Research Article

Antimicrobial Susceptibility Patterns of Helicobacter pylori Among Minia University Hospital Patients

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Abstract
The successful treatment rate of Helicobacter pylori is facing many challenges, mainly due to antimicrobial resistance as a result of antibiotics abuse. To assess the burden of resistance-associated treatment failure, the efficacy of culture-based antimicrobial susceptibility was tested for all antimicrobial used as treatment guidelines for eradication of Helicobacter pylori infection. Methods: In this study, incidence of Helicobacter pylori infection in Minia University hospital was determined by testing a random sample of 40 patients undergoing upper gastrointestinal endoscopy for Helicobacter pylori infection. Then, the agar dilution method was used to determine the minimal inhibitory concentration of 6 alternative antibiotics, patterns of antimicrobial resistance and efficacy of commercial antibiotics in treatment of Helicobacter pylori infection. Results: Prevalence of Helicobacter pylori infection was 47.5% among our patients. Antibiotic resistance rates were alarmingly high, up to 100% in amoxicillin, rifampicin, and levofloxacin, 94.7% in tetracycline and clarithromycin, and 89.4% in metronidazole. Discussion: This Study showed unprecedented antimicrobial resistance rates of Helicobacter pylori. New alternative treatment regimens should be considered and investigated to replace the current ineffective therapies.

Keywords: Helicobacter pylori infection, antimicrobial resistance, ineffective therapies

Introduction
Helicobacter pylori (H. pylori) is a flagellated, spiral shaped Gram-negative bacterium that persistently colonizes the human stomach of approximately half the population worldwide [1,2]. H. pylori is linked with a wide variety of diseases, including gastro-duodenal diseases as chronic gastritis, gastric ulcers and mucosa associated lymphoid tissue (MALT) lymphoma, and gastric cancer. H. pylori was the first bacterial species proven to cause cancer and is classified as a group I carcinogen by the International Agency for Research on Cancer [3].

H pylori eradication can improve the outcomes of the related diseases and complications. But the H pylori eradication rates by proton-pump inhibitor (PPI) based triple therapy have declined to unprecedented levels in many areas mainly because of increased clarithromycin, levofloxacin and metronidazole resistance [5].

Increasing H. pylori resistance to treatment regimen is noticed all over the World: amoxicillin 11% (95% CI: 6%-18%), levofloxacin 21% (95% CI: 14%-29%), and metronidazole 67% (95% CI: 58%-75%). The clarithromycin 11% (95% CI: 6%-18%) and many strains show resistance to more than one antimicrobial agent in Europe [5]. On the other hand, in developing countries in Asia and Africa, the resistance rates have markedly increased over the last decades. Resistance to Clarithromycin and metronidazole are 39.3 and 94.6%, respectively. Also, increasing resistance to levofloxacin, almost about 66.1% [6,7].

And even though H. pylori treatment was only prescribed to patients with clinical manifestations over the last decades, the recent H. pylori treatment guidelines recommends treatment for all positively infected patients [8].

Therefore, an evaluation of the efficacy of the currently used antibiotics for treatment of H. pylori must be done and effective new therapy regimen needs to be established because the inappropriate use of antibiotics causes antimicrobial resistance and triggers chronicity.
and complications in the already established infections.

Material and Methods

Subjects
This study was conducted from December 2018 till June 2019 after permission was granted from Minia university ethical committee. This study included 40 patients referred to Minia University hospital unit of Gastroenterology and Endoscopy in Minia University, complaining of dyspepsia, unexplained heartburn, nausea, vomiting, hematemesis, or melena. All patients who agreed to participate in the study were preparing to undergo upper gastrointestinal endoscopy. Full personal and medical histories were taken from the patients. Patients older than 70 years old, younger than 5 years old, receiving antibiotics during the past 2 weeks or having any bleeding disorder were excluded from the study.

Informed consent
The patients were 100% compliant to the study. For confidentiality, their names were omitted and replaced by numerical codes.

Clinical specimens
1 to 3 gastric biopsies was collected during upper gastrointestinal endoscopy, using biopsy probe through the inner endoscopic channel for bacterial culture and antibiotic sensitivity testing. Gastric Biopsy specimens were immersed in Brain-Heart infusion broth (CM1135, Oxoid Co. UK) supplemented with Bovine albumin fraction V powder (A-00395, Oxford lab chem Co. India) and then transported immediately to the microbiology laboratory on Medical Microbiology and Immunology Department, Faculty of Medicine, Minia University.

Microbiological culture
Gastric biopsies were grind and cultured immediately on Columbia blood agar (CM331, Oxoid Ltd, Basingstoke, UK) with Dents H. pylori selective supplement (SR0147E, Oxoid Co. UK), containing Vancomycin, Trimethoprim, Cefsulodin and Amphotericin B. Plates were incubated under a microaerophilic atmosphere (5.5% CO₂, 5% O₂, 85% N₂) using CO₂ incubator or CampyGen™ microaerophilic sachets (CN0025A, Oxoid Co.UK) in a gas pack system at 37°C for 3-5 days. The microorganism was identified as H. pylori by colony morphology, darting cellular motility, Gram staining, and positive urease, catalase and oxidase tests.

Antimicrobial susceptibility testing:
The minimal inhibitory concentrations (MIC) were determined for each isolate. The MICs of 6 antibiotics for the H pylori isolates were examined by 2-fold agar dilution method. The susceptibility testing reference was according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST)⁹¹⁰. The used antibiotics are Amoxicillin, Clarithromycin, Levofloxacin, Metronidazole, Rifampicin, and Tetracyclene. The bacterial suspensions were adjusted to 2 McFerl and standards (1 × 10⁷ colony-forming units). The Antibiotics were mixed with Mueller-Hinton agar supplemented with 5% defibrinated blood and the bacteria were inoculated directly onto an antibiotic-containing agar dilution plate. The MICs were determined after 72 hours of incubation under microaerophilic conditions. The range of concentrations used to evaluate resistance to amoxicillin was 0.016 to 1 mg/L, clarithromycin was 0.065 to 4 mg/L, levofloxacin was 0.125 to 8 mg/L, metronidazole was 1 to 64 mg/L, rifampicin was 0.125 to 8 mg/L, and for tetracycline was 0.125 to 8 mg/L. The resistance breakpoints for amoxicillin, clarithromycin, and levofloxacin were defined as ≥0.125, ≥0.5, and ≥1 mg/L, respectively. The breakpoint for metronidazole, rifampicin, and tetracycline were defined as >8, >1, and >1 mg/L according to the CLSI guidelines.

Results

Culture of H. pylori from gastric biopsy:
Our study included 26 men and 14 women with ages ranged from 17 to 69 years (mean age 51.7 years). All patients were complaining of dyspepsia, unexplained heartburn, nausea, vomiting, hematemesis, or melena. The percentage of patients’ biopsies that cultured positive for H. pylori was 47.5% (19/40).

Antimicrobial susceptibility testing results:
The MIC results were available for all isolates. The antimicrobial resistance rates were shockingly high. The rates of resistance to amoxicillin, rifampicin, and levofloxacin were
100% (19/19). The rates of resistance to tetracycline and clarithromycin were 94.7% (18/19). The rates of resistance to metronidazole were 89.4% (17/19).

**Discussion**

Recent studies report concerning values of *H. pylori* antimicrobial resistance, confirming that eradication rates have been declining while the incidence of antibiotic resistance has been markedly increasing. In populations with high clarithromycin resistance, Bismuth quadruple therapy and concomitant therapy are now prescribed for 10 days according to European and North American guidelines and for 14 days according to the Canadian guidelines. And in populations with high clarithromycin and metronidazole resistance, bismuth quadruple therapy is the regimen of choice. The clarithromycin-containing triple therapy is recommended in patients without previous exposure to macrolides. However, the introduction of mass eradication programs can drive the decreasing effectiveness of first-line therapy by increasing clarithromycin resistance.

In this study, the first report of antimicrobial sensitivity testing using MIC determinations based on 2-fold agar dilution testing in Upper Egypt. The antimicrobial resistance trends of 19 cases included in this study are similar to the antibiotic resistance rates reported in a previous study in Cairo in 2013. This indicates an alarming pattern of antimicrobial resistance of *H. pylori* to first-line therapy regimen or single antibiotic-based regimens. Further investigation is needed to determine the efficacy of combined or second-line therapy regimens for *H. pylori* in Egypt.

European and Japanese new treatment guidelines recommend alternative treatment regimens as third of fourth-line salvage therapies; these include sequential, concomitant, or bismuth-containing quadruple therapy in regions with high levels of antimicrobial resistance. New antimicrobials are also proposed as trial antimicrobials against *H. pylori*, such as Rifabutin as a Rifabutin-containing triple therapy (PPI + Rifabutin + Amoxicillin). Also the use of Rifaximin, garenoxacin and sitafloxacin which are novel therapies for *H. pylori* infection to overcome the wildly expanding antimicrobial resistance, should be investigated to replace the current ineffective therapies.

**References**