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Short Communication

MOST OF THE *HELICOBACTER PYLORI* ISOLATES ARE RESISTANT TO LEVOFLOXACIN IN NORTH INDIA

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ABSTRACT

Objective: *Helicobacter pylori* related gastro-duodenal diseases can be cured by proper treatment therapy. In India, the commercially available classic treatment therapy (proton pump inhibitor, amoxicillin, and clarithromycin) for bacterial eradication is available. However, antibiotic resistance to the commonly used triple regimen is increasing very rapidly. Considering that treatment including levofloxacin may be an alternative to the classic regimen. Therefore, we aimed to verify *H. pylori* isolates susceptibility to levofloxacin in India.

Methods: *H. pylori* were cultured from 56 patients suffering from different gastro-duodenal diseases. Minimum Inhibitory concentration to levofloxacin was determined by agar dilution method.

Results: The clinical diagnosis of 56 patients who were *H. pylori* culture positive were Gastro Esophageal Reflux Disease (GERD) (n=23), Non-erosive reflux Disease (NERD) (n=22), Non Ulcer Dyspepsia (NUD) (n=3), Antral Gastritis (n=2), Duodenal ulcer (n=1) and others (n=5). Of the 56 *H. pylori* isolates, the prevalence of levofloxacin resistance was found in 41 *H. pylori* isolates (73.2%).

Conclusion: We found that three-fourth of the isolated *H. pylori* strains of North India showed resistance to levofloxacin which is used for *H. pylori* treatment in other countries. Therefore, the conventional triple therapy comprising amoxicillin and clarithromycin is more appropriate for anti *H. pylori* management in India.

Keywords: *Helicobacter pylori*, Levofloxacin, Resistance

Helicobacter pylori (H. pylori) associated gastroduodenal diseases can be cured by proper eradication therapy [1]. Based on this knowledge, numerous treatments have been tested and the currently recommended regimen is a proton pump-inhibitor-based triple therapy with two antibiotics between clarithromycin, amoxicillin or metronidazole which is also called 'classic' regimen [2]. In India, the classic triple therapy regimen including clarithromycin and amoxicillin has been used as the first choice therapy [3, 4]. Treatment regimens that replace the clarithromycin with metronidazole compounds are questionable, as evidence in our country from the previous reports showed a low eradication efficacy due to the high prevalence of metronidazole-resistant bacteria [5-7]. Levofloxacin containing triple therapy should be preferred when this treatment fail which have shown good eradication rates in other countries [1, 8]. Overall, a worldwide increase in *H. pylori* resistance to metronidazole, clarithromycin has been observed, causing a decrease in the efficacy of the classic regimen [9, 10]. In India, this decrease occurred unevenly due to the size of the country and socio-economic differences between regions [5-7, 11]. Considering the increasing resistance to the commonly used antibiotics, our aim is to assess the susceptibility of *H. pylori* isolates from the patients suffering from various gastroduodenal diseases to the levofloxacin for choosing the effective therapy for *H. pylori* eradication.

In this study, clinical *H. pylori* strains were isolated from a gastric biopsy of a patient suffering from gastro-duodenal diseases seeking care at Yashoda Super speciality Hospital, Ghaziabad. Patients who had previous gastric surgery and used bismuth, antimicrobial agents, H_2 receptor antagonists, Proton pump inhibitors within four weeks prior to endoscopic examination; or having any of several concomitant medical illnesses including cardiac, respiratory, renal and liver diseases were excluded from the study. This study was approved by the local institutional ethical research review committee (AUUP/UEC/2011). All the patients gave their written informed consent for participation in the study.

Frequency Scale for the Symptoms of GERD (FSSG) questionnaire was administered to all the patients by another person. Gastro-Esophageal reflux disease (GERD) is defined as FSSG questionnaire score is >7 with endoscopic evidence of reflux esophagitis, Non Erosive Reflux Disease (NERD) has FSSG Score is>7 with no endoscopic evidence of reflux esophagitis and Non-ulcer dyspepsia (NUD) has FSSG Score is <7 with no evidence of reflux esophagitis [12]. One biopsy from the antrum of each patient was used for *H. pylori* culture. The biopsy specimens were cultured on brain heart infusion agar (BHIA) medium (Becton Dickinson, Sparks, MD, USA) supplemented with 5% Horse Serum; 0.4% Isovitale X (Becton Dickinson, Sparks, MD, USA), amphotericin B (8 µg/ml), trimethoprim (5 µg/ml) and vancomycin (6 µg/ml) incubated at 37 °C for 3-6 d under microaerophilic atmospheres (5% O_2; 10% CO_2; 85% N_2) (Double gas incubator, Hera cell 150i). *H. pylori* were identified by colony morphology, gram staining and positive urease, oxidase and catalase tests. All the *H. pylori* stocks were kept in BHI broth containing 20% glycerol at-80 °C.

The minimum inhibitory concentration (MIC) breakpoint for Levofloxacin (Sigma, St Louis, MO, USA), was >1 µg/ml determined by agar dilution method as described by European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [13]. Two-fold serial dilution of levofloxacin antibiotic (0.2-2 µg/ml) was used. Three microliter of the adjusted inoculum from the BHI broth yielding a viable cell count of 1 x 10^8 CFU/ml (equivalent to 2 McFarland turbidity standard unit) [14] was delivered as a spot to BHI agar with 5% horse serum plates containing the various concentrations of antibiotic (fig. 1). Antibiotic-free control plates were inoculated at the beginning and end of each series of plates. All plates were incubated under microaerophilic conditions at 37 °C for 3 d. The MIC was defined as the lowest concentration of the drug that inhibited the visible growth of the bacteria. Differences between groups were statistically evaluated by using the chi-square test.

Differences were considered significant at the 5% probability level. Statistical analysis was performed using SPSS ver. 20 software.

A total of 56 *H. pylori* strains isolated from patients between 18-86 y of age were included in this study. Out of these, 30 were males (45.0±17.9 y) and 26 were females (41.5±15.1 y). Clinical diagnosis in these patients revealed GERD (n=23), NERD (n=22), NUD (n=3), antral gastritis (n=2), duodenal ulcer (n=1) and others (n=5) (table 1).

Resistance to levofloxacin was found in 73.2% (41/56; MIC >1 µg/ml) of *H. pylori* isolates. Further testing identified different levels of levofloxacin resistance, MIC < 0.2 µg/ml (3/56; 5.4%), 0.5 µg/ml (1/56; 1. 8%), 1 µg/ml (10/56; 17.9%), 2 µg/ml (1/56; 1.8%) (fig. 2).

The distribution of levofloxacin resistance according to gender, age, disease outcome was shown in table 1. As for association of levofloxacin resistance with gender, age and the clinical outcome no significant difference was found (p > 0.05).

Though, the resistance to levofloxacin was higher in patients with NUD (100%) than patients GERD (78.3%) and NERD (77.3%), but these differences were not significant (p > 0.05) (table 1).

Table 1: Correlation of Levofloxacin resistance of 56 *H. pylori* isolates according to their clinical information

Clinical information		Levofloxacin-resistant (%)	p-value
Gender	Male (n=30)	22 (73.3)	0.99
	Female (n=26)	19 (73)	
Age	18-40 (n=25)	20 (80)	0.91
	41-60 (n=21)	14 (66.7)	
	61-86 (n=10)	7 (70)	
Disease diagnosis	GERD (n=23)	18 (78.3)	0.90
	NERD (n=22)	17 (77.3)	
	NUD (n=3)	3 (100)	
	AG (n= 2)	1 (50)	
	DU (n=1)	0 (0)	
	Others (n=5)	2 (40)	

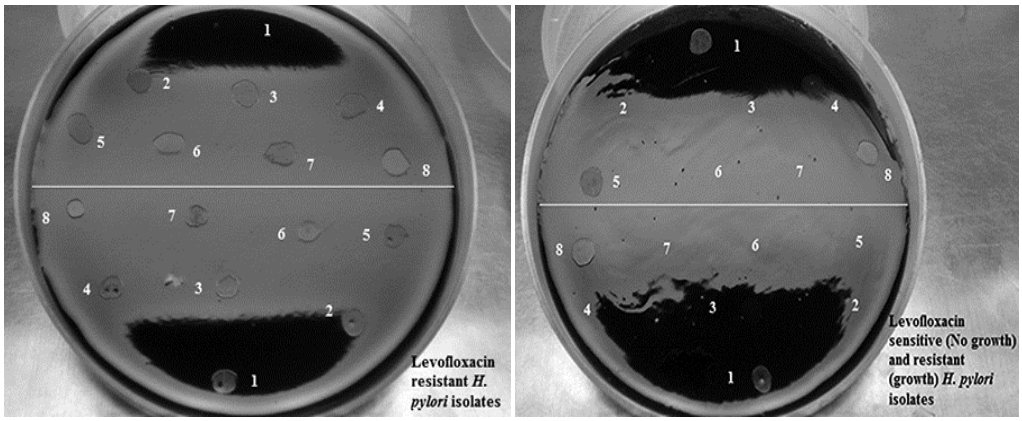

Fig. 1: Antibiotic Susceptibility test: Brain heart infusion agar (BHIA) media plates containing a specific concentration of antibiotic levofloxacin. Three micro liters of the *H. pylori* inoculum in BHI broth yielding the cell count of 1 × 10^8 CFU/ml delivered as a spot to the BHIA media plate. One to eight numbering indicates different *H. pylori* isolates. The test was done in duplicates in each media plate. Growth of culture shows the levofloxacin-resistant *H. pylori* isolate and no growth shows levofloxacin-sensitive *H. pylori* isolate

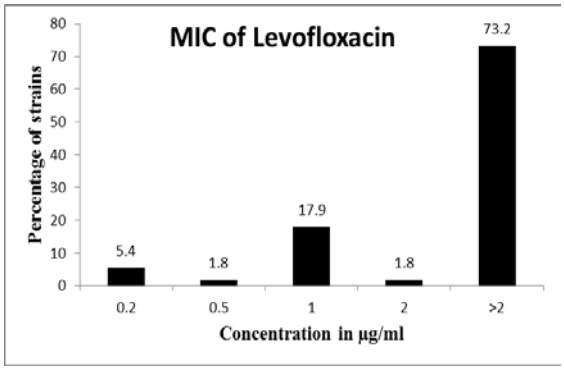

Fig. 2: Distribution of minimum inhibitory concentration (MIC) among the levofloxacin-resistant *Helicobacter pylori* strains isolated from North India

The results are shown from three independent experiments

Therapeutic failures are ascribed to increasing resistance to antibiotic. Antibiotic susceptibility results show controversial findings even in the same geographical areas. This may be due to not all the therapeutic trials considered the bacterial and host factors which finally affects the therapeutic outcome.

The resistance of *H. pylori* to commonly used antibiotic metronidazole in classic triple therapy has been reported very high in India viz. Lucknow (68%), Hyderabad (100%), Chennai (88.2%) [5], Gangetic belt of North India (100%) [6], Kolkata (85%) [11] and Gujarat (83.2%) [7]. According to the Maastricht III Consensus Conference report, metronidazole resistance rate of *H. pylori* over 40%, the effectiveness of triple therapy with metronidazole may decrease. However, the efficiency of metronidazole containing therapy can be enhanced by increasing its dosage, extending its duration or adding bismuth salts [15, 16].

Another drug of choice in triple therapy is clarithromycin used for the treatment for eradication of *H. pylori* with an efficacy of 90% [17]. However, the resistance of *H. pylori* to clarithromycin varies in our country viz. Kolkata (0%) [11], Lucknow (4%) [5], Mumbai (91%) [5], Hyderabad (96%) [5] and Gujarat (58.8%) [7]. According to the recommended guidelines if the clarithromycin resistance is over 15%-20%, therapies with clarithromycin should not be used as firsthand treatment. However, therapies with combined clarithromycin and bismuth can improve the bactericidal activity of clarithromycin.

Recently levofloxacin, a broad spectrum antibiotic with a strong antimicrobial activity, has been used in eradicating *H. pylori* in many countries. The guidelines suggest that levofloxacin-containing therapies as the first line therapy have good efficacy. The resistance rate of levofloxacin in other countries ranges between 12%-33% viz. France (15%), Germany (22%), Taiwan (12%), South Korea (33%) [8], Beijing (29.1%), Xi'an (21.7%) and Shanghai (32.6%) [18]. To our knowledge in India prevalence of its resistance has been determined in only a limited number of studies including Gujarat where the resistance of *H. pylori* to levofloxacin is 13.8%. In contrast, our present study showed very high resistance rate of *H. pylori* to levofloxacin in North India (73.2%), indicating that eradication of *H. pylori* may fail and should not be used in the treatment of *H. pylori.* According to the recommended guidelines, levofloxacin is not the good choice for therapeutic regimen if its resistance is over 20% in susceptibility test of *H. pylori* to this antibiotic [19, 20].

Antimicrobial susceptibility testing is an effective method that tests if *H. pylori* strains are resistant to some antibiotics which improve the rate of response to therapy and is cost saving. However this could not performed every time at all the places because of the culture of *H. pylori* is time-consuming and costly.

In this study, we found that three-fourth of the isolated *H. pylori* strains showed resistance to levofloxacin which is used for *H. pylori* treatment in other countries very effectively. Therefore, the conventional triple therapy comprising amoxicillin and clarithromycin is more appropriate for anti-*H. pylori* management in India. Treatment strategies should be made and changed according to the resistance of *H. pylori* to antibiotics. Further study is needed in more centers.

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COMPETING INTERESTS

None to declare

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