Prevalence of *Helicobacter pylori* resistance to clarithromycin determined by 23S ribosomal RNA analysis in Jordan

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Abstract

Background: Antimicrobial resistance is a growing problem in *Helicobacter pylori* treatment. This study was intended to evaluate the prevalence of clarithromycin resistance, using a polymerase chain reaction (PCR) technique on gastric specimens, from adult Jordanian patients with *H. pylori* infection.

Materials and Methods: Gastric biopsy specimens were taken from gastric antrum and body during routine upper gastrointestinal endoscopies, and were tested with Rapid Urea test for *H. pylori*. Only specimens that were positive for *H. pylori* by the rapid Urea test were included in the study. A total of 50 specimens tested positive for *H. pylori*, and were further tested using a dual priming oligonucleotide(DPO) PCR methodology, to determine the frequency of point mutations in 23s rRNA gene, known to confer resistance to clarithromycin (A2142G and A2143G point mutations).

Results: Out of a total of 50 gastric specimens that tested positive for *H. pylori* by rapid urease test, 49 were confirmed positive for *H. pylori* by PCR technique. Point mutations were found in 11 specimens (8 had A2143G point mutation, and 3 had A2142G point mutation).

Conclusion: Prevalence of clarithromycin resistant strains of *H. pylori* in Jordan was 22.4%. A2143G was the most prevalent point mutation. This high rate of clarithromycin resistant strains should be taken into consideration when prescribing eradication regimens. To our best knowledge, this is the first study to investigate *H. pylori* resistance to clarithromycin in Jordan.

Keywords: *H. pylori*, Clarithromycin resistance, Gene mutation, Duplex PCR

Introduction

Helicobacter pylori is the most successful human pathogen infecting an estimated 50% of the global population [1]. The prevalence of the infection in the developing countries is greater than 80% in adults over 50 years of age [2]. *H. pylori* is a Gram-negative bacterium that infects the human gastric mucosa. It plays an important role in the pathogenesis of chronic gastritis, peptic ulcer diseases, gastric carcinomas, and gastric marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue type [1].

Eradication of *H. pylori* infection has been reported as an effective strategy in the treatment of peptic ulcers, gastric mucosa-associated lymphoid tissue lymphoma [3], as well as in the prevention of gastric cancer [4].

Triple treatment including proton pump inhibitor, amoxicillin and clarithromycin, proposed at the first Maastricht conference was, until recently, globally accepted as the empiric regimen of choice to treat *H. pylori* infection [5]. However, with the emergence of new data, the efficacy of legacy triple regimens containing clarithromycin has been seriously challenged and eradication rates lower than 70% are now reported in many countries [6]. These elusive success rates preclude acceptability under Maastricht consensus [80% in intention to treat (ITT) analysis] and fall short of what it should be expected for an infectious disease, for which a 95% per protocol (PP) efficacy is warranted [7].

The 4th edition of the Maastricht consensus recommended a threshold of 15–20% to separate regions of high and low clarithromycin resistance. In areas of low clarithromycin resistance, treatments containing clarithromycin are recommended as a first-line empirical treatment. Whereas in areas of high clarithromycin resistance (>20%), bismuth-containing quadruple treatments (bismuth sub-salicylate, PPI, tetracycline, and metronidazole) are recommended as a first-line empirical treatment. After failure of the quadruple therapy, levofloxacin-

containing triple therapy is recommended. After failure of the second line of treatment, subsequent treatment methods should be guided by antimicrobial susceptibility testing whenever possible [1].

Antibiotic resistance is a constantly evolving process and numerous studies have shown that the prevalence of *H. pylori* antibiotic resistance varies significantly from country to country, and even between regions within the same country [8].

The present study aimed at investigating the prevalence of *H. pylori* resistance to clarithromycin, using dual priming oligonucleotide (DPO)-PCR kit, designed to detect the presence of the wild type of 23S rRNA, and two types of point mutations causing clarithromycin resistance, A2143G, A2142G [9].

This is the first study to explore the prevalence of clarithromycin resistance in Jordan, taking into consideration that clarithromycin based triple therapy is the most commonly used regimen for *H. pylori* eradication in this country.

Methods

The study was conducted over the period from June 2015 to January 2016, at Khaldi Medical Center, a 140-bed, tertiary care center in Amman Jordan. Approval for the study was obtained from the hospital Institution Review Board. Written, signed consents were obtained from all participants.

Gastric biopsies were obtained from 68 consecutive patients undergoing routine upper gastrointestinal endoscopy. 2 sets of biopsies were taken from gastric body and antrum, respectively. The first set was tested by rapid urease test (Helicotest) for the presence of *H. pylori*. The second set was placed in 0.9 normal saline solution. Only patients with positive Helicotest on gastric biopsies were included in the study (50 patients).

Characteristics of patients

There were 27 males and 23 females, 41 patients presented with epigastric pain and 9 with chronic

heartburn. Endoscopic findings were: 20 patients with non-ulcer dyspepsia, 13 with non-erosive reflux disease, 12 with reflux esophagitis and 5 with peptic ulcer disease.

DNA preparation and PCR

Genomic DNA was extracted and purified from gastric biopsies by using QIAamp DNA Mini Kit, (QIAGEN, Inc., Valencia, California). After DNA extraction, PCR amplification was done using The Seeplex® ClaR-*H. pylori*ACE detection kit, a dual priming oligonucleotide(DPO) methodology from Seegene Inc., Seoul, Korea. The amplified PCR products were then electrophoresed on a 2% agarose gel for 30 minutes, to detect A2142G and A2143G point mutations and the wild type of 23S rRNA.

The DPO detection kit includes 3 primer pairs with a DPO structure which allows amplification of the *H. pylori* 23S rRNA (621 bp amplicon) and detection of A2142G and A2143G point mutations (194 bp and 475 bp, respectively). The kit also includes a primer pair for internal control. DPO-PCR is a multiplex PCR that can be performed in any standard thermocycler[9].

Results

Of the 68 gastric samples studied, 50 tested positive for *H. pylori* by rapid urease test (74%). Of those 50 samples, 49 were confirmed positive for *H. pylori* using PCR technique (98% concordance) (Table 1).

After gene amplification and PCR testing, point mutations were found in 11 patients (8 had A2143G point mutation, and 3 had A2142G point mutation). Using this method, a high prevalence of clarithromycin resistance was detected in our local Jordanian strains (22.4%). A2143G was the most prevalent point mutation (8 out of 11 mutations, (73%).

Gene mutation was present in 8 males (A2143G in 5, A2142G in 3), and 3 females (all had A2143G mutation) (Table 2).

Table 1. Characteristics of 50 investigated patients

	Number of patients (%)
Age (years)	
<30	10(20)
30-40	17(34)
40-50	14(28)
>50	9(18)
Sex	
Male	27(54)
Female	23(46)
Indication for Endoscopy	
Epigastric pain	41(81)
Chronic heartburn	9(18)
Endoscopic findings	
Non-ulcer dyspepsia	20(40)
Peptic ulcer	5(10)
Reflux esophagitis	12(24)
No-erosive reflux disease	13(26)

Table 2. HP status and point mutations detected in 50 investigated patients

	Number of patients (%)
Tests for <i>H. pylori</i>	
RUT positive	50 (100)
DPO-PCR positive	49 (98)
Point Mutations (11/49)	
A2143G	8 (5 males, 3 females) (16.3)
A2142G	3 (all males) (6.1)
Any mutation	11 (22.4)

HP: *Helicobacter pylori*; RUT: Rapid Urease Test
DPO-PCR: Dual Peptide Oligonucleotide-Polymerase Chain Reaction

Discussion

Helicobacter pylori resistance to antibiotics is a major global challenge, and this problem seems to be increasing yearly in most parts of the world, especially in countries where there is no proper control on prescription of antibiotics [8]. Several antibiotic regimens are used worldwide for the eradication of

H. pylori, but the regimen mostly prescribed is the triple regimen that includes a proton pump inhibitor, amoxicillin and clarithromycin [10].

Resistance to amoxicillin is rare (1%), while resistance to clarithromycin varies in different geographical areas (1.5-86.4%), and is correlated with the consumption of antibiotics in the general population [11]. *H. pylori* infection was cured less frequently in patients with pure resistant strains (46%) than those infected with hetero-resistant strains (78.5%) or susceptible strains (94.5%) [12]. Therefore resistance to clarithromycin has significant impact on the outcome of *H. pylori* therapy. Venerito *et al.*, have shown that standard triple therapies successfully eradicated 88% and 14% of clarithromycin-sensitive and clarithromycin-resistant *H. pylori* strains respectively (risk difference = 0.75, 95%CI: 0.63-0.87) [13].

The availability of a simple, accurate, and fast test to detect resistant strains would have a significant impact on the outcome of *H. pylori* therapy. Therefore it has become imperative that each country studies the prevalence of clarithromycin resistance, and come out with specific therapeutic recommendations suitable for that geographical area [11]. Although several studies from neighboring middle eastern countries have shown a variable rate of *H. pylori* resistance to clarithromycin (**Table 3**), none has been published from Jordan [14-21]. Our study is the first to show

a significantly high prevalence of *H. pylori* primary resistant strains to clarithromycin in Jordan.

Antimicrobial resistance is traditionally assessed by *H. pylori* culture and antimicrobial susceptibility testing. While *H. pylori* culture allows an evaluation of antibiotic resistance irrespective of the intrinsic mechanism involved, *H. pylori* is a fastidious bacterium and culture is often time-consuming, difficult, and has a low sensitivity [22]. Recently, developed molecular tests offer an attractive alternative to culture and allow for the rapid molecular genetic identification of *H. pylori* and resistance-associated mutations directly from biopsy samples or bacterial culture material. As such, it provides the opportunity for rapid analysis, enabling same-day diagnosis [8,9,23]. Resistance to clarithromycin is due to point mutations in the 23S ribosomal subunit encoded by the 23S rRNA gene, which affect the binding of clarithromycin to the bacterial ribosome [24]. Three major point mutations in domain V of the 23S rRNA gene have been linked to macrolide resistance: A2142G, A2143G and, less frequently, A2142C [25].

Dual priming oligonucleotide (DPO)-PCR is a recently developed multiplex PCR assay that increases specificity and sensitivity of detection compared to conventional PCR. It detects *H. pylori* and A2142G, A2143G mutations, which account for the majority of primary clarithromycin resistance in western countries [12,26]. In our study, this PCR technique detected *H. pylori* point mutations in 22.4% of our patient population. The predominant point mutation found in our study was the A2143G mutation (73%), which is in harmony with the medical literature. Indeed, the lowest eradication rate (30.7%) has been observed when the phenotypic bacterial resistance was genetically linked to A2143G, suggesting this mutation, rather than the A2142G and A2142C, may significantly affect the therapeutic outcome [12].

There was a male preponderance in point mutations of 8 to 3 in this study, whereas most studies

Table 3. Clarithromycin resistance rates in neighboring middle-eastern countries

Country	Year	Resistance rate (%)	Reference
Egypt	2016	57.7	14
Iran	2015	22.4	15
Iraq	2015	16.2	16
KSA	2013	65	17
Lebanon	2002	4	18
Tunisia	2010	15.4	19
UAE	2010	19.2	20
Jordan	2016	22.4	Current Study

have shown a female preponderance in clarithromycin resistance [27].

As the most recent Maastricht IV consensus guidelines recommend that clarithromycin should not be used to empirically treat *H. pylori* if resistance rates are above 15%-20%[1], surveillance of primary antibiotic resistance is warranted to guide clinicians in their choice of therapy[8]. Antibiotic resistance is the most important cause of failure to eradicate *H. pylori*. Clarithromycin containing triple therapy has been seriously challenged recently by the increasing rate of clarithromycin resistance.

The Toronto Consensus for the treatment of *Helicobacter pylori* infection (2016), concluded that optimal treatment of *H. pylori* infection requires careful attention to local antibiotic resistance and eradication patterns. Nonbismuth quadruple therapies (proton pump inhibitor (PPI) + amoxicillin + metronidazole + clarithromycin) and traditional bismuth quadruple therapy (PPI + bismuth + metronidazole + tetracycline) should play a more prominent role in eradication of *H. pylori* infection, and all treatments should be given for 14 days [28].

In conclusion, since *H. pylori* resistance to clarithromycin exceeded 20% in our study, then it is concluded that clarithromycin based triple therapy should not remain standard therapy in Jordan. Alternative strategies should be explored, including the development of novel, more effective, empirical treatments, and the use of tailored therapeutic approach based on pre-treatment determination of *H. pylori* therapeutic susceptibility.

We recommend that local resistance patterns should be studied in every country and therapeutic regimens should be adapted accordingly.

Disclosure statement

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