



***In-vitro* Studying of the Efficacy of Highly Used Antibiotics in Syria for *Helicobacter pylori* Treatment**

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ABSTRACT

There was an increasing in therapy failure for *Helicobacter pylori* (*H. pylori*) in the last few years using the Standers triple therapy [proton pump inhibitor (PPI), amoxicillin and clarithromycin] in my country. To determine the most efficient therapy for *H. pylori* in vitro, we compared the efficacy of the following antibiotics: Amoxicillin, Levofloxacin, Tetracycline, Rifampicin, Ciprofloxacin, Metronidazole and Azithromycin, with the failure of Clarithromycin by disk diffusion method. We took 2 biopsies from the Antrum or Corpus of gastric for culture and isolation of *Helicobacter pylori* by using selective media (Pylori Agar), and then made Antibiotic Susceptibility test by disk diffusion method (Kirby-bauer) on Muller-Hinton Agar after adding 5% horse serum and antibiotics (Vancomycin 6µg/ml, Nalidixic Acid 20µg/ml and Amphotericin B 2µg). We isolated 51 strains of *Helicobacter pylori*. We found that the most effective antibiotics were Levofloxacin and Amoxicillin (90.2%, 86.3% of *Helicobacter pylori* were sensitive to both respectively), and the susceptibility to metronidazole, tetracycline, rifampicin, ciprofloxacin, azithromycin, and clarithromycin were 39.2%, 45.1%, 51%, 52.9%, 54.9%, and 56.9%, respectively. Our study suggests using levofloxacin-based protocols and Amoxicillin as a first line therapy of *Helicobacter pylori* infection in Syria instead of clarithromycin containing therapy, unless antibiotic susceptibility test is done and the bacteria were sensitive to other antibiotics.

Keywords: *Helicobacter pylori*, disk diffusion method, amoxicillin, clarithromycin, levofloxacin, Syria.

INTRODUCTION

About 20 years ago, *Helicobacter pylori* was discovered by Robin Warren and Barry Marshal. The two researchers isolated and cultured spiral bacteria from human gastric, latterly named *Helicobacter pylori* (*H. pylori*).¹ They were awarded of Noble Price in 2005.² *H. pylori* causes gastritis, peptic ulcer and cancer in the stomach.³ The World Health Organization (WHO) has classified *H. pylori* as class I carcinogen.⁴ This bacterium infects more than 50% of population, but the disease occurs in only 10-20% of infected people.⁵ *H. pylori* infection usually occurs in childhood and continues for a lifetime, unless a specific antibiotic regimen is administered. The standard triple therapy for treatment is proton pump inhibitor (PPI), Amoxicillin and clarithromycin.⁶ However, the eradication rate of this regimen has decreased to less than 80%,⁷ because of the increasing resistance to clarithromycin, this resistance became over 20%,⁶ and in some European countries the resistance reaches 75%,⁸ leading subsequently to the decreased usage of this therapy.⁶ This problem promotes gastroenterologists to administer alternative antimicrobial therapy, many studies concern about the usage of Quinolone-or Rifabutin-based therapy, which gives high efficacy and good toleration.⁹ Levofloxacin is a broad spectrum fluoroquinolone (active against *H. pylori*) which has bactericidal effect by inhibiting bacterial DNA Gyrase, and the drug's activity against *H. pylori* increases when its co-administrated with a PPI.¹⁰ Levofloxacin

containing therapy is as rescue regimen, especially when the resistance to clarithromycin is over 20%, because it has a higher eradication rate and causes less side effects.⁷

MATERIALS AND METHODS

Two Biopsies were taken from the antrum or the corpus (if the participants had taken proton pump inhibitors, it has caused *Helicobacter pylori* to migrate from the antrum to the corpus,⁶ so the gastritis increases in the corpus and decreases in the antrum¹¹) of gastric. During Upper Gastric Endoscopy in Almoasaa and Al-Assad University hospitals in Damascus University, between June and December, 2011, 150 participants (60% men; 40% women; mean age 45 years; range from 16-83). To enter our study, the participant must not have been taking antibiotics for at least one month before the endoscopy. Local ethics committee of the participating hospitals approved the study protocol and a fully informed written consent was obtained from each of the patients included in the study before the endoscopy. Two biopsies are taken from each of the 150 participants for isolation, culture of *H.pylori*, and antibiotic susceptibility test. Biopsies were put in Portagerm Pylori (Biomeriux, France) used as transport media, which was put in the temperature from (2-8°C) for 2 days maximum until working on them. We took the biopsies from Portagerm pylori by sterile loop and rubbed them on the surface of Pylori agar (Biomeriux, France) and incubated for 3-7 days in the temperature of 37°C¹² in the candle jar that provides Microaerophilic condition to isolate *H.pylori*.¹⁰



We started watching the growth after 2 days, and we considered the growth is positive if one or more small, gray colonies have appeared.¹³ We obtained 51 strains, and we carried out the Gram stain, Catalase test and Oxidase test, to identify and confirm *H. pylori*. We used the Brain Heart infusion (Abtek, UK) slant agar (BHIA) after adding 5% horse serum,¹⁴ and antibiotics which were vancomycin 6µg/ml, amphotericin B 20µg/ml, and nalidixic Acid 20µg/ml, (VAN)¹⁵ to store the strains. We took a colony from *H. pylori* that grew on Pylori agar by sterile loop and cultured in a zekzak way on the surface of BHIA and incubated for 38 hours in a temperature of 37°C in the candle jar, after seeing the growth of *H. pylori* we kept them in the refrigerator as a source of bacteria.¹⁶ We took colonies from BHIA by sterile loop and cultured them in nutrient broth agar with 5% horse serum and incubated for 18-20 hours in the temperature of 37°C in the candle jar. Then we got a bacterial suspension with a density equivalent to a McFarland number 4 standard (12×10^8 colony forming units/ml). After making 3 dilutions we added the suspension to Petri dishes that contain Muller Hinton agar (Abtek, UK) after adding 5% horse serum, and VAN, after that we put the antibiotic disks: amoxicillin (Amx, 25µ), clarithromycin (clm, 15 µ), azithromycin (azm, 15µ), tetracycline (tet, 30 µ), metronidazole (mtz, 5 µ), levofloxacin (Lev, 5 µ), ciprofloxacin (cpr, 5 µ), rifampicin

(Rif, 30µ), according to Kirby-bauer method (disk diffusion method) and we returned the Petri to the candle jar and incubated it for 24 hours in the temperature of 37°C.

RESULTS

Biopsies were cultured in pylori agar from all 150 participants. A total of 56 (37.3%) were positive, and 94 (62.7%) negative by culture. After measuring inhibition zone of 51 bacteria (5 bacteria died), the results were: *H. pylori* had very high resistance to 6 out of 8 antibiotics; higher resistance was to tetracycline (39.2%), metronidazole (45.1%), rifampicin (51.0%), ciprofloxacin (52.9%), azithromycin (54.9%), and then clarithromycin (56.9%). There were two antibiotics levofloxacin and Amoxicillin, that *H. pylori* were very sensitive to, the sensitivities were 90.2% and 86.3%, respectively. There were 2 antibiotics, azithromycin and tetracycline, that *H. pylori* were intermediate to them, as table (1) is presented the result.

H. pylori that's sensitive to amoxicillin and clarithromycin (most common used combination) were only 26 (51%), it is very low especially that we had 18 (35.3%) of *H. pylori* sensitive to amoxicillin but resistant to clarithromycin. For new treatment that contains levofloxacin and amoxicillin, the sensitivity of *H. pylori* to both antibiotics were very high 43 (84.3%).

Table 1: *H. pylori* sensitivity to the antibiotics used in our study

Antibiotics	Number of bacteria				Percentage			
	R ^o	I ^o	S ^o	Total	R ^o	I ^o	S ^o	Total
Amoxicillin	7	0	44	51	13.7%	0%	86.3%	100
Levofloxacin	5	0	46	51	9.8%	0%	90.2%	100
Clarithromycin	22	0	29	51	43.1%	0%	56.9%	100
Azithromycin	21	2	28	51	41.2%	3.9%	54.9%	100
Tetracycline	26	2	23	51	51.0%	3.9%	45.1%	100
Rifamycin	25	0	26	51	49.0%	0%	51.0%	100
Metronidazole	31	0	20	51	60.8%	0%	39.2%	100
Ciprofloxacin	24	0	27	51	47.1%	0%	52.9%	100

R^o: resistance, I^o: intermediate, S^o: sensitive

DISCUSSION AND CONCLUSION

Levofloxacin was the most effective, 90.2% of isolated *H. pylori* were sensitive to it. That is because of the little use of this antibiotic in Syria. Almost the same result was reported in Carothers et al study in USA, where 91.2% of *H. pylori* were found to be sensitive to levofloxacin.¹⁷ Amoxicillin was effective in comparison with other antibiotics which were used in our study, 86.3% of *H. pylori* were sensitive to it, but comparing to other studies such as Wueppenhorst et al. study⁹, in Germany, and Kim et al. study¹⁸, in Korea, there were no isolated *H. pylori* resistant to Amoxicillin, this difference in result is belonged to the random use of Amoxicillin in our country. *H. pylori* isolates demonstrated sensitivity less than 80%

against antibiotics, rendering them ineffective in eradication therapy⁷, so all other antibiotics appeared to be ineffective in therapy because of their sensitivity which was less than 80%. The sensitivity of *H. pylori* to metronidazole, tetracycline, rifampicin, ciprofloxacin, azithromycin, and clarithromycin was 39.2%, 45.1%, 51%, 52.9%, 54.9%, and 56.9%, respectively. The high resistance to metronidazole could partially be explained by its excessive use in some parasite and anaerobic infections in Syria. For tetracycline, high resistance could partially be attributed to the frequent prescription for acne and non-gonococcal urethritis¹⁹. For Syrian physicians, ciprofloxacin is prescribed as the first choice to treat urinary tract infection, hence the high resistance



reported in this study. For azithromycin and clarithromycin, high resistance could partially be attributed to the frequent prescription for respiratory infections, and cross resistance. Most common therapy to *H. pylori* in Syria is standard triple therapy which contains clarithromycin, amoxicillin, and PPI, and we have noticed from our study that only 26 (51%) of *H. pylori* were sensitive to both antibiotics, which is very low in comparing with levofloxacin and amoxicillin, where *H. pylori* were sensitive to 43 (84.3%) of both.

Recommendation and Suggestion

We are advised to replace clarithromycin with levofloxacin in *H. pylori* infection therapy.

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