Increasing antimicrobial resistance of *Helicobacter pylori* infection among Saudi patients undergoing upper gastrointestinal endoscopy

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ABSTRACT

There is growing concern about *Helicobacter pylori* resistance strains being the main agent contributing to eradication failure. In this study, we evaluated the prevalence and antimicrobial susceptibility trends of *H. pylori* over a period of time. Over a period of seven years a total of 384 biopsy specimens were sent to the microbiology department and evaluated for culturing *H. pylori* and assess its susceptibility. Biopsy specimens that cultured and where bacterial susceptibility was determined were regarded as *H. pylori* positive, and specimens that failed to culture were considered *H. pylori* negative. A total of 221 patients with *H. pylori* positive were assessed and antimicrobial susceptibility was determined. The overall prevalence of *H. pylori* infections among Saudis undergoing upper gastrointestinal endoscopy, with indicated specimens, was 57.6%. Antimicrobial susceptibility was evaluated; overall clarithromycin resistance was found to be 4.5% and metronidazole resistance 52.5%. The number of clarithromycin resistant strains has increased significantly from 2.5% in 2008 to 10% in 2014 (P<0.0001), and metronidazole resistance strains from 47% in 2008 to 65% in 2014 (P<0.001). *H. pylori* infection is very common among Saudi patients with peptic ulcer disease. Resistance of *H. pylori* against clarithromycin and metronidazole has increased significantly over the seven-year period. This suggests a need to monitor the annual antimicrobial susceptibility pattern.

Introduction

Helicobacter pylori discovery and the recognition of its clinical impact on peptic ulcer diseases have been considered a major development in clinical practice of medicine. It is a microaerobic, Gram-negative spiral bacterium that grows in the stomach of more than half of the world's population.[1] *H. pylori* infections are closely associated with a range of peptic diseases such as chronic gastritis, peptic ulcer disease, and linked to the development of gastric cancer.[1,2] Elimination of the infection definitely improves peptic ulcer disease. Therefore, experts' opinion and international guidelines recommend eradication of *H. pylori* for patients with symptoms.[3,4] Several treatment regimens are available and widely used with different success rates, mostly consisting of two antimicrobials and one proton pump inhibitor.[5,6] Failure of therapy is the result of not only non-adherence but also of the resistance of the bacterium to the prescribed antimicrobials.[7] The presence of resistant strains substantially decreases the success rate of *H. pylori* eradication.[8,9]

The focus of this study is to determine the prevalence of *H. pylori* infection and the antimicrobial susceptibility pattern over a period of time.

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Materials and Methods

All patients undergoing upper gastrointestinal endoscopy in the endoscopy unit at King Abdulaziz Medical City (KAMC), Riyadh, Saudi Arabia, and who satisfied the following inclusion and exclusion criteria, were retrospectively recruited into this study. The study was approved by the ethical committee, King Abdullah International Medical Research Center (KAIMRC). Patients met the following criteria were

eligible for enrolment: being aged ≥18 years and above with symptoms of dyspepsia, and no previous antimicrobial therapy to eradicate *H. pylori* infection. The following exclusion criteria were applied: previous gastric surgery; any use of bismuth, antimicrobial agents, H2-receptor antagonists, proton pump inhibitors, or sucralfate within four weeks prior to endoscopic examination. The main indications for performing endoscopy are the presence of upper abdominal complaints, anemia, or reflux complaints. One biopsy specimen from the gastric antrum or duodenal ulcer for detection of *H. pylori* was collected during upper endoscopy, when it is clinically indicated. The biopsy specimens were sent immediately and processed at the microbiology department in the KAMC. A selective media (Columbia agar with 7% horse blood and *H. pylori* selective supplement, Oxoid, Basingstoke, UK) was used to culture *H. pylori* from the biopsy specimens under microaerophilic conditions produced by a gas-generating system (CampyGen, Oxoid, Basingstoke, UK) for three to six days.

Statistical analysis

A Chi-square test was used to assess the potential association of *H. pylori* infection with the following proportions: age, gender, body mass index, smoking, and peptic ulcers. The risks were estimated using the odds ratios and 95% confidence interval. A multivariate logistic regression model was used to determine the independent risk factors associated with *H. pylori* infection. The Cochran-Armitage test for linear trends was applied to analyze an antimicrobial-resistance time trend over a seven-year period, using STATA 12.0 software. All tests were two-sided, and the results were considered significant at a P value of <0.05.

Results

A total of 384 patients underwent endoscopy where biopsy specimens were obtained and sent to the microbiology department for culturing and processing during the study period (from January 2008 to December 2014). Of the 384 biopsy specimens, 221 were cultured and bacterial susceptibility was successfully identified; these patients served as the *H. pylori* case group, and *H. pylori* negative patients were used as the control group. The mean age of the case group was 65.4 years (±16) and 129 (58.4%) were female (Table 1). The overall prevalence of *H. pylori* infection in our study was 57.6%. The prevalence of *H. pylori* among females was higher than in the male population but was not statistically significant (P=0.392). The prevalence of *H. pylori* infection in patients aged 40-59 years was significantly higher than in other age groups (P=0.021). The proportion of *H. pylori* positive cases who were smokers was 37.6% - statistically significant when compared to the proportion of smokers in the *H. pylori* negative control group - 20.2% (P<0.001). No associa-

Table 1. Demographic characteristics and prevalence of *Helicobacter pylori* infection.

Variables	*H. pylori* positive Cases n=221 (%)	*H. pylori* negative Control n=163 (%)	OR (95%CI)	P value
Age (years)				
≤39 23	(10.4)	28 (17.2)	1.00	1.000
40-59	167 (75.6)	100 (61.3)	2.03 (1.11-3.72)	0.021
≥60	31 (14.0)	35 (21.5)	1.08 (0.52-2.25)	0.840
Gender				
Male	92 (41.6)	75 (46.0)	1.00	1.000
Female	129 (58.4)	88 (54.0)	0.84 (0.87-1.08)	0.392
BMI				
Underweight (BMI<18.5)	17 (7.7)	10 (6.1)	1.00	1.000
Healthy (BMI=18.5-24.9)	70 (31.7)	53 (32.5)	1.29 (0.55-3.04)	0.550
Overweight (BMI=25-29.9)	58 (26.2)	42 (25.8)	1.01 (0.65-1.65)	0.975
Obese (BMI≥30)	76 (34.4)	58 (35.6)	1.10 (0.62-1.78)	0.844
Smoking				
No	138 (62.4)	130 (79.8)	1.00	1.000
Yes	83 (37.6)	33 (20.2)	2.37 (1.48-3.79)	<0.001
Gastrointestinal diseases				
Peptic ulcer				
No	62 (28.1)	119 (73.0)	1.00	1.000
Yes	159 (71.9)	44 (27.0)	6.94 (4.41-10.92)	<0.001

OR, odds ratios; CI, confidence interval; BMI, body mass index.

tion was observed between the rate of *H. pylori* infection and body mass index categories. *H. pylori* infection was observed significantly in peptic ulcer patients (P<0.001).

In the logistic regression model analysis, performed to determine the independent risks, we found a statistical significant correlation between smoking and *H. pylori* infection (P<0.001); *H. pylori* infection was also identified as a predominant risk in patients suffering from peptic ulcer disease (P<0.001) (Table 2).

The overall antimicrobial resistance rates were as follows: amoxicillin 0.9%, tetracycline 1.4%, clarithromycin 4.5%, and metronidazole 52.5%. Table 3 represents the trends of clarithromycin-resistance rates and metronidazole-resistance rates over a seven-year period. The rate of clarithromycin-resistance was significant elevated (P<0.0001). The rate increased four-fold over the seven-year period.

Discussion and Conclusions

The overall prevalence of *H. pylori* infection among the Saudi population that underwent endoscopy over the seven-year period was 57.6%. The relatively low prevalence rate of *H. pylori* infection in this study may be attributed to the design of the study in which only patients who had a biopsy taken and sent to laboratory were included in the study. In a preliminary report, a similar prevalence rate of *H. pylori* was found in patients undergoing bariatric surgery.[10] Age as a risk factor was analyzed and it was found that *H. pylori* infection was higher in the middle-age category than in other age categories. Similarly, Zhu *et al*. found the same prevalence.[11] However, several studies have showed that the prevalence of *H. pylori* infections decreased with increased age.[12] Several studies in the Middle East Countries have reported different prevalence rates of *H. pylori* infections.[13,14] The contributing factors responsible for these differences may include socioeconomic, geographic, or life style, ethnicity or the location of different population.[15,16]

H. pylori resistance to antimicrobials is attributed to several factors, such as frequent consumption of antimicrobials, misuse of antimicrobials, and lack of control in prescribing and dispensing. In our study, the resistance of *H. pylori* against clarithromycin increased four-fold over the seven-year period, but still remained low with an overall rate of 4.5%, while resistance against metronidazole was reported as 52.5%. However, the rate of clarithromycin resistance in our study is much lower than what has been reported in different regions of Saudi Arabia with resistance percentage of 27.7% and 21%.[17,18] Worldwide, a rising trend in the emergence of resistance to clarithromycin, levofloxacin and metronidazole among *H. pylori* strains has been noticed.[19,20] The phenomenon of antimicrobial resistance is expected to rise with widespread use of antimicrobials for eradication of *H. pylori* infection.[21] In a recent literature review authors conclude that resistance to clarithromycin and levofloxacin has increased during the last 6 years.[20] Polish researchers found that the primary resistance to clarithromycin was seen in 19-21% of the isolates.[22] Similarly, the resistant rates among Pakistani popula-

Table 2. Logistic multivariate regression analysis of *Helicobacter pylori* infection.

Variable	OR (95% CI)	P value
Age	0.81 (0.84-3.31)	0.142
Smoking	2.42 (1.43-4.09)	0.001
Peptic ulcer	7.01 (0.61-0.83)	<0.001

OR, odds ratios; CI, confidence interval.

Table 3. Antimicrobial resistance rates over a seven-year period.

Year	Clarithromycin-resistance rate (95%CI)	Metronidazole-resistance rate (95%CI)
2008	2.5% (0.1-13.9)	47.5% (28.0-74.4)
2009	2.8% (0.1-15.5)	52.8% (31.8-82.4)
2010	2.8% (0.1-15.5)	47.2% (27.5-75.6)
2011	3.0% (0.1-16.9)	48.5% (27.7-78.7)
2012	6.5% (0.8-23.3)	54.8% (31.9-87.8)
2013	8.0% (1.0-28.9)	60.0% (33.6-99.0)
2014	10.0% (1.2-36.1)	65.0% 34.6-111.2)
P value	<0.0001	<0.001
Overall	4.5 (2.2-8.3)	52.5 (43.4-63.0)

CI, confidence interval.

tion were higher: 89% for metronidazole, 36% for clarithromycin, 37% for amoxicillin, 18.5% for ofloxacin, and 12% for tetracycline. Furthermore, clarithromycin resistance increased from 32% in 2005 to 38% in 2008.[23] However, researchers from Malaysia reported no resistance against clarithromycin. This was possibly due to the restriction applied to macrolides prescribing in the general practice.[24] Instead, in most of the countries in which clarithromycin susceptibility has been studied, resistance has risen over time. According to the results of our study, metronidazole should not be used to eradicate *H. pylori* infections.

Our conclusion is that the resistance of *H. pylori* against clarithromycin and metronidazole has increased significantly over the seven-year period in Saudi Arabia. The use of regimens containing metronidazole in the eradication of *H. pylori* infection should be avoided. Continuous monitoring of antimicrobial susceptibility patterns for *H. pylori* is essential in order to select appropriate agents to achieve higher eradication rates.

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