# Increased Resistance of Fluoroquinolones among *H. pylori* isolates from Patients with Gastroduodenal Diseases in Jordan

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*Abstract:* -. Recently, *Helicobacter pylori* has been connected to more than 80% of chronic active gastritis and other gastroduodenal diseases worldwide. Treatment of H. pylori is routinely dependent on the use of multiple antimicrobial agents however, recent data showed the emergence of resistance among clinical strains especially against metronidazole and clarithromycin. This study was conducted to investigate the rate of resistance to different antibiotics that are routinely used in the first line and second line therapies including ciprofloxacin and levofloxacin, tetracycline, amoxicillin, clarithromycin and metronidazole among *H. pylori* strains isolated from patients with gastroduodenal diseases in Jordan. Both antral and corpus mucosal biopsies from the stomach of patients with positive results of *H. pylori* stool antigen and urease breath tests were used for the isolation of *H. pylori* on selective culture media. The standard agar diffusion method was performed to determine the sensitivity of *H. pylori* clinical isolates against ciprofloxacin and levofloxacin according to CLSI. Among 62 *H. pylori* clinical strains isolated from gastric biopsies, 21% and 11% were resistant to levofloxacin and ciprofloxacin respectively. Resistance to metronidazole and clarithromycin was found in 90% and 11% respectively. No resistance was observed against amoxicillin, tetracycline and gemifloxacin. The following $MIC_{90}$ (mg/L) of resistant strains results were obtained at neutral pH 7.3, 64 for metronidazole, 2 for clarithromycin, 2 for ciprofloxacin and 1 for levofloxacin. The present study reported the emergence of increased resistance of fluoroquinolones among H. pylori clinical isolates in Jordan. Concern should be taken into consideration when triple and quadruple therapy regimens are applied for the management of *H. pylori* infections in our region

*Key-Words:* - Helicobacter pylori, Antibiotic resistance, fluoroquinolones, MIC, CLSI, Agar diffusion method

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## 1 Introduction

Recently, interest in *Helicobacter pylori* related a disease is increasing. Several studies have been discussing the key role of H. pylori in the development of chronic gastritis and peptic ulcer worldwide [1, 2]. The ability of *H. pylori* to colonize the gastric mucosa constitutes a major risk factor in the pathogenesis of gastric cancer and gastric mucosa-associated lymphoid tissue (MALToma) [3-5]. *H. pylori* possess a variety of biological factors that enable this microaerophilic bacterium to withstand the extreme harsh conditions of human stomach [6]. Consequently, the eradication of *H. pylori* would primarily contribute to improve the clinical conditions of the patients infected with this bacterium including accelerating peptic ulcer healing and minimizing the recurrence of gastric cancer. In general, the current approaches for the treatment of diseases caused by H. pylori rely basically on the effective eradication of this microorganism by the aid of at least two antibiotics and a proton pump inhibitor (triple and more recently quadruple therapy) [7]. This is achieved routinely by amoxicillin and clarithromycin in combination with a proton pump inhibitor given for a week [8]. Other treatments include a four-drug combination consisting of a PPI combined with bismuth (120 mg) and tetracycline (500 mg) four times daily and metronidazole (500 mg) three times daily [9]. However, due to the emergence of antibiotic resistance among *H. pylori* clinical strains particularly against metronidazole and clarithromycin, higher rates of failure to common regimens of were reported [10, 12]. Significantly, antimicrobial resistance patterns have not been uniformly reported [13]. Resistance rates reported vary from 10% to 90% for metronidazole, from 0% to 45% for clarithromycin, from 0% to 33% for

amoxicillin, from 5% to 59% for tetracycline, and from 6% to 21% for levofloxacin [14, 15, 16].

As there is an ongoing demand to determine resistance rates locally and to monitor these changing susceptibility patterns to recommend optimal therapy, this study is conducted to determine the prevalence of *H. pylori* resistance to various antibiotics especially those recently increasingly used such as fluoroquinolones and tetracycline of *H. pylori* isolates collected from patients with gastroduodenal diseases in Jordan.

# 2 Methods

## 2.1 Biopsy specimens and isolation of *H. pylori*

Antral and corpus mucosal gastric biopsies from stomach of patients with H. pylori related clinical manifestations including manifestations active gastritis, peptic ulcer or gastric cancer and positive results of stool antigen of H. pylori and urease breath test were obtained from the pathology department of Alpha medical diagnostic laboratories in Jordan. Gastric biopsy specimens were stored in cysteine Freezing medium at -80OC C before processing in a sterile tissue grinder with heat-inactivated fetal bovine serum. Aliquots of 100 µl of the homogenate of each sample were directly sub-cultured onto Columbia blood agar base containing 7 % laked horse blood and selective supplement containing trimethoprim, vancomycin and polymyxin B (Oxoid, UK). All of the plates were incubated at 37°C under microaerophilic conditions using CampyGen atmosphere generating system (Oxoid, UK) for 3-5 days. Growth of H. pylori was confirmed on the basis of positive catalase, oxidase, and urease reactions; typical uniform, small, translucent colonies; curved Gram-negative bacilli on Gram-stained smears; susceptibility to cephalothin (30mg); and resistance to nalidixic acid (30mg) and subsequently by standard PCR of 16S rDNA test [26]. H. pylori cultures were stored at -70°C in Trypticase soy broth (Oxoid, UK) containing 10% v/v fetal calf serum (PAA, Austria) and 15% glycerol.

## 2.1 Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) were measured by agar dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) as described by Abu-Qatouseh et al., 2017). In brief, bacterial suspensions were prepared to the 2 McFarland's standard and inoculated onto antibiotic-containing Mueller Hinton agar supplemented with 7% laked sheep blood. The MIC of each antibiotic was determined after 72 h of incubation. *H. pylori* NCTC 11916 was used as control strain.

The susceptibility of the isolates was tested using the E-test method (Epsilometer test; AB Biodisk, Solna,Sweden) as recommended by the British Society for antimicrobial Chemotherapy (BSAC). The breakpoints used to classify strains as susceptible or resistant according to MIC are listed in Table 1. The breakpoints for clarithromycin and quinolones were interpreted according to CLSI recommendations. The breakpoints for amoxicillin, metronidazole, and tetracycline were interpreted according to the BSAC recommendations (not published by CLSI) [17].

# 3 Results and Discussion

A total of 62 *H. pylori* isolates were collected from endoscopic specimens of patients with gastroduodenal diseases. $MIC_{50}$ and $MIC_{90}$ values for the seven antibacterial agents used in this study are shown in Table 1. Best in vitro antimicrobial activity against *H. pylori* isolates was observed for amoxicillin, tetracycline and gemifloxacin were no resistance was reported.

The highest resistance was observed in metronidazole where only 6 strains were sensitive. Clarithromycin resistance was reported in 10 strains (16%). For fluoroquinolones, increased resistance to levofloxacin compared to ciprofloxacin was significantly reported with 15 (24%) and 10 (16%) strains are phenotypically resistant respectively (Table 2).

Table1: Minimum inhibitory concentration for the antimicrobial agents tested against 62 *H. pylori* isolates recovered in this study

| Antimicrobial agent | Inhibitory concentration (mg/L) | | |
|---|---|---|---|
| | $MIC_{50}$ | $MIC_{90}$ | MIC range |
| Amoxicillin | 0.03 | 0.125 | 0.008-0.25 |
| Tetracyclin | 0.25 | 1 | 0.008-1 |
| Metronidazole | 32 | 128 | 1-256 |
| Clarithromycin | 0.06 | 0.25 | 0.008-4 |
| Ciprofloxacin | 0.5 | 1 | 0.016-32 |
| Levofloxacin | 0.25 | 1 | 0.03-8 |
| Gemifloxacin | 0.125 | 0.5 | 0.008-0.5 |

Table2: Rates of resistance to conventional antimicrobial agents among *H. pylori* isolates recovered in this study

| Antimicrobial agent | Resistance rate (%) | Breakpoints criteria (mg/L) | |
|---|---|---|---|
| | | S | R |
| Amoxicillin | 0.0 | $\leq 1$ | $\geq 2$ |
| Tetracyclin | 0.0 | $\leq 2$ | $\geq 4$ |
| Metronidazole | 90 | $\leq 4$ | $\geq 8$ |
| Clarithromycin | 16 | $\leq 0.25$ | $\geq 1$ |
| Ciprofloxacin | 16 | $\leq 1$ | $\geq 2$ |
| Levofloxacin | 24 | $\leq 1$ | $\geq 2$ |
| Gemifloxacin | 0.0 | $\leq 1$ | $\geq 2$ |

The present study provides important updates regarding the prevalence of drug-resistant H. pylori in Jordan. According to our investigation, it has been revealed that amoxicillin, tetracycline, and gemifloxacin still show excellent in vitro anti-H. *pylori* activity. Approximately nine tenth and one fourth of isolates of *H. pylori* were resistant to Metronidazole and clarithromycin respectively. While our updated resistance rates to quinolones show a notable increase of resistance rate reported for this region (24% and 16% for both levofloxacin and ciprofloxacin respectively), rates for clarithromycin and metronidazole have minor increase over the last decade. Hence, more frequent failure of H. pylori eradication would be anticipated when metronidazole is used in first-line regimens in areas where metronidazole resistance is prevalent, such as Jordan and other countries [13, 18, 19]. Since there is an increasing yet fluctuating patterns of antimicrobial resistance of *H. pylori* isolates, susceptibility testing (either phenotypic or genotypic) of clinical isolates of *H. pylori* in Jordan prior to empirical treatment would be optimal in terms of achieving higher eradication rates and better results.

It is unfortunately common in Jordan not to perform antimicrobial susceptibility testing of *H. pylori* to guide initial therapy; physicians should note that metronidazole resistance has been consistently associated with extra-gastric infections caused by other pathogenic microorganisms as well as due to the misuse of this antimicrobial agent. In addition, since metronidazole resistance rates depend primarily on the overall metronidazole consumption rates, its future role in empiric H. pylori therapy will continue to evolve depending on changing susceptibility rates. In contrast, amoxicillin showed excellent in vitro activity, indicating that amoxicillin should still be the first choice for treatment of H. pylori in Jordan. Clarithromycin has increased resistance rates which makes its use in the empiric clarithromycin-based triple therapy cautious. This is in agreement with most of the reports from similar geographical region however, the reported MIC values of clarithromycin resistant H. pylori in our study were higher. [20, 21]. In a study conducted by PCR for the detection of point mutations associated with clarithromycin resistance, similar rates were reported in Jordan [22].

Interesting in this study is the observation of minimal in vitro resistance to older drugs such as tetracycline. In addition, our investigation revealed that newer generation fluoroquinolones such as gemifloxacin were superior to ciprofloxacin and levofloxacin against clinical H. pylori isolates as in agreement with a previous report [23]. Higher resistance rates were observed to levofloxacin although MIC values are still near the lower breakpoints of resistance. Furthermore, ciprofloxacin resistance in *H. pylori* is increasing with higher MIC values. Whether gemifloxacin provides better clinical outcomes than other fluoroquinolones through fewer iterative failures warrants further studies. Nevertheless, these drugs may potentially be used as salvage therapy for patients for whom the clarithromycin-based standard therapy fails.

In Jordan, current eradication regimens available for *H. pylori* include triple drug combinations comprising a proton pump inhibitor and two antibiotics, amoxicillin plus metronidazole, clarithromycin plus metronidazole, or clarithromycin plus amoxicillin, with eradication rates of 60%, 50%, and 75%, respectively. Testing of fluoroquinolones susceptibility is not routinely performed for antibiotic resistance among *H. pylori* strains however, our results are in agreement with data from other countries which reported higher rates for fluoroquinolones resistance among *H. pylori* clinical strains [24, 25]. Although it is not possible to link eradication rates to the primary resistance rates to individual agents alone, we do believe that potential for synergism between various agents is implicated. Previous studies have demonstrated in vitro synergy between various combinations of amoxicillin, clarithromycin, and metronidazole with a proton-pump inhibitor for antibiotic-resistant strains of *H. pylori* [26, 27]. The interactions of fluoroquinolones especially levofloxacin with a second antibiotic, especially for clarithromycin-resistant strains, showed good antimicrobial synergism for the combination of levofloxacin (instead of amoxicillin) and clarithromycin. However, this depends on the MIC values of the clarithromycin where MIC values >1 mg/L would not have any useful effect of such drug combination. Nevertheless, a mounting evidence for testing the genetic determinants of antimicrobial susceptibility of clarithromycin is favoured.

# 4 Conclusion

In this study, we conclude that the rates of resistance among H. pylori clinical strains in Jordan for Metronidazole and fluoroquinolones are dramatically increasing. Caution should be taken for physicians when using these conventional antimicrobial agents in the treatment of H. pylori since it is expected to have higher treatment failure rates due the emerging resistance in H. pylori toward fluoroquinolones and metronidazole. In addition, it is recommended to perform antimicrobial susceptibility testing for H. pylori prior to use triple and quadruple therapies to minimize the increasing rates of resistance to the antimicrobial agents used in the medical practice and to minimize the undesirable outcomes associated with failure of treatment associated with *H. pylori* infections.

Future work is necessary to determine the mechanisms of resistance for fluoroquinolones in H. pylori since this can help in controlling spread of resistance between the clinical isolates especially if plasmids are expected to be the responsible factors for the resistance phenotypes and genotypes.

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## Contribution of individual authors to the creation of a scientific article (ghostwriting policy)

Luay Abu-Qatouseh, is the main author responsible for writing the manuscript.
Mona Bustami and Eyad Mallah, carried out data collection and statistical analysis.
Luay Abu-Qatouseh and Emad Al-khateed have organized and executed the experiments.

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