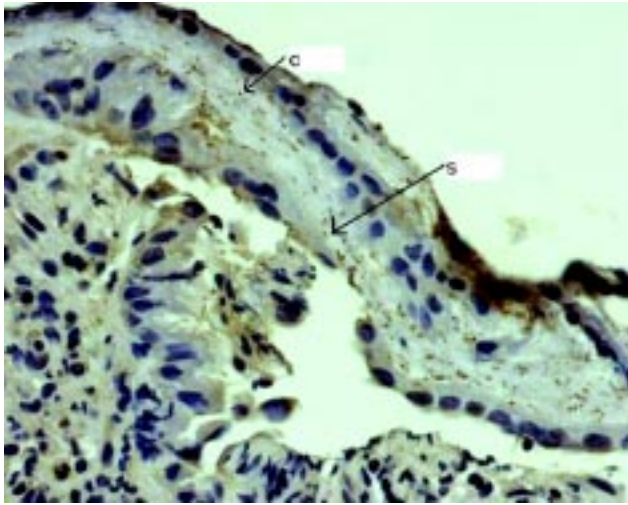
D.R. Saha

*Helicobacter pylori* is of major concern today because of its causal relationship with gastroduodenal diseases. The bacteria are prevalent worldwide and more than half of the world population are infected with *H. pylori*. Though the bacteria have direct casual relationship in the disease process, actual pathogenesis is still not clear. The prevalence of *H. pylori* related chronic gastritis, duodenal and gastric ulcer is quite high in eastern India. Long term infection with *H. pylori* can lead to the dreadful diseases like gastric carcinoma and MALT lymphoma. MALT lymphoma regresses in about half of the cases when *H.pylori* infection is eradicated with antimicrobial agents. Two important bacterial factors *CagA* and *VacA* are associated with *H.pylori* infection which are of importance in public health would have implication for strategies of control and therapy.

This project was undertaken to determine the association and tissue response to *Helicobacter pylori* having different genetic markers like *CagA*, *VacA* etc. with different diseased conditions as well as in healthy controls. Endoscopic biopsy samples were collected from fundus and antrum of the stomach of the patients having the history of upper gastrointestinal complains, who attended S.S.K.M hospital Kolkata. Five bits

of tissue were taken - one piece was used for Rapid urease test, two peaces were kept in Brucella broth with 15% glycerol for culture and two pieces were kept in buffered formalin for histopathological examination. The biopsy samples were obtained from patients suffering from duodenal ulcers, gastric ulcers, non ulcer dyspepsia and from healthy volunteers. Formalin treated tissues were embedded in paraffin and sectioned into serial thin pieces. These pieces were stained by Haematoxyline and Eosine (H&E) for light microscopic examination. Few special stains like modified Giemsa and Immunohistochemical stains were also used for better visualization of *H.pylori* in biopsy specimens. Histological examination revealed that the bacteria had good colonizing ability in the damaged gastric mucosal. Modified Giemsa stain helped to better visualization of the morphology of *H.pylori*. The bacteria usually stays in spiral and coccoid forms and Modified Giemsa and immuno peroxidase stains used in the study also helped to detect spiral and coccoid forms of the organism. The immuno stain containing polyvalent antibody against *H.pylori* is considered to be specific for *H.pylori*. In addition to histology, biopsy specimen was cultured for isolation of *H.pylori* and PCR examination was done for genotyping of the strains. Activity was frequently observed in *H.pylori* associated gastritis, which was evaluated by the presence of inflammatory cells in lamina propria and in glands. Persistent infection and recurrence after eradication therapy is a great problem in *H.pylori* infection. A detailed ultrastructural study of the host cell and bacterial interaction, the consequent cytotoxic and inflammatory activities are in progress to better explore the actual bacterial effect in the disease pathology.





**Fig. 8.1.1** spiral and coccoid form of *H.pylori* by immuno stain -40x

**8.2 Most *Helicobacter pylori* strains of Kolkata are resistant to metronidazole but susceptible to other drugs commonly used for eradication and ulcer therapy**

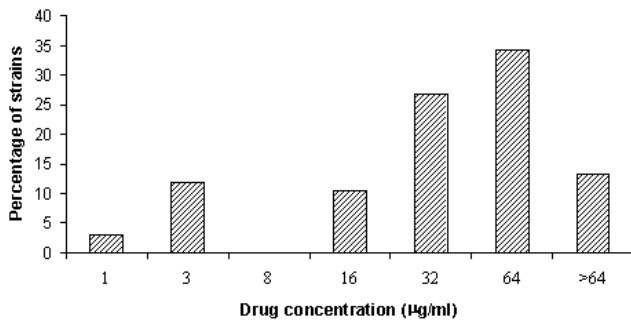
Investigator:

A.K. Mukhopadhyay

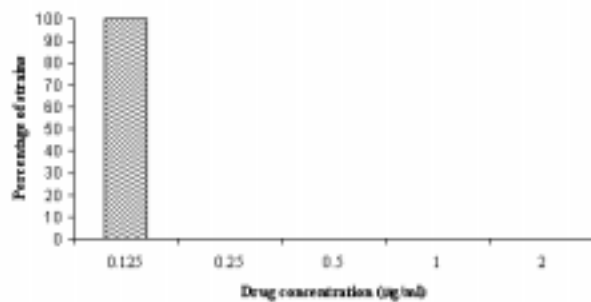
*Helicobacter pylori* infection is the major cause of peptic ulcer disease as well as a contributor to other illness ranging from childhood malnutrition to gastric cancer, and to increased

susceptibility to other food and water-borne pathogens. Since virulence markers of *H. pylori* are not associated with diseases in all the geographical regions, eradication of *H. pylori*, if infection is detected, provides the most effective treatment for *H. pylori* associated diseases. Resistance to some of the potentially useful antimicrobial agents, such as metronidazole (Mtz), is unfortunately quite common, and the search for an optimal treatment has resulted in a wide range of eradication regimens, often with contradictory results. The increased prevalence of antibiotic resistant *H. pylori* strains seriously complicates the efforts to eradicate infection.

The prevalence of *H. pylori* infection in India is extremely high: some 70% to 90% of patients with duodenal ulcer, and also 50% to 80% of patients with non-ulcer dyspepsia or healthy asymptomatic adults. Even though many treatment schedules for *H. pylori* eradication are used or have been proposed in India, there is a paucity of comprehensive reliable *in vitro* *H. pylori* drug susceptibility data except for Mtz. One recent multicentric study in India had reported 80% amoxicillin (Amx) resistance and 96% Clarithromycin resistance among *H. pylori* isolates from Hyderabad, a city in southern India, which contrasts alarmingly with the prevalence reported from most other studies, with the exception of one from Mumbai, in Western India. These high frequencies in resistance, if correct and general for the Indian subcontinent, would be of immense concern, given the more than one billion



**Fig. 8.2.1A** MIC for Metronidazole.



**Fig. 8.2.1B** MIC for Clarithromycin.



Indians and many additional millions of people in neighboring countries. This concern and uncertainty motivated us to conduct the present study.

A total of 103 consecutive patients, between 21 and 71 years of age, were enrolled in this study. Of these 103 patients, 69.9% (72) were male and 30.1% were female. Sixty-nine patients were diagnosed as peptic ulcer (PU) and the other 34 patients had non-ulcer dyspepsia (NUD). Sixty nine percent (71/103) of the enrolled subjects were positive for *H. pylori* by culture. Of the 71 isolates, a total of 67 were available for antimicrobial susceptibility testing. Among these strains, 64% (43/67) were isolated from patients presenting with PU and

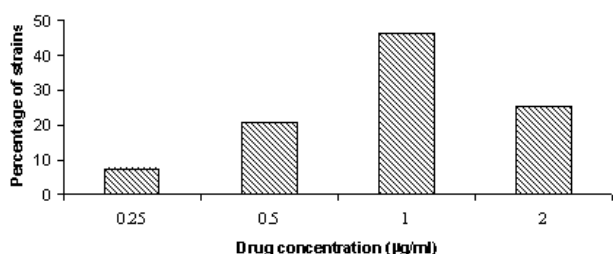


Fig.8.2.1C MIC for Furazolidone.

36% (24/67) from patients with NUD. Susceptibilities to Mtz, Cla, Amx, tetracycline (Tet) and furazolidone (Fz) were determined by the agar dilution method. Fifty seven (85%) of the 67 strains were resistant to at least 8 µg/ml of Mtz. Further testing identified several different levels of Mtz resistance, with MICs of 16 (10.4%), 32 (26.9%), 64 (34.3%) and >64 µg/ml (13.4%) (Fig.8.2.1A) Among the ten Mtz sensitive strains, 2 were susceptible to lower concentrations (1 µg/ml) and these strains were also unusual in its very low Mtz<sup>R</sup> mutant frequency: <math>10^{-8}</math>, in contrast  $10^{-4}$  with other sensitive strains, the remaining 8 strains were sensitive to 3 µg/ml of the drug. In contrast to high Mtz resistance, all 67 Kolkata strains tested were sensitive to Cla, Fz and Amx (Fig. 8.2.1B-

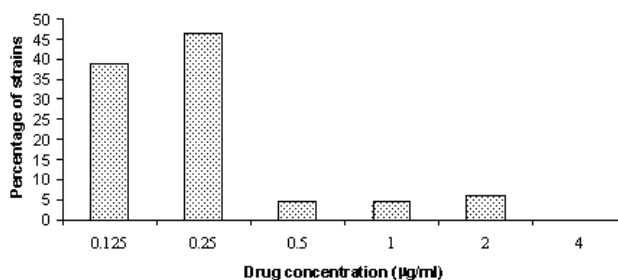


Fig. 8.2.1D MIC for Amoxicillin

D). Five of these 67 strains, however, showed low level of resistance to Tet (2 µg/ml, but not with 4 µg/ml) (Fig. 8.2.1E).

The genetic basis of the Mtz susceptibility of ten *H. pylori* strains was studied by sequential inactivation of the *rdxA* and *frxA* genes, which could each contribute to susceptibility in other populations. Inactivation of *rdxA* alone was sufficient to confer Mtz<sup>R</sup> phenotype in 8 strains that had exhibited high rates ( $\sim 10^{-4}$ ) of mutation to resistance, but not the other two, which had only rarely ( $< 10^{-8}$ ) mutated to Mtz<sup>R</sup>. On the other hand, inactivation of *frxA* alone had little if any effect on Mtz susceptibility of any of these ten isolates. Subsequently, all *rdxA* deficient strains were subjected to *frxA* inactivation and the sensitivity to Mtz was determined. This increased the MIC for Mtz from 32 µg to 64 µg/ml for each of the first 8

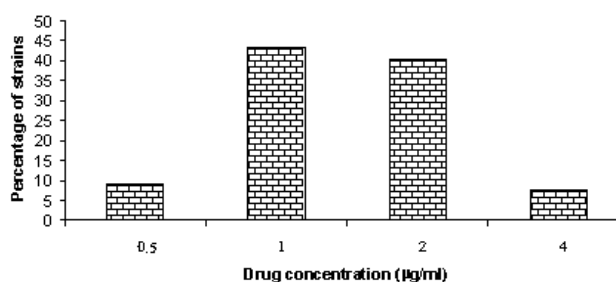


Fig. 8.2.1E MIC for Tetracycline



strains, and also to 64 µg/ml for the final two that had remained Mtz<sup>S</sup> after *rdxA* inactivation. Thus, two types were distinguished among the very rare Mtz<sup>S</sup> strains of Kolkata. Loss of function mutations in *rdxA* was sufficient to cause Mtz resistance in one group of Mtz<sup>S</sup> *H. pylori* strains, which are the predominant group and another group of Mtz<sup>S</sup> strains become Mtz<sup>R</sup> only if *rdxA* and *frxA* are both inactivated. *frxA* alone does not contribute to the normal Mtz<sup>S</sup> phenotype of wild-type *H. pylori* strains but the MIC level of Mtz<sup>R</sup> (*rdxA* deficient) strains can be increased by mutation in *frxA* gene. Inactivation of *rdxA* is always needed for Mtz<sup>S</sup> strain to become Mtz<sup>R</sup>.

This huge variation in the sensitivity patterns among *H. pylori* strains from different parts of India could be due to the possibility that these strains acquired resistance to antibiotics differently. It is possible because inhabitants from different parts of India bear distinct genetic traits, which may allow this long-term colonizer of human gastric niche to co-evolve with different host populations. However, we do not ignore the possibility of interpreting antibiotic sensitivity patterns for *H. pylori* falsely due to variation in the assay technique and interpretation criteria. Based on our results, Cla, Fz and Amx stand as the drug of choice in the line of treatment of *H. pylori* infection at least in Kolkata, eastern part of India. The wide discrepancy in the antibiotic sensitivity profiles of *H. pylori* within the same country undoubtedly suggest the urgent need for a systematic and consensus approach to determine the antibiogram of the strains before trying out drug regimens. Our results also support the need for susceptibility testing to define the resistance patterns of *H. pylori* in particular geographical area before the general use of an eradication schedule. Given the threat that antimicrobial resistance poses to currently available treatment regimens, this would greatly assist in optimizing antibiotic use in patients infected with this important gastric pathogen.



*Weighting of baby at home by the field workers*

