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 assesse 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 [4].

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 manifesttations 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 *H. pylori* eradication therapy 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 grinded 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% CO_2, 5% O_2, 85% N_2) using CO_2 incubator or CampyGen^{TM} microaerophilic sachets (CN0025A, Oxoid Co.UK) in a gas pack system at 37^{o}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)[9],[10]. The used antibiotics are Amoxicillin, Clarithromycin, Levofloxacin, Metrnidazole, Rifampicin, and Tetracyclin. The bacterial suspensions were adjusted to 2 McFerl and standards (1×10^7 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 64mg/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[11]. 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[2] and for 14 days according to the Canadian guidelines[11]. 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[2]. However, the introduction of mass eradication programs can drive the decreasing effectiveness of first-line therapy by increasing clarithromycin resistance[12].

In this study is 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[13]. This indicates an alarming pattering 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[14]. New antimicrobials are also proposed as trial antimicrobials against *H. pylori*, such as Rifabutin as a Rifabutin-containing triple therapy (PPI + Rifabutin + Amoxicillin)[15]. 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[6],[16].

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