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Antimicrobial susceptibility profiles of *Helicobacter pylori* isolated from patients in North India

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ABSTRACT

Helicobacter pylori-related gastroduodenal diseases are very common in India. Antibiotic resistance to commonly used antibiotics against *H. pylori* is increasing very rapidly. The aim of this study was to determine the antimicrobial susceptibility patterns of *H. pylori* strains from India against commonly used antibiotics in *H. pylori* treatment. *Helicobacter pylori* were cultured from 68 patients suffering from various gastroduodenal diseases in North India. Minimum inhibitory concentrations (MICs) to different antibiotics were determined by agar dilution. The clinical diagnosis of the 68 patients who were *H. pylori* culture-positive were gastro-oesophageal reflux disease (GERD) ($n = 23$), non-erosive reflux disease (NERD) ($n = 22$), non-ulcer dyspepsia (NUD) ($n = 13$), antral gastritis ($n = 3$), duodenal ulcer ($n = 2$) and others ($n = 5$). Of the 68 *H. pylori* isolates, 20 (29.4%) showed no resistance. The prevalence of drug resistance was 70.6%, including resistance to metronidazole (48.5%), furazolidone (22.1%), amoxicillin (17.6%), tetracycline (16.2%) and clarithromycin (11.8%). Dual and multiple drug resistance were found in 26.5% and 8.8% of cases, respectively. In conclusion, more than two-thirds of the isolated *H. pylori* strains showed resistance to at least one of the antibiotics for *H. pylori* treatment. Metronidazole resistance was most prevalent amongst the isolates tested. Emergence of dual and multidrug resistance is of great concern and there is an urgent need for regular antibiotic resistance surveillance studies. Amoxicillin- and clarithromycin-based anti-*H. pylori* regimens commonly prescribed for triple therapy in India show least resistance and hence are appropriate for anti-*H. pylori* management in India.

1. Introduction

Helicobacter pylori is a Gram-negative gastric pathogen that causes chronic gastritis, peptic ulcer disease, gastric carcinoma and B-cell mucosa-associated lymphoid tissue (MALT) lymphoma [1]. In India, almost 80% of the population is infected with *H. pylori* [2] and these rates have not changed since 1991 [3].

Helicobacter pylori infection is usually eradicated by the combination of a proton pump inhibitor (PPI) with multiple antibiotics [4]. The commonly used antibiotics against *H. pylori* are metronidazole, furazolidone, clarithromycin, amoxicillin and tetracycline. Antibiotic resistance is a major problem with *H. pylori* and it is rapidly increasing and reaching ominous proportions. Resistance to the most frequently used antibiotics such as metronidazole (10–90%), clarithromycin (0–15%), furazolidone (0–13%), tetracycline (1–2%) and amoxicillin (0–1%) has been shown to be variable and dissimilar in different region within the country [5–9]. Because of antibiotic resistance amongst *H. pylori*, commonly available antibiotics may not be effective in *H. pylori* eradication. This is of vital significance as it is important to select the most potent and effective antibiotic combination in a given community. The prevalence of dual resistance and multidrug resistance has also increased significantly in many countries and has become a major obstacle in eradicating *H. pylori* infection [10]. Therefore, antibiotic resistance surveillance studies are necessary to determine which antibiotics to use for *H. pylori* eradication.

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Since there are very few studies on the antibiotic resistance profiles of *H. pylori* in India, therapeutic recommendations in India are mostly based on either insufficient data or on data obtained from other geographically unrelated regions. In India, there is also a lack of a regional surveillance programme for antimicrobial susceptibility amongst *H. pylori*.

In view of the above, the present study aimed to evaluate the prevalence of antimicrobial resistance to commonly used antibiotics of *H. pylori* strains isolated from Indian patients.

2. Materials and methods

2.1. Patients and strains

In this study, *H. pylori* strains were isolated from gastric biopsies of patients suffering from gastroduodenal disease seeking care at Yashoda Superspeciality Hospital (Ghaziabad, India) and Maulana Azad Medical College and Associated Hospital (New Delhi, India) according to the following inclusion and exclusion criteria. Inclusion criteria included: age ≥18 years; with symptoms of duodenal or gastric ulcer/gastritis/gastric adenocarcinoma/non-ulcer dyspepsia (NUD); and no antimicrobial therapy to eradicate *H. pylori* infection. Exclusion criteria included: previous gastric surgery; any use of bismuth, antimicrobial agents, H_2 receptor antagonists or PPIs within 4 weeks prior to endoscopic examination; or any of several concomitant medical illnesses including cardiac, respiratory, renal and liver diseases. This study was approved by the Local Institutional Human Research Review Committee (Amity University, Noida, Uttar Pradesh, India; and Yashoda Superspeciality Hospital, Ghaziabad, India). All patients were clinically evaluated and upper gastrointestinal endoscopy was performed by certified gastroenterologists (PK and KD). All patients were also administered the Frequency Scale for the Symptoms of GERD (FSSG) questionnaire by another person (VG or SM). Gastro-oesophageal reflux disease (GERD) is defined as an FSSG questionnaire score >7 with endoscopic evidence of reflux esophagitis; non-erosive reflux disease (NERD) has an FSSG score of >7 with no endoscopic evidence of reflux esophagitis; and NUD has an FSSG score of <7 with no evidence of reflux esophagitis [11]. *Helicobacter pylori* was cultured on brain–heart infusion (BHI) agar (Becton Dickinson, Sparks, MD) supplemented with 5% horse serum, 0.4% IsoVitaleX™ (Becton Dickinson), amphotericin B (8 μg/mL), trimethoprim (5 μg/mL) and vancomycin (6 μg/mL) incubated at 37 °C for 3–6 days under a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) (Heracell™ 150i double gas incubator; Thermo Electron LED GmbH, Langenselbold, Germany). *Helicobacter pylori* was identified by colony morphology, Gram staining, and positive urease, oxidase and catalase tests. Suspensions of *H. pylori* were stored at −80 °C in BHI broth containing 20% glycerol.

2.2. Minimum inhibitory concentration (MIC) determination

MICs for metronidazole, furazolidone, amoxicillin, tetracycline (Sigma, St Louis, MO) and clarithromycin (Abbott Laboratories, Abbott Park, IL) were determined by the agar dilution method as described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [12]. Isolates were considered to be resistant when the MIC was >8 μg/mL for metronidazole [12], >2 μg/mL for furazolidone [13], >0.12 μg/mL for amoxicillin [12], >1 μg/mL for tetracycline [12] and >0.5 μg/mL for clarithromycin [12]. Two-fold serial dilutions of antibiotics were used: metronidazole, 0.2–64 μg/mL; furazolidone, 0.2–2 μg/mL; amoxicillin, 0.12–2 μg/mL; tetracycline, 1–4 μg/mL; and clarithromycin, 0.125–2 μg/mL. Three microliters of the adjusted inoculum from the BHI broth yielding a viable cell count of 1×10^8 CFU/mL (equivalent to a McFarland 2 turbidity standard unit) [14] was delivered as a spot onto the BHI agar with 5% horse serum plates containing the various concentrations of antibiotics. Antibiotic-free control plates were inoculated at the beginning and end of each series of plates. All plates were incubated under microaerobic conditions at 37 °C for 72 h. The MIC was determined as the lowest concentration of the antibiotics at which the growth of the inoculum was completely inhibited.

2.3. Statistical analysis

Differences between groups were evaluated statistically using the χ^2 test. Differences were considered significant at the 5% probability level. Statistical analysis was performed using IBM SPSS Statistics for Windows v.20.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Incidence of Helicobacter pylori

A total of 68 *H. pylori* strains isolated from patients between 18–86 years of age were included in this study during the years 2011–2014. Of these, 36 were male [mean ± standard deviation (S.D.) age, 45.0 ± 17.9 years] and 32 were female (mean ± S.D. age, 41.5 ± 15.1 years). The clinical diagnosis in these patients was GERD (n = 23), NERD (n = 22), NUD (n = 13), antral gastritis (n = 3), duodenal ulcer (n = 2) and others (n = 5).

3.2. Antimicrobial susceptibility and the emergence of multidrug resistance

Of the 68 *H. pylori* isolates, resistance to metronidazole (MIC > 8 μg/mL) was observed in 33 isolates (48.5%). Amongst these 33 metronidazole-resistant strains, 11, 17 and 5 showed single, dual and multidrug resistance, respectively (Table 1). Metronidazole was the common drug in 17 of 18 dual-drug resistant *H. pylori* isolates. Further testing identified different levels of metronidazole susceptibility: MIC < 3 μg/mL (n = 29); MIC = 8 μg/mL (n = 6); MIC = 16 μg/mL (n = 4); MIC = 32 μg/mL (n = 11); MIC = 64 μg/mL (n = 15); and MIC > 64 μg/mL (n = 3) (Fig. 1).

Resistance to furazolidone (MIC > 2 μg/mL) was observed in 15 (22.1%) of the 68 *H. pylori* isolates. Of the 15 furazolidone-resistant strains, 3, 6 and 6 showed single, dual and multiple resistance, respectively (Table 1). Furazolidone was the common drug in all six of the multidrug-resistant *H. pylori* isolates. The remaining 53 susceptible strains showed different level of furazolidone susceptibility: MIC = 0.2 μg/mL (n = 18); MIC = 0.5 μg/mL (n = 5); MIC = 1 μg/mL (n = 13); and MIC = 2 μg/mL (n = 17) (Fig. 1).

Resistance to amoxicillin (MIC > 0.12 μg/mL) was observed in 12 (17.6%) of the 68 isolates. Of the 12 amoxicillin-resistant strains, 2, 6 and 4 showed single, dual and multidrug resistance, respectively (Table 1). The remaining 56 susceptible strains had an MIC of 0.12 μg/mL (Fig. 1).

Resistance to tetracycline (MIC > 1 μg/mL) was observed in 11 (16.2%) of the 68 isolates. Of the 11 tetracycline-resistant strains, 6, 4 and 1 showed single, dual and multiple resistance, respectively (Table 1). The remaining 57 susceptible strains had MIC of 1 μg/mL (Fig. 1).

Resistance to clarithromycin (MIC > 0.5 μg/mL) was found in 8 (11.8%) of the 68 isolates. Of the eight clarithromycin-resistant strains, two, three and three showed single, dual and multidrug resistance, respectively (Table 1). The remaining 60 susceptible strains showed varying MICs: MIC = 0.125 μg/mL (n = 53); MIC = 0.25 μg/mL (n = 6); and MIC = 0.5 μg/mL (n = 1) (Fig. 1).