Five Alternative Helicobacter pylori Antibiotics to Counter High Levofloxacin and Metronidazole Resistance in the Dominican Republic

by Muhammad Miftahussurur
Five Alternative *Helicobacter pylori* Antibiotics to Counter High Levofloxacin and Metronidazole Resistance in the Dominican Republic

Running head: *H. pylori* antibiotic susceptibility in Dominican Republic

Muhammad Miftahussurur*1,2*, Modesto Cruz*3,4*, Dalla Doohan2,3, Phawinee Subsomwong5, José A. Jiménez Abreu6, Celso Hosking7, Langgeng Agung Waskito2,5, Yoshio Yamaoka5,7,8
Abstract
The prevalence of *Helicobacter pylori* resistance to levofloxacin and metronidazole was high in the Dominican Republic. We used two-fold agar dilution method to determine the minimum inhibitory concentration of five alternative antibiotics in 63 Dominican strains. We also assessed the genetic mutations associated with the antibiotic resistance using next-generation sequencing. We revealed that all 63 strains were sensitive towards sitafloxacin, furazolidone, and rifabutin. In contrast, the prevalence of rifaximin and garenoxacin resistance were high (82.5% and 34.9%, respectively). Patients more than or equal to 60 years old had the highest risk of double-antibiotic resistance (7/9, 77.8%, OR = 31.5, P = 0.009) and garenoxacin resistances (8/9, 88.9%, OR = 45.33, P = 0.002) with an increasing risk simultaneously by age (P = 0.004, r = 0.357). Almost all rifaximin resistant strains possessed multiple mutations with more than three mutations within *rpoB* including the most frequent novel mutations of S352L, I2726L, and V2465A. There was a significant association between vacA genotype and rifaximin resistance (P = 0.042). Among 23 levofloxacin-resistant strains, 82.6% (19/23, P < 0.001) were also resistant to garenoxacin, and 39.1% (9/23) had a high minimal inhibitory concentration $\geq 8$ μg/mL with positive trend correlation (P = <0.001, r = 0.84). Among 19 garenoxacin resistant strains, 16 (84.2%) contained mutations at D91 and N87 of gyrA. In conclusion, sitafloxacin, rifabutin, and furazolidone might be considered as alternative antibiotics to be included in *H. pylori* eradication regimen in regions with high prevalence of levofloxacin and metronidazole resistance, such as the Dominican Republic.

Introduction
*Helicobacter pylori*, causative agent in the pathogenesis of gastroduodenal disease including gastric cancer, highly colonized Latin American populations thus brought this region to be the second highest infection rate worldwide after African populations. [1] In addition, Latin America is a region with significant burden of gastric cancer (9.7 cases per 100,000 people per year; GLOBOCAN 2012; [http://globochan.iarc.fr](http://globochan.iarc.fr)). Moreover,
meta-analysis reported a higher recurrence rate of *H. pylori* infection in this region (7.9, 11.2, and 6.2 cases per 100 person-years, first year and in subsequent years, respectively) than the estimated global (4.5/100 person-years) [2]. Several countries in Latin America showed an emerging *H. pylori* antibiotics resistance rate; including 16% for clarithromycin, 33% for tetracycline and 83% for metronidazole in Colombia, 15% for amoxicillin in Brazil, and 37% for levofloxacin in Peru [3].

The Dominican Republic is a sovereign state located in the Caribbean Sea region with an approximately total population of 11 million, located in the western portion of Hispaniola Island. The population density is the highest in the southern area, where the capital city of Santo Domingo is located (https://www.cia.gov/). Although the gastric cancer risk in the Dominican Republic is reported to be low (7.3 per 100,000 year) based on http://globocan.iarc.fr/, the infection rate of *H. pylori* was reported to be high (58.9%) [4]. In addition, *H. pylori* contained virulent types (cagA-positive/vacA s1m1) were predominant, which induced more severe gastritis [4]. Previously, we reported that clarithromycin and amoxicillin resistance against *H. pylori* in the Dominican Republic strains were low (3.1% and 1.6%, respectively) [5]. Maastrict V consensus for management of *H. pylori* infection mentioned that clarithromycin-based therapy should be abandoned in case the clarithromycin resistance rate in the region is more than 15% [6].

Thus, we recommended that initial treatment for *H. pylori* infection with clarithromycin-based standard triple therapy might still applicable in the Dominican Republic [5]. Nevertheless, we have to take an important note that the emerging resistance to metronidazole and levofloxacin were demonstrated (82.8% and 35.9%, respectively).

Both antibiotics-based regiments would be insufficient as primary or secondary *H. pylori* antibiotic regiments in the Dominican Republic [5]. Other appropriate antibiotics including novel regiments are necessary to counter multiple side effects due to a repeated treatment course.

Furazolidone is a synthetic nitrofuran derivative which is utilized for an alternative drug with high efficacy but low cost for *H. pylori* eradication therapy as a component of the
multidrug regimens, particularly in association with bismuth [7, 8]. Novel quinolones become potential candidate agents including garenoxacin and sitafloxacin, which are known to have high affinity to DNA gyrase and low minimum inhibitory concentration (MIC) against H. pylori, thus superior rather than levofloxacin-based regimen, especially against gyrA mutations [9]. Rifaximin, a semisynthetic rifamycin derivates which act as a surface antibiotic against H. pylori including clarithromycin-resistant strain. It becomes alternative drug due to rare primary resistance, poor absorbance to the blood and the bactericidal effect is not significantly affected by an acidic environment [10]. Rifabutin, the rifamycin-S derivates which formed spiro-piperidyl-rifamycin [11], become a promising H. pylori treatment which demonstrated high in vitro activity, chemically stable and not share resistance to clarithromycin [12]. Mutation in the rpoB gene was reported to have association with the H. pylori resistance of rifabutin and rifaximin, which mostly encoded the DNA-directed RNA polymerase.

The Dominican Republic could be used as a model of the country with high metronidazole and levofloxacin resistances. Further investigation about an alternative regimen is important to decide better regimen especially after the first-line therapy failure.

The aim of the present study was to examine the susceptibility of five alternative antibiotics to the H. pylori isolates from the Dominican Republic. We also analyzed the DNA sequences of genes involved in antibiotic resistance to identify candidate mutations that might play a role in the antibiotic resistance mechanism.
Materials and methods

Patients and *H. pylori*

We utilized 64 strains which was isolated from homogenized antral biopsy specimens of patients who underwent endoscopy examination at the Digestive Disease Center, Dr. Luis E. Aybar Health and Hygiene City, Santo Domingo, Dominican Republic [5]. Importantly, all strains we used had information about resistance rate of standard *H. pylori* antibiotics (amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin) from our previous study [5]. All *H. pylori* stock had been stored in Brucella Broth (Difco, NJ, USA) with 10% dimethyl sulfoxide and 10% horse serum at the -80°C. We also had information about *H. pylori* virulence factors (cagA and vacA genotypes), and about clinical outcomes which were diagnosed by endoscopic observation and histologic examination [5]. All participants submitted the written informed consent and we obtained study protocol approval from the ethics committees of Dr. Luis E. Aybar Health and Hygiene City, Institute of Microbiology and Parasitology, Autonomous University of Santo Domingo, Santo Domingo, the Dominican Republic; and the Oita University Faculty of Medicine, Japan.

Antibiotic susceptibility testing

The MICs of five antibiotics including rifaximin (Tokyo Chemical Company, Tokyo, Japan), furazolidone (Tokyo Chemical Company, Tokyo, Japan), rifabutin (Sigma Aldrich, St. Louis, US), sitafloxacin (Haoyuan Chemexpress, Shanghai, China), and garenxacin (Sigma Aldrich, US) were tested by using the two-fold agar dilution method. Prior the susceptibility test, the frozen stocks were subcultured twice on the Mueller-Hinton II agar contained 10% horse blood to recover the bacteria from the frozen environment and ensure the growth of the bacteria. The cultured bacteria were diluted in Brucella broth and were adjusted to the OD of 0.1. The prepared bacterial suspension was inoculated on Mueller-Hinton II agar contained with blood and antibiotic. Then the plates were incubated at 37°C under microaerophilic conditions (10% O2, 5% CO2, and 85% N2). The MICs
were determined after 72-h incubation. As a quality control, an *H. pylori* strain from American Type Culture Collection (catalog #43504) was tested along with each batch of susceptibility test. The resistance breakpoint was determined on the MIC of each antibiotic tested: ≥4 μg/mL for rifaximin, ≥4 μg/mL for furazolidone, and ≥1 μg/mL for rifabutin, sitafloxacin, and garenexacin, as previously described [13-16]. The rifaximin and furazolidone concentration were ranged between 0.25 μg/mL to 32 μg/mL, while the concentration range of rifabutin, sitafloxacin, and garenexacin were between 0.064 μg/mL to 8 μg/mL.

**Molecular analysis of resistant strains**

*H. pylori* genomic DNA was extracted by using commercial DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) and then stored at -20°C. The next-generation sequencing (MiSeq next-generation sequencer; Illumina, San Diego, CA) was used to analyze the full-length *rpoB*. The BLAST algorithm implemented in the CLC Genomic Workbench software (ver. 11; Qiagen, Venlo, Netherlands) was used to analyze the MiSeq output data. The sequences of *hp1198* of the strain 26695 were used as queries to obtain the *rpoB* sequence from the sequencing data. The variants in the *rpoB* were determined by comparing the full-length *rpoB* sequence of the strain 26695 (GenBank accession number AE000511.1) with the *rpoB* sequences of rifaximin-resistant strains and five randomly selected rifaximin-sensitive strain sequences. Then, all the sequences were aligned at the codon level by using MAFFFT software, after confirming the absence of insertion or deletions leading to frameshift mutation. By comparing each codon of the resistant and sensitive strains, variants of codons were checked. The variants found in both sensitive and resistant strains were considered as normal variant. The variants found in the resistant strains but not in the sensitive strains were considered as variant-related to antibiotic resistance.

**Statistical analysis**
We analyzed the discrete variables by the chi-square test, whereas the continuous variables by Mann-Whitney U and t-tests. A binary logistic regression model was used to calculate the odds ratio (OR). All determinants with P values of <0.10 were entered together in to the full logistic regression model, and the model was reduced by excluding variables with P values of >0.10. The OR and 95% confidence interval (CI) were used to estimate the risk. The statistically significant was determined by P values <0.05. All statistical analysis in this study was using SPSS statistical software package version 23.0 (SPSS, Inc., Chicago, IL).

Results

Five Antibiotics Susceptibility

Among 64 strains included, 63 H. pylori were successfully cultured (strain Dominica151 was not grown) and sub-cultured for agar dilution test including 19 strains from male and 44 from female patients. Rifaximin had extremely high prevalence of resistance (52/63, 82.5%). A high prevalence of garenoxacin resistance was also observed (22/63; 34.9%). In contrast, agar dilution test exhibited there was no resistant strain towards furazolidone, rifabutin, and sitafloxacin. The distribution of five antibiotics resistance pattern is shown in Table 1 and Figure 1. Male had higher prevalence of rifaximin resistance, but female had higher prevalence of garenoxacin resistance; however, there was no statistically significant association (both \( P > 0.05 \)). Patients more than or equal to 60 years old had the highest risk of garenoxacin resistances (8/9, 88.9%, \( \text{OR} = 45.33 \) [CI 4.055-506.836], \( P = 0.002 \)). There was no association between antibiotic resistance and clinical outcomes (\( P > 0.05 \)). The correlation between age and the garenoxacin resistance based on the Spearman’s rank correlation model showed that an increasing age reflects the increased prevalence of garenoxacin resistance (\( P = 0.004 \), \( r = 0.357 \)). The association between demographic and clinical outcome to rifaximin and garenoxacin of double-antibiotic resistance pattern is shown in Table 2. Eighteen strains had double-antibiotic resistance to rifaximin and garenoxacin (18/63; 28.6%). Patients more than or equal to 60 years old had the highest
prevalence of double-antibiotic resistance (7/9, 77.8%, OR = 31.5 [CI 2.350-422.299], P = 0.009) than the patients less than 30 years old. There was no association between double-antibiotic resistance with sex and clinical outcome (P = 0.385 and P = 0.099, respectively). We also found a relatively high prevalence of quadruple-resistance strains (15/63; 23.8%). Almost all of the quadruple-resistance strains had resistance towards rifaximin, clarithromycin, levofloxacin, and metronidazole (14/15; 93.3%).

Table 1. Distribution of antibiotic resistance in the Dominican Republic

| Characteristic | N   | Rifaximin | Furazolidone | Rifabutin | Garen oxacin | Sitafloxacin |
|---------------|-----|-----------|--------------|-----------|-------------|--------------|
| Total         | 63  | 52/63 (82.5) | 0/63 (0.0)  | 0/63 (0.0) | 22/63 (34.9) | 0/63 (0.0)  |
| Sex           |     |            |              |           |              |              |
| Male          | 19  | 17/19 (89.5) | 0/19 (0.0)   | 0/19 (0.0) | 4/19 (21.0)  | 0/19 (0.0)  |
| Female        | 44  | 35/44 (79.5) | 0/44 (0.0)   | 0/44 (0.0) | 18 (40.9)    | 0/44 (0.0)  |
| Age           |     |            |              |           |              |              |
| <30           | 10  | 8/10 (80.0)  | 0/10 (0.0)   | 0/10 (0.0) | 2/10 (20.0)  | 0/10 (0.0)  |
| 30-39         | 13  | 11/13 (84.6) | 0/13 (0.0)   | 0/13 (0.0) | 4/13 (30.8)  | 0/13 (0.0)  |
| 40-49         | 20  | 17/20 (85.0) | 0/20 (0.0)   | 0/20 (0.0) | 3/20 (15.0)  | 0/20 (0.0)  |
| 50-59         | 11  | 8/11 (72.7)  | 0/11 (0.0)   | 0/11 (0.0) | 5/11 (45.4)  | 0/11 (0.0)  |
| >59           | 9   | 8/9 (88.9)   | 0/11 (0.0)   | 0/11 (0.0) | 8/9 (88.9)*  | 0/11 (0.0)  |
| Clinical outcome |   |            |              |           |              |              |
| Gastritis     | 47  | 41/47 (87.2) | 0/47 (0.0)   | 0/47 (0.0) | 19/47 (40.4) | 0/47 (0.0)  |
| PUD**         | 16  | 11/16 (68.8) | 0/16 (0.0)   | 0/16 (0.0) | 3/16 (18.8)  | 0/16 (0.0)  |

* P = 0.002, OR = 45.33 [CI 4.055-506.836] ** PUD; peptic ulcer disease

Table 2. Association between demographic and clinical outcome to rifaximin and garen oxacin double-antibiotic resistance in the Dominican Republic

| Characteristic | N   | Rifaximin + | Crude OR | 95% CI | P value |
|---------------|-----|-------------|----------|--------|---------|
|               |     |             |          |        |         |
| Garenoxacin (%) |
|-----------------|
| Total           | 63 | 18/63 (28.6%) |
| Sex             |    |               |
| Male            | 19 | 4/19 (21.0%)  |
| Female          | 44 | 14/44 (31.8%) |
| Age             |    |               |
| <30             | 10 | 1/10 (10.0%)  |
| 30-39           | 13 | 3/13 (23.1%)  |
| 40-49           | 20 | 3/20 (15.0%)  |
| 50-59           | 11 | 4/11 (36.4%)  |
| >59             | 9  | 7/9 (77.8%)   |
| Clinical outcome|    |               |
| Gastritis       | 47 | 16/47 (34.0%) |
| PUD             | 16 | 2/16 (12.5%)  |

*P <0.05**, PUD: peptic ulcer disease

**Susceptibility Comparison with Standard Antibiotic**

We compared our previous results with the resistance of five standard antibiotics (Table 3) [5]. Sitafloxacin, furazolidone, and rifabutin become a potential drug to be used as alternative *H. pylori* eradication antibiotic due to 100% sensitive especially as the second line in the high prevalence of levofloxacin and metronidazole resistance. Among 23 levofloxacin-resistant strains, almost all (19/23, 82.6%, P <0.001) were also resistant to garenoxacin, and 39.1% (9/23) had a high MIC ≥8 μg/mL with positive trend correlation (P = <0.001, r = 0.84). There was no association between the resistance of sitafloxacin and levofloxacin (P = 0.992). Although there was an association between the resistance of rifaximin and amoxicillin (P = 0.028), only one strain was resistant to amoxicillin. In contrast, there was no association between the resistance of rifaximin and clarithromycin, levofloxacin and metronidazole (P = 0.512, P = 0.991, and P = 0.945, respectively).
Table 3. Comparison susceptibility test of alternative vs. standard antibiotics

| Standard Regiment | Resistant rate (%)* | Alternative Regiment            | Resistant rate (%)** |
|-------------------|---------------------|---------------------------------|-----------------------|
| Clarithromycin    | 2/64 (3.1%)         | Garenoxacin                     | 22/63 (34.9%)         |
| Amoxicillin       | 1/64 (1.6%)         | Sitafloxacin                    | 0/63 (0.0%)           |
| Metronidazole     | 53/64 (82.8%)       | Furazolidone                    | 0/63 (0.0%)           |
| Tetracycline      | 0/64 (0.0%)         | Rifabutin                       | 0/63 (0.0%)           |
| Levofloxacin      | 23/64 (35.9%)       | Rifaximin                       | 52/63 (82.5%)         |

* This number is corresponded to our previous study

** This number is corresponded to our current study

Multiple resistances in standard and alternative antibiotics

We combined the standard antibiotics susceptibility data from our previous study [5] with alternative antibiotics susceptibility. The pattern of multiple antibiotic resistance is shown in Supplemental Table 1. There was 1 strain that sensitive to all 10 antibiotics tested.

Thirteen of 63 strains (20.6) were resistant to only 1 antibiotic. Double antibiotic resistance was present in 25 strains (39.7%). Interestingly, there were 14 strains (22.2%) that had quadruple resistance towards rifaximin, garenoxacin, levofloxacin, and metronidazole.

There was only 1 strain (1.6%) that had quintuple resistance towards rifaximin, garenoxacin, levofloxacin, and metronidazole, and clarithromycin.

MIC and mutations among Fluoroquinolone Group

We compared the level of MIC between levofloxacin-resistant strains with the other fluoroquinolone group. Most of the strains possessing gyrA or gyrB mutations had considerably higher MIC level to levofloxacin (8-128 times higher) and garenoxacin (8-64
times higher), but around the same level with sitafloxacin. Among 19 garenoxacin resistant
strains, 16 (84.2%) contained mutation at D91 and N87 (Table 4). Of nine garenoxacin
resistant strains with high MIC ≥8 μg/mL, 8 (88.9%) contained an amino acid changes at
91 and 87 locations.

| Strains     | gyra mutation | gyrb mutation | Levofloxacin MIC | Status | Garenoxacin MIC | Status | Sitafoxacin MIC | Status |
|-------------|---------------|---------------|------------------|--------|----------------|--------|----------------|--------|
| Dominica08  | None          | None          | 0.25             | S      | <0.063         | S      | 0.125           | S      |
| Dominica32  | None          | None          | 0.25             | S      | 0.125          | S      | <0.063          | S      |
| Dominica57  | None          | None          | 0.25             | S      | 0.125          | S      | <0.063          | S      |
| Dominica95  | None          | None          | 0.25             | S      | 0.125          | S      | <0.063          | S      |
| Dominica9   | None          | None          | 16               | R      | 8              | R      | 0.5             | S      |
| Dominica10  | N87I          | None          | 32               | R      | 8              | R      | 0.5             | S      |
| Dominica14  | D91G, E193D,  | None          | 2                | R      | 1              | R      | 0.125           | S      |
|             | I194F, A197F  |               |                  |        |                |        |                 |        |
| Dominica18  | N87I, D91N    | None          | 16               | R      | 8              | R      | 0.5             | S      |
| Dominica43  | N87I, D91N    | None          | 2                | R      | 0.5            | S      | 0.125           | S      |
| Dominica44  | N87I, A97V    | None          | 16               | R      | 8              | R      | 0.25            | S      |
| Dominica49  | N87A          | D435N, S479G  | 32               | R      | 8              | R      | 0.5             | S      |
| Dominica50  | E58G, G75V,   | None          | 2                | R      | 1              | R      | 0.125           | S      |
|             | D91G, Q98L    |               |                  |        |                |        |                 |        |
| Dominica51  | D91N          | None          | 2                | R      | 1              | R      | 0.125           | S      |
| Dominica60  | A134V, D145N  | None          | 2                | R      | 0.5            | S      | <0.063          | S      |
| Dominica62  | D91N          | S479G         | 2                | R      | 1              | R      | 0.125           | S      |
| Dominica71  | N87I          | S479G         | 32               | R      | 8              | R      | 0.5             | S      |
| Dominica73 | None   | None   | 32 | R   | 4    | R    | 0.125 | S   |
| Dominica75 | N87I   | None   | 32 | R   | 8    | R    | 0.5   | S   |
| Dominica96 | None   | None   | 4  | R   | 4    | R    | 0.25  | S   |
| Dominica103 | N87I, D91N, I162T | None   | 16 | R   | 8    | R    | 0.5   | S   |
| Dominica115 | N87K   | None   | 2  | R   | 1    | R    | 0.125 | S   |
| Dominica142 | N87T, D91N | None   | 4  | R   | 1    | R    | 0.25  | S   |
| Dominica146 | N87T   | None   | 16 | R   | 8    | R    | 0.25  | S   |
| Dominica147 | D91Y   | None   | 2  | R   | 2    | R    | 0.125 | S   |
| Dominica148 | None   | None   | 2  | R   | 0.25 | S    | <0.063 | S  |
| Dominica150 | N87T, D91N, F149V | None   | 16 | R   | 0.25 | S    | <0.063 | S  |
| Dominica152 | N87I   | Y514F  | 8  | R   | 4    | R    | 0.5   | S   |

MIC: Minimal Inhibitory Concentration, in µg/mL. S: sensitive; R: resistant. N87I means isoleucine replaced asparagine in amino acid position 182. We ignored mutation that were present in both sensitive and resistant strains.

**Rifaximin resistance and rpoB mutations**

We analyzed the rpoB sequence from 15 rifaximin-resistant strains (MIC ≥4 µg/mL). We found a total of 78-point mutations in rpoB categorized as missense mutation (Suppl. Table 1). All of the resistant strains possessed multiple mutations with more than 3 mutations within rpoB. We could not find mutation in V657I as mentioned in previous studies [17-19]. Of the 15 strains, S352L and I2726V were the most common novel point mutations (6/15, 40.0% and 6/15, 40.0%, respectively, Table 5). V2465A point mutation appeared in four resistant strains (4/15, 26.7%), while A761V, M1628I, and T1929A point mutations appeared in 3 strains (3/15, 13.3%).

**Table 5. The frequency of the 15 most common mutations in rpoB**

| No. | Point Mutation | Total number (%) |
|-----|----------------|------------------|

12
S352L | 6/15 (40.0)
I2726V | 6/15 (40.0)
V2465A | 4/15 (26.7)
A761V | 3/15 (20.0)
M1628I | 3/15 (20.0)
T1929A | 3/15 (20.0)
S2889P | 3/15 (20.0)
D2381E | 2/15 (13.3)
A735T | 2/15 (13.3)
D1163N | 2/15 (13.3)
K1166R | 2/15 (13.3)
S2525G | 2/15 (13.3)
T2534M | 2/15 (13.3)
A2884T | 2/15 (13.3)
N2888G | 2/15 (13.3)

S352L: means leucine replaced serine in amino acid position 182. We ignored mutation that were present in both sensitive and resistant strains.

Virulence genes and antibiotic resistance

We picked up the information of virulence factors (cagA and vacA) from our previous study [5]. The distribution and association between virulence genes and antibiotic resistance is presented in Table 6. There was a significant association between vacA genotypes and rifaximin resistance ($P = 0.033$). The vacA s1/m1 was tended to have rifaximin resistance ($P = 0.076$) and garenoxacin resistance ($P = 0.090$). There was no significant association between cagA positivity with both rifaximin and garenoxacin resistance ($P = 0.207$ and $P = 0.883$, respectively).

Table 6. Association between virulence genes and resistance pattern
| Virulence genes | Rifaximin (%) | Garenoxacin (%) |
|-----------------|--------------|-----------------|
| Number of strains | S 11 | R 52 | P value 0.207 |
| cagA | | | |
| Positive | 10/11 (90.9) | 38/52 (73.1) | 31/41 (75.6) | 17/22 (77.3) | 0.883 |
| Negative | 1/11 (9.1) | 14/52 (26.9) | 10/41 (24.4) | 5/22 (22.7) | 0.116 |
| vacA | | | |
| s region | 0.035* | 0.181 |
| s1* | 11/11 (100) | 36/52 (69.2) | 28/41 (68.3) | 19/22 (86.4) |
| s2* | 0/11 (0.0) | 16/52 (30.8) | 13/41 (31.7) | 3/22 (13.6) |
| m region | 0.115 | 0.09 |
| ml | 10/11 (90.9) | 35/52 (67.3) | 27/41 (65.9) | 18/22 (81.8) |
| m2 | 1/11 (9.1) | 17/52 (32.7) | 14/41 (34.1) | 4/22 (18.2) |
| s1/m1 | 10/11 (90.9) | 33/52 (63.5) | 0.076 | 25/41 (61.0) | 18/22 (81.8) | 0.09 |
| s1/m2 | 1/11 (9.1) | 3/52 (5.8) | 0.618 | 3/41 (7.3) | 1/22 (4.5) | 0.667 |
| s2/m1 | 0/11 (0.0) | 2/52 (3.8) | 0.509 | 2/41 (4.9) | 0/22 (0.0) | 0.292 |
| s2/m2 | 0/11 (0.0) | 14/52 (26.9) | 0.051 | 11/41 (26.8) | 3/22 (13.6) | 0.23 |

*N P < 0.05

**Nucleotide sequencing**

Nucleotide sequence data from this study are available under DDBJ accession numbers LC425694–LC425711 (rpoB).

**DISCUSSION**

We confirmed the low prevalence of sitafloxacin resistance in the Dominican Republic. Moreover, all the strains possessed very low MIC, indicating that sitafloxacin was a potent antibiotic to be used as alternative antibiotic in the Dominican Republic. Our result was in agreement with previous studies reported that sitafloxacin were potential drugs to be included in *H. pylori* eradication regimen [20, 21]. Sitafoxacin was reported to be combined with other antibiotics, such as amoxicillin [20] and metronidazole [21] with
relatively equal results [22]. However, due to the high prevalence of metronidazole-resistant strains in the Dominican Republic [5], it is not recommended to combine sitafloxacin with metronidazole. In contrast, the prevalence of amoxicillin-resistant strain is very low [5]. Thus, the combination of sitafloxacin and amoxicillin seems to be more reasonable to be used in the Dominican Republic. Currently, sitafloxacin is still not available in the Dominican Republic. But with its recent availability in Thailand after in Japan, hopefully it will be widely distributed. Sitafoxacin is already reported not only to be effective as H. pylori eradication therapy in Japan, but also for treatment of complicated urinary tract infection and pyelonephritis, it might be important to provide sitafloxacin in the Dominican Republic.

Our study revealed all strains were sensitive towards rifabutin, supporting the possibility to recommend rifabutin as potential antibiotic to combat H. pylori infection. Previous study reported that rifabutin-based therapy was highly effective and reliable as an alternative therapy, especially after two times eradication failure [23, 24]. Rifabutin is known to be a broad spectrum of antimicrobial activity and has high activity towards varieties of Gram-positive and -negative bacteria, including Mycobacterium [11]. A history of rifampicin usage, primarily in tuberculosis treatment should be taken into consideration before prescribing rifabutin for H. pylori eradication therapy. Combination of clarithromycin along with rifabutin should be considered carefully, as the combination between these antibiotics was showing the evidence of the inhibition of rifabutin metabolism, suggesting the possibility of toxicity [25]. Therefore, the combination of clarithromycin and rifabutin in the Dominican Republic should be carefully prescribed, even though the clarithromycin-resistant strain was low [5].

Similar to sitafloxacin and rifabutin, furazolidone resistance showed to be low in the Dominican Republic. Furazolidone is one of the antibiotic recommended by several guidelines to be included in eradication regimen [8, 26]. Furazolidone is included in quadruple-therapy regimen, substituting metronidazole and combined with the addition of bismuth [27] to increase the efficacy of the therapy. Although the bismuth-quadruple
containing furazolidone was shown to be effective [28, 29], the unavailability of bismuth
make this regimen could not be applied in many population.

Levofoxacin resistance is widely known to be associated with the mutations in
\textit{gyrA} and/or \textit{gyrB}. Our previous study also mentioned that more than third-quarter of the
levofloxacin-resistant strains in the Dominican Republic had amino acid substitution
associated with \textit{gyrA} mutations [5]. Our current result showed that despite the occurrence
of mutations in \textit{gyrA}, sitafoxacin was still highly effective. This result was in concordance
with previous study which reported that sitafoxacin might overcome the antibiotic
resistance of \textit{H. pylori} with \textit{gyrA} mutation [9]. Although garenoxicin is included in the
same fluoroquinolone with sitafoxacin without significant side effect, we found the high
prevalence of garenoxicin resistance. Suggesting a less effect of garenoxicin to inhibit the
gyrase function, thus, it may not suitable as second line regimen in the Dominican
Republic. The increasing drug resistance in the older age might be related to the improper
antibiotic usage and also the spreading of resistant strain in their environment such as
nursing home, which might be a reservoir for multidrug-resistance strain [30].

Compared to other four antibiotics tested in this study, rifaximin was shown to be
the one with exceptionally very high resistance prevalence. Although our study revealed
low susceptibility rate of rifaximin as single-antibiotic therapy, a better result was obtained
by combining rifaximin with another antibiotic, such as clarithromycin [10, 31, 32]. The
remarkable safety of rifaximin allows the high-dose regimen or longer duration of therapy,
make it a promising drug to be used as alternative antibiotic in \textit{H. pylori} eradication
regiment and to be tested in clinical trial phase [10]. Moreover, rifaximin might become a
potential drug to be used to combat \textit{H. pylori} infection in childhood, due to the high safety
properties [33, 34]. While rifaximin resistance was high in the Dominican Republic, further
study is needed to examine the efficacy of rifaximin-base combination therapy in clinical
trial.

The \textit{rpoB} mutations is widely known to have important role in the
rifamycin-group drug resistance mechanism in various microorganism, such as \textit{Clostridium}
difficile [35], Mycobacterium tuberculosis [36-38], and Escherichia coli [39]. In this study, we found the occurrence of more than 3 mutations within rpoB which was rarely reported. In addition, although numerous mutations in rpoB, most of them only appeared in one resistant strain including novel mutations: S352L, I2726L, and V2465A, suggesting molecular antibiotic susceptibility testing is not applicable for rifaximin.

There are several limitations in this study. First, sample number is relatively small, and we collected samples only from the capital city of the Dominican Republic, thus our results could not be generalized across Latin America. However, we believe that our current data would become important data to decide a general policy for eradicating H. pylori in this region. Second, out of thousands H. pylori genes, we only analyzed few genes that might be related to antibiotic resistance mechanism. Since we have large data from our next-generation sequencing, detailed genome-wide association study of H. pylori genome is now in progress. Third, we only determined resistance rate of five alternative antibiotics by in vitro study. Clinical trial should be required to examine the efficacy of five alternative antibiotics-based combination therapy.

**CONCLUSIONS**

Sitafloxacin, rifabutin, and furazolidone might be considered as an alternative antibiotic to be included in the therapy to H. pylori eradication regiment in regions with high prevalence of levofloxacin and metronidazole resistance such as the Dominican Republic.

**Potential competing interests**

The authors declare that they have no competing interests.

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**Author contributions**

Conceived and design the experiments: MM, MC, and YY. Performed the experiments: LAW, DD, PS. Analyzed the data: MM, DD, LAW, KAF, YY. Contributed reagents/material/data acquisition: JA (José A. Jiménez Abreu) and CH. Wrote the paper: DD, MM, YY. YY and MC revised the manuscript and added important content. All authors read and approved the final version of the manuscript.
References

1. Hooi JKY, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-9. Epub 2017/05/01. doi: 10.1016/j.gastro.2017.04.022. PubMed PMID: 28456631.

2. Corral JE, Mera R, Dye CW, Morgan DR. Helicobacter pylori recurrence after eradication in Latin America: Implications for gastric cancer prevention. World journal of gastrointestinal oncology. 2017;9(4):184-93. Epub 2017/04/30. doi: 10.4251/wjgo.v9.i4.184. PubMed PMID: 28451066; PubMed Central PMCID: PMCPMC5390804.

3. Camargo MC, Garcia A, Riquelme A, Otero W, Camargo CA, Hernandez-Garcia T, et al. The problem of Helicobacter pylori resistance to antibiotics: a systematic review in Latin America. The American journal of gastroenterology. 2014;109(4):485-95. doi: 10.1038/ajg.2014.24. PubMed PMID: 24589670; PubMed Central PMCID: PMC4268803.

4. Shiota S, Cruz M, Abreu JA, Mitsui T, Terao H, Disla M, et al. Virulence genes of Helicobacter pylori in the Dominican Republic. J Med Microbiol. 2014;63(Pt 9):1189-96. doi: 10.1099/jmm.0.075275-0. PubMed PMID: 24965801; PubMed Central PMCID: PMCPMC4140083.

5. Mitiahussacur J, Cruz M, Subsoumang P, Jimenez Abreu JA, Hosking C, Nagashima H, et al. Clarithromycin-Based Triple Therapy is still useful as an initial treatment for Helicobacter pylori infection in the Dominican Republic. Am J Trop Med Hyg. 2017;96(5):1060-9. doi: 10.4269/ajtmh.16-0729. PubMed PMID: 28193745; PubMed Central PMCID: PMCPMC5417194.

6. Malfortheimer P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection: the Maastricht V/Florence Consensus Report. Gut. 2017;66(3):30-60. Epub 2016/11/02. doi: 10.1136/gutjnl-2016-312288. PubMed PMID: 27707777.

7. Hunt RH, Xixo SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, et al. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. Journal of gastrointestinal and liver diseases : JGLD. 2011;20(3):239-304. PubMed PMID: 21961099.

8. Coelho LG, Leon-Barua R, Quigley EM. Latin American Consensus Conference on Helicobacter pylori infection. Latin American National Gastroenterological Societies affiliated with the Inter-American Association of Gastroenterology (AIAGE). Am J Gastroenterol. 2000;95(10):2688-91. Epub 2000/10/29. doi: 10.1111/j.1572-0241.2000.03174.x. PubMed PMID: 11051336.

9. Suzuki H, Nishizawa T, Murakai H, Hibi T. Sulfaphoxacin and garenoxacin may overcome the antibiotic resistance of Helicobacter pylori with gyrA mutation. Antimicrobial agents and chemotherapy. 2009;53(4):1720-1. Epub 2009/02/04. doi: 10.1128/aac.00049-09.
10. Gasbarrini A, Gasbarrini G, Pelosini I, Scarpignato C. Eradication of Helicobacter pylori: are rifaximin-based regimens effective? Digestion. 2006;73 Suppl 1:129-35. Epub 2006/02/25. doi: 10.1159/000093788. PubMed PMID: 16498261.

11. Kunin CM. Antimicrobial activity of rifabutin. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1999;22 Suppl 1:S3-13; discussion S-4. Epub 1999/04/01. PubMed PMID: 8785253.

12. Heep M, Rieger U, Beck D, Lehn N. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both Helicobacter pylori and Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2000;44(4):1076-7. Epub 2000/03/18. PubMed PMID: 10722516. PubMed Central PMCID: PMCPMC39817.

13. Adachi JA, DaPonte HL. Rifaximin: a novel nonabsorbed rifamycin for gastrointestinal disorders. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003;37(4):541-7. Epub 2006/01/20. doi: 10.1086/436950. PubMed PMID: 16421799.

14. Murakami K, Okimoto T, Kodama M, Tanahashi J, Fujikura T, Ikeda F, et al. Site-specific sites of resistance to rifamycins in Helicobacter pylori isolates, including those with gyrA mutations. Antimicrobial agents and chemotherapy. 2000;44(7):3097-9. Epub 2000/04/22. doi: 10.1128/aac.44.7.3097-9. PubMed PMID: 10658513. PubMed Central PMCID: PMCPMC98458.

15. Nishizawa T, Suzuki H, Matsuzaki J, Muraoka H, Tsugawa H, Hirata K, et al. Helicobacter pylori resistance to rifabutin in the last 7 years. Antimicrobial agents and chemotherapy. 2011;55(1):3374-5. Epub 2011/05/09. doi: 10.1128/acj.05437-11. PubMed PMID: 21803015. PubMed Central PMCID: PMCPMC3155021.

16. Ogata SK, Geist AC, Kawakami E. Antimicrobial susceptibility testing for Helicobacter pylori isolates from Brazilian children and adolescents: comparing agar dilution, E-test, and disk diffusion. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]. 2014;45(4):1439-48. Epub 2014/01/01. PubMed PMID: 2576302.

17. Holton J, Vaira D, Menegatti M, Barbara J. The susceptibility of Helicobacter pylori to rifaximin, rifaximin. The Journal of antimicrobial chemotherapy. 1996;35(4):545-9. Epub 1995/04/01. PubMed PMID: 7528989.

18. Heep M, Odenbreit S, Beck D, Decker J, Prohaska E, Rieger U, et al. Mutations at Four Distinct Regions of the rpoB Gene Can Reduce the Susceptibility of Helicobacter pylori to Rifamycins. Antimicrobial agents and chemotherapy. 2000;44(6):1713-5. PubMed PMID: 1089941.

19. Heep M, Beck D, Bayerk€¼rffer E, Lehn N. Rifampin and Rifabutin Resistance Mechanism in Helicobacter pylori. Antimicrobial agents and chemotherapy. 1999;43(6):1497-9. PubMed PMID: 1033030.

20. Hirata Y, Ohmae T, Yamas A, Sakitan K, Hayakawa Y, Yoshida S, et al. Sitaflaxacin
resistance in Helicobacter pylori isolates and sitafloxacin-based triple therapy as a third-line regimen in Japan. International journal of antimicrobial agents. 2012;39(4):352-5. Epub 2012/02/11. doi: 10.1016/j.ijantimicag.2011.12.002. PubMed PMID: 22831792.

21. Sugimoto M, Sahara S, Ichikawa H, Kagami T, Uotani T, Furuta T. High Helicobacter pylori cure rate with sitafloxacin-based triple therapy. Alimentary pharmacology & therapeutics. 2015;42(4):477-83. Epub 2015/06/16. doi: 10.1111/apt.13280. PubMed PMID: 26075059.

22. Furuta T, Sugimoto M, Kodaira C, Nishino M, Yamada M, Uotani T, et al. Sitafloxacin-based third-line rescue regimens for Helicobacter pylori infection in Japan. J Gastroenterol Hepatol. 2014;29(3):487-93. Epub 2013/11/15. doi: 10.1111/jgh.12442. PubMed PMID: 24224808.

23. Borody TJ, Pang G, Wettstein AR, Clancy R, Herdman K, Surace R, et al. Efficacy and safety of rifabutin-containing ‘rescue therapy’ for resistant Helicobacter pylori infection. Alimentary pharmacology & therapeutics. 2006;23(4):481-8. Epub 2006/01/31. doi: 10.1111/j.1365-2073.2006.02793.x. PubMed PMID: 16441468.

24. Lim JIC, Lee YJ, An B, Lee SW, Lee YC, Moon BS. Rifabutin-based high-dose proton-pump inhibitor and amoxicillin triple regimen as the rescue treatment for Helicobacter pylori. Helicobacter. 2014;19(6):455-61. Epub 2014/09/19. doi: 10.1111/hel.12147. PubMed PMID: 25231089; PubMed Central PMCID: PMC4284035.

25. Gisbert JP, Calvet X. Review article: rifabutin in the treatment of refractory Helicobacter pylori infection. Alimentary pharmacology & therapeutics. 2012;35(2):209-21. Epub 2011/12/02. doi: 10.1111/j.1365-2073.2011.04937.x. PubMed PMID: 22129228.

26. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, et al. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. Journal of gastrointestinal and liver diseases : JGLD. 2011;20(3):299-304. Epub 2011/10/01. PubMed PMID: 21961099.

27. Graham D, Mohammadi M. Synopsis of Antibiotic Treatment. In: Kim, Naysong (editor): Helicobacter pylori (Part VII, Chapter 40). Springer. 2016:p417-26.

28. Cheng H, Hu FL, Furazolidone, amoxicillin, bismuth and rabeprazole quadruple rescue therapy for the eradication of Helicobacter pylori. World J Gastroenterol. 2009;15(7):860-4. Epub 2009/02/21. PubMed PMID: 19230048; PubMed Central PMCID: PMC2655387.

29. Segura AM, Gutierrez O, Otero W, Angel A, Genta RM, Graham DY. Furazolidone, amoxicillin, bismuth triple therapy for Helicobacter pylori infection. Alimentary pharmacology & therapeutics. 1997;11(3):529-32. Epub 1997/06/01. PubMed PMID: 9218077.

30. Buwari YD. Antibiotic Resistance in the Elderly. Journal of Aging Research and Healthcare. 2017;Vol. 01(Issue No. 4). doi: DOI: 10.4302/issn.2474-7785.jahr.16:1356.

31. De Giorgio R, Stanghellini V, Barbara G, Guerrini S, Ferrieri A, Corinaldesi R.
Rifaximin and Helicobacter pylori eradication. European review for medical and pharmacological sciences. 1997;1(4):105-10. Epub 1997/07/01. PubMed PMID: 9558774.

32. Gasbarrini A, Lauritano EC, Nista EC, Candelli M, Gabrielli M, Santoro M, et al. Rifaximin-based regimens for eradication of Helicobacter pylori: a pilot study. Digestive diseases (Basel, Switzerland). 2006;24(1-2):195-200. Epub 2006/05/16. doi: 10.1159/0000900330. PubMed PMID: 16699278.

33. Nizheviche AA, Shcherbakov PL, Akhmadeeva EN. [Rifaximin in complex treatment of Helicobacter pylori infection in children (a pilot study)]. Ekspериментальная и клиническая гастроэнтерология = Experimental & clinical gastroenterology. 2009(3):98-100. Epub 2009/11/26. PubMed PMID: 19828007.

34. Nizheviche AA, Shcherbakov PL, Akhmadeeva EN, Khasanov R. Rifaximin in combined treatment of the Helicobacter pylori infection in childhood. Ekспериментальная и клиническая гастроэнтерология = Experimental & clinical gastroenterology. 2011(1):85-7. Epub 2011/05/13. PubMed PMID: 21560396.

35. O'Connor JR, Galang MA, Sambol SP, Hecht DW, Vedantam G, Gerding DN, et al. Rifaximin and rifaximin resistance in clinical isolates of Clostridium difficile. Antimicrobial agents and chemotherapy. 2008;52(8):2813-7. Epub 2008/06/19. doi: 10.1128/aac.00342-08. PubMed PMID: 18559647; PubMed Central PMCID: PMCPMC2493101.

36. Wichelhaus TA, Schäfer V, Brade V, Bodinghaus B. Molecular characterization of rpoB mutations conferring cross-resistance to rifamycins on methicillin-resistant Staphylococcus aureus. Antimicrobial agents and chemotherapy. 1999;43(11):2813-6. Epub 1999/10/30. PubMed PMID: 10543773; PubMed Central PMCID: PMCPMC89569.

37. Williams DL, Spring L, Collins L, Miller LP, Heifets LB, Gangadharan PR, et al. Contribution of rpoB mutations to development of rifamycin cross-resistance in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 1998;42(7):1853-7. Epub 1998/07/14. PubMed PMID: 9661035; PubMed Central PMCID: PMCPMC105697.

38. Yang B, Koga H, Ohno H, Ogawa K, Fukuda M, Hirakata Y, et al. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and rpoB mutations of Mycobacterium tuberculosis. The Journal of antimicrobial chemotherapy. 1998;42(5):621-8. Epub 1998/12/16. PubMed PMID: 9848446.

39. Kohary V, Scherl EJ, Bosworth B, Jiang ZD, Dupont HL, Harel J, et al. Rifaximin resistance in Escherichia coli associated with inflammatory bowel disease correlates with prior rifaximin use, mutations in rpoB, and activity of Phe-Arg-beta-naphthylamide-inhibitable efflux pumps. Antimicrobial agents and chemotherapy. 2013;57(2):811-7. Epub 2012/11/28. doi: 10.1128/aac.02163-12. PubMed PMID: 23183443; PubMed Central PMCID: PMCPMC3553721.
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Helicobactor pylori, 2003.
Publication

www.diva-portal.org
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Toracchio, S.. "Rifabutin based triple therapy for eradication of H. pylori primary and secondary resistant to tinidazole and clarithromycin", Digestive and Liver Disease,
Chih-Cheng Lai, Chi-Chung Chen, Ying-Chen Lu, Yin-Ching Chuang, Hung-Jen Tang. "The clinical significance of silent mutations with respect to ciprofloxacin resistance in MRSA", Infection and Drug Resistance, 2018

Farideh Siavoshi, Parastoo Saniee, Reza Malekzadeh. "Effective antimicrobial activity of rifabutin against multidrug-resistant", Helicobacter, 2018

Lim, Hyun Chul, Yong Jae Lee, ByoungRak An, SeungWoo Lee, Yong Chan Lee, and Byung Soo Moon. "Rifabutin-based High-dose Proton-pump Inhibitor and Amoxicillin Triple Regimen as the Rescue treatment for Helicobacter pylori, 2000."
Kellie J. Goodlet, Fatima Z. Benhalima, Michael D. Nailor. "A systematic review of single-dose aminoglycoside therapy for urinary tract infection: Is it time to resurrect an old strategy?", Antimicrobial Agents and Chemotherapy, 2018

Heep M.. "Detection of Rifabutin Resistance and Association of rpoB Mutations with Resistance to Four Rifamycin Derivatives in Helicobacter pylori", European Journal of Clinical Microbiology & Infectious Diseases, 02/01/2002

Lanckriet, A., L. Timbermont, M. De Gussem, M. Marien, D. Vancraeynest, F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. "The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model", Avian Pathology, 2010.

K. Murakami. "Sitafloxacin activity against Helicobacter pylori including isolates with gyrA mutations", Antimicrobial Agents and Chemotherapy, 04/20/2009
| Page | Reference |
|------|-----------|
| 46   | *Helicobacter pylori, 1998.* Publication |
| 47   | Z. Samra. "Resistance of Helicobacter pylori isolated in Israel to metronidazole, clarithromycin, tetracycline, amoxicillin and cefixime", *Journal of Antimicrobial Chemotherapy*, 06/01/2002 Publication |
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fluoroquinolone resistance: Antibiotic resistance of H. pylori", Journal of Gastroenterology and Hepatology, 2012.

"Abstracts of the European Helicobacter Study Group XXVIth International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer", Helicobacter, 2013.