

Emerging Antimicrobial Resistance in *Helicobacter pylori* Strains Isolated from Gastric Disease Patients in Karachi, Pakistan

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Abstract. In the assessment of the antimicrobial susceptibility of *Helicobacter pylori* strains isolated from gastric biopsies of patients with gastric diseases against commonly prescribed antibiotics in Pakistan, 31 strains were subjected to antimicrobial susceptibility testing using disk diffusion method against seven antimicrobial agents. Most of the isolates showed resistance to metronidazole (93.5%), while only 6.5% isolates were resistant to ofloxacin. The isolates also exhibited variable resistance to other five antibiotics including clindamycin (61.3%), tetracycline (48.4%), erythromycin (41.9%), clarithromycin (38.7%) and amoxicillin (29.0%). Multiple drug resistance in local *H. pylori* isolates was also observed.

Keywords: antimicrobial resistance, *H. pylori*, culture, disk diffusion

Introduction

Helicobacter pylori is a gram negative, microaerophilic spiral bacterium that colonizes the stomach of approximately half of the world population (Dunn *et al.*, 1997). Infection with *H. pylori* is associated with chronic gastritis and peptic ulceration and the bacterium is also considered a risk factor for the development of gastric adeno-carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Blaser, 1993; Parsonnet *et al.*, 1991).

H. pylori once acquired persists usually for life unless eradicated by antimicrobial therapy. Treatment regimens against *H. pylori* have been evolving since the early 1990s. Antimicrobial treatment for *H. pylori* infection is a complicated issue. Currently used regimen for *H. pylori* treatment includes: two antibiotics and an anti-secretory drug like a proton pump inhibitor, to which bismuth salt can be added (Malfertheiner *et al.*, 2005). Commonly prescribed antibiotics are metronidazole, clarithromycin and amoxicillin (Malfertheiner *et al.*, 2002), while tetracycline is used in rescue therapy (Gisbert and Pajares *et al.*, 2001).

Antimicrobial resistance in *H. pylori* is a serious problem and it has become an important cause, leading to eradication failure. Information on antimicrobial susceptibility is important for selection of an appropriate treatment regimen. It is necessary to have information about the frequency of resistance to particular antimicrobial agents before the selection of an optimum treatment regimen.

Successful treatment of *H. pylori* infection not only results in eradication of the pathogen, but often also cures and prevents the development of associated diseases (Kuipers, 1997). However, the increasing incidence of resistance to antibiotics is largely responsible for the decline in eradication rates which result in financial losses as well as frustration for the patient. Resistance rates reported vary from 10-90% for metronidazole, 0-45% for clarithromycin, 0-33% for amoxicillin and 5-59% for tetracycline (Thyagarajan *et al.*, 2003; Boyanova *et al.*, 2002; Al-Qurashi *et al.*, 2001; Kim *et al.*, 2001; Wang *et al.*, 2000). Increasing resistance of *H. pylori* to metronidazole, clarithromycin and amoxicillin have been reported from different parts of the world (Megraud, 2004). The increase in prevalence of antimicrobial resistance in *H. pylori* strains has

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serious implications as, apart from patient compliance, antimicrobial resistance is the most important factor in determining the outcome of antibiotic treatment (Tom *et al.*, 2006). Many studies from Pakistan have emphasized the clinical pattern of gastroduodenal diseases, detection of *H. pylori* infection and comparison of efficacies of different treatment regimens against this pathogen (Yakoob *et al.*, 2004; Khokhar 2002; Abbass *et al.*, 2001). None, to our knowledge, provides information on resistance to commonly used antibiotics for *H. pylori* eradication. High level of resistance to metronidazole (93.5%) and the emerging resistance to other antibiotics, currently included or prescribed for treatment of *H. pylori* infections is a matter of great concern in view of the increasing incidence of *H. pylori* infections and their complications. These observations strongly suggest the necessity of re-evaluating the eradication treatment regimens in local setting and taking immediate measures to reduce self prescription practices as well as increasing the patient compliance. The present study was conducted to determine the antibiotic resistance of *H. pylori* isolates against commonly prescribed antibiotics in Pakistani population.

Materials and Methods

Patients. The study included 110 patients experiencing upper gastrointestinal symptoms including abdominal pain, diarrhoea, nausea and heartburn undergoing upper gastroduodenal endoscopy during December 2005 to June 2006 at Medical Unit II, Civil Hospital, Dow University of Health Sciences, Karachi, Pakistan. Patients with previous *H. pylori* eradication treatment failure or using antibiotics and/or gastric acid inhibitors were excluded from the study. The population consisted of 73 males and 37 females with mean age of 37.7 years and range, 10 to 85 years.

Endoscopy and biopsy sampling. Biopsy specimens were collected from each patient for culture, dipped in 20% glucose solution (Christopher and Harry, 1997) and transported to the Immunology and Infectious Disease Research Laboratory (IIDRL), Department of Microbiology, University of Karachi, Karachi, Pakistan, in a light-proof insulated box containing ice packs and cultured within an hour.

Culture, growth conditions and identification of *H. pylori*. Gastric biopsy specimens were chopped and inoculated on Columbia Blood Agar (CM 0331B, Oxoid) containing laked horse blood (SR0048, Oxoid) 7% and

H. pylori selective supplement Dent (SR 0147E, Oxoid,) containing Vancomycin 5 mg/2 mL, Trimethoprim 2.5 mg/2 mL, Cefsulodin 2.5 mg/2 mL and Amphotericin B 2.5 mg/2 mL. Plates were incubated in a moist microaerophilic atmosphere containing CO₂ 10%, O₂ 5% and N₂ 85% by providing Campylobacter gas generating kit (BR 0056A, Oxoid) at 37 °C for 7 days. After incubation for 3 days, the plates were examined for growth daily. If no growth of *H. pylori* was observed after incubation of 7 days, the plates were discarded. Small rounded colonies were picked and sub-cultured twice to obtain a pure culture. *H. pylori* strains were identified on the basis of morphology by Gram stain and using oxidase, catalase and rapid urease tests (Enroth *et al.*, 1999).

Antimicrobial susceptibility testing. Seven antimicrobial agents metronidazole, amoxicillin, clarithromycin, erythromycin, tetracycline, clindamycin and ofloxacin were tested against each *H. pylori* isolate using Kirby Bauer disk diffusion procedure (McNulty *et al.*, 2002; Debets-Ossenkopp *et al.*, 1999). Briefly, inoculum from a fresh subculture was made equivalent to 3 McFarland standard and spread using a cotton swab onto blood agar plates. The antibiotic disks were placed and then the plates were incubated at 37 °C in a microaerophilic environment for 3-4 days. Antibiotic susceptibility was determined by measuring the growth inhibition zone around the disk.

Statistical analysis. Statistical tests were carried out using SPSS software, version 15 (SPSS Inc., Chicago, USA). The group differences were evaluated using the Pearson chi-square for categorical variables; $P < 0.05$ was accepted as statistically significant.

Results and Discussion

Among 110 symptomatic patients, aged 10-85 years, 73 were male (66.4%) and 37 were female (33.6%). Overall, 32.7% patients were found to be positive for *H. pylori* infection by culture. Among the infected patients, there were 37.0% male and 24.3% female. No statistical difference was detected between the two sexes in the overall prevalence of *H. pylori* infection ($P = 0.181$), (Table 1). As shown in Table 1, 24(34.3%), 9(32.1%) and 2(28.6%) individuals were *H. pylori* positive in the age groups of 19-44, 45-64 years and 65+ years; the incidence was comparatively higher than that in the age group of 13-18 years with non-statistically significant differences.

Antimicrobial susceptibility profiles of 31 *H. pylori* isolates were determined by Kirby Bauer disc diffusion procedure. Out of 31 isolates, 25 were from male and 6 from female patients in the age range of 18 to 68 years. Majority of the isolates were found to exhibit high level of resistance to metronidazole (93.5%) followed by resistance to clindamycin (61.3%), tetracycline (48.4%), erythromycin (41.9%), clarithromycin (38.7%) and amoxicillin (29.0%). However, the resistance to ofloxacin was comparatively low (6.5%). Resistance to multiple antibiotics was also observed; it was found that double drug resistance pattern for metronidazole and amoxicillin was 12.9% and that for metronidazole and tetracycline was 9.6%. Triple drug resistance to metronidazole, clindamycin and tetracycline was in 12.9% of the isolates and 19.3% multiple resistance was also observed for metronidazole, clindamycin, erythromycin and clarithromycin. Two (6.5%) of *H. pylori* isolates were found resistant to all the used antibiotics.

The geographical variation in the antimicrobial resistance of *H. pylori* is considered to be related to the level of use of the antibiotics in different communities (Graham, 1998). In Pakistan, there is indiscriminate use of antibiotics, especially that of metronidazole, clarithromycin, amoxicillin, erythromycin and tetracycline, for treatment of various infections. In addition, self medication is encouraged by free access to drugs due to un-restricted over-the counter-sale of such antibiotics. This could be a contributing factor for the increased antimicrobial resistance among the *H. pylori* isolates to metronidazole, clarithromycin, tetracycline, clindamycin, erythromycin and amoxicillin, observed

in this study. High prevalence of metronidazole resistance might also be due to the frequent use of this antibiotic for other intestinal disorders.

The resistance pattern as reported here is quite similar to what has earlier been reported from India with the exception of tetracycline in which case higher resistance rate (48.4%) was observed as compared to 4.2% (Sengupta *et al.*, 2002) and 28.5% reported by other investigators (Misra *et al.*, 2006). The resistance rate against the two related antibiotics, clarithromycin and erythromycin, was similar (38.7% and 42.0%, respectively), while resistance against ofloxacin was very low (6.5%) indicating that it could be used in combination therapy (Schrauwen *et al.*, 2009). Similar to other reports (Falsafi *et al.*, 2004; Torre *et al.*, 2001), multiple drug resistant *H. pylori* isolates were also found which reflects the indiscriminate use of antibiotics in Pakistan.

Resistance to antimicrobial agents among *H. pylori* isolates is prevalent worldwide and it complicates the efforts to eradicate infection. There are reports that prevalence of resistance varies according to the population studied, with resistance to metronidazole ranging from 10 to 90% and to clarithromycin from 0 to 45%. The associative resistance to metronidazole and clarithromycin is particularly important clinically because these two drugs are frequently prescribed together in a combined therapy. An ideal treatment regimen should be simple, with minimal side effects and most importantly, should consider the local antimicrobial resistance patterns. Accordingly, clinicians could use some help from culture and susceptibility testing before prescribing the best possible treatment

Table 1. *H. pylori* infection according to sex and age

Features	Patients	<i>H. pylori</i> + (%)	OR(95% CI)	<i>p</i> value
Status	110	36(32.7)		
Sex				
Male	73	27(37.0)	0.54(0.22-1.33)	0.181
Female	37	9(24.3)	1.00(1.00-1.00)	1.000
Age category				0.918 ^a
Adolescent: 13-18 years	5	1(20.0)	1.00(1.00-1.00)	1.000
Adult: 19-44 years	70	24(34.3)	2.08(0.22-19.72)	0.513
Middle aged: 45-64 years	28	9(32.1)	1.89(0.18-19.48)	0.586
Aged: 65+ years	7	2(28.6)	1.60(0.10-24.70)	0.735

Note: ^a χ^2 test among all groups, OR: odds ratio, CI: confidence interval.

regimen for the patient depending upon the geographical area.

The antimicrobial resistance pattern among the *H. pylori* isolates was determined first time in Pakistan. The increased resistance could be explained, most probably due to high antibiotics consumption here as compared to other countries. Finally, a high rate of resistance to metronidazole, a considerable resistance to clarithromycin, tetracycline, clindamycin, erythromycin, amoxicillin and the least resistance to ofloxacin has been documented. This study provides a basis for future detailed investigations to understand the resistance pattern of *H. pylori* strains isolated from Pakistani population and stresses the need for search of novel antibacterial factors to combat this highly prevalent infection successfully. The recommended treatment regimens in local practice need to be evaluated carefully in the light of studies highlighting the development of resistance in *H. pylori* isolates. Culture and susceptibility testing is recommended where the first-line and/or the rescue therapy has failed and the patient needs to be cured to reverse the pathophysiological changes associated with *H. pylori* infection.

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