Antimicrobial susceptibility and clarithromycin resistance patterns of *Helicobacter pylori* clinical isolates in Vietnam

Camelia Quek¹, Son T. Pham², Kieu T. Tran⁴, Binh T. Pham⁵, Loc V. Huynh⁴, Ngan B.L. Luu⁴, Thao K.T. Le⁴, Kelly Quek³, Van H. Pham⁴-⁶

¹Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Australia  
²Sydney Medical School, University of Sydney, Sydney, Australia  
³Department of Thoracic Head/Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, USA  
⁴Department of Research and Development, NK-Biotek, Ho Chi Minh, Vietnam  
⁵School of Medicine, University of Medicine and Pharmacy, Ho Chi Minh, Vietnam  
⁶School of Medicine, Tan Tao University, Duc Hoa, Vietnam

**Abstract**

*Helicobacter pylori* is a gastric pathogen that causes several gastroduodenal disorders such as peptic ulcer disease and gastric cancer. Eradication efforts of *H. pylori* are often hampered by antimicrobial resistance in many countries, including Vietnam. Here, the study aimed to investigate the occurrence of antimicrobial resistance among *H. pylori* clinical isolates across 13 hospitals in Vietnam. The study further evaluated the clarithromycin resistance patterns of strains. In order to address the study interests, antimicrobial susceptibility testing, epsilometer test and PCR-based sequencing were performed on a total of 193 strains isolated from patients, including 136 children (3–15 years of age) and 57 adults (19–69 years of age). Antimicrobial susceptibility testing showed that the overall resistance to amoxicillin, clarithromycin, levofloxacin, metronidazole, and tetracycline was 10.4%, 85.5%, 24.4%, 37.8%, and 23.8% respectively. The distribution of minimum inhibitory concentrations (MICs) of clarithromycin-resistant strains was 85.5% with MIC >0.5 μg/mL. The majority of the clarithromycin resistant isolates (135 of 165 subjects) have MICs ranging from 2 μg/mL to 16 μg/mL. Furthermore, sequencing detection of mutations in 23S rRNA gene revealed that strains resistant and susceptible to clarithromycin contained both A2143G and T2182C mutations. Of all isolates, eight clarithromycin-resistant isolates (MIC >0.5 μg/mL) had no mutations in the 23S rRNA gene. Collectively, these results demonstrated that a proportion of clarithromycin-resistant *H. pylori* strains, which are not related to the 23S rRNA gene mutations, could be potentially related to other mechanisms such as the presence of an efflux pump or polymorphisms in the CYP2C19 gene. Therefore, the present study suggests that providing susceptibility testing prior to treatment or alternative screening strategies for antimicrobial resistance is important for future clinical practice. Further studies on clinical guidelines and treatment efficacy are pivotal for successful eradication of *H. pylori* infection.
Corresponding author: Van H. Pham (van.pham@ttu.edu.vn)

How to cite this article: Quek C, Pham ST, Tran KT et al. Antimicrobial susceptibility and clarithromycin resistance patterns of Helicobacter pylori clinical isolates in Vietnam [version 1; referees: 2 approved] F1000Research 2016, 5:671 (doi: 10.12688/f1000research.8239.1)

Copyright: © 2016 Quek C et al. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Grant information: This work was supported by the Nam Khoa-Biotek and Nguyen Tri Phuong hospital.

Competing interests: The authors declare that they have no competing interests.

First published: 13 Apr 2016, 5:671 (doi: 10.12688/f1000research.8239.1)
Introduction

Helicobacter pylori is a Gram-negative bacterium that plays a causative role in the development of gastric adenocarcinoma, peptic ulcer disease and chronic gastritis. The prevalence of H. pylori infection is more than half of the world’s population, comprising of >80% in developing countries and approximately 40% in the United States. In Vietnam, the prevalence of H. pylori is approximately 80% in adults and 26%–71.4% in children.

Eradication therapy of symptomatic H. pylori infection substantially prevents the recurrence and reduces the risk of developing gastroduodenal-associated diseases. Recommended therapy, triple-therapy regimen, composed of two antimicrobial agents (e.g. amoxicillin, metronidazole, tetracycline, levofloxacin, and clarithromycin) in combination with a proton pump inhibitor (PPI), has been widely used to eliminate the bacteria. However, H. pylori antimicrobial resistance is increasing worldwide, contributing to the main factor that affects the efficacy of current therapeutic regimens. Resistance to clarithromycin is believed to be the major factor in treatment failure. In Vietnam, many studies showed that H. pylori is highly resistant to clarithromycin; 33%–34% primary and 74% secondary resistance. The majority of clarithromycin-resistant strains are identified based on point mutations in the peptidyltransferase region of domain V of 23S rRNA, which affects the binding of macrolides to the bacterial ribosome.

The common 23S rRNA point mutations (e.g. A2143G, A2142C/G and T2182C) are recommended for rapid routine diagnostic procedures, as compared to the time-consuming bacterial culture. A plethora of studies have evidently reported the association of minimum inhibitory concentrations (MICs) of clarithromycin-resistance strains to the respective point mutation. For example, A2142C/G mutations are associated with MIC >256 μg/mL, and mutations such as A2143G and T2182C are associated with MIC >0.5 μg/mL. However, it is unclear whether such association between point mutation and MIC can be utilised as predictors for strains resistant to clarithromycin. Here, the present study evaluated the antimicrobial resistance of H. pylori strains isolated from patients in Vietnam with the following antimicrobial agents: amoxicillin, metronidazole, tetracycline, levofloxacin and clarithromycin. The strains resistant to clarithromycin were further investigated to assess the point mutations in the 23S rRNA gene and MIC values as predictors for screening H. pylori strains. The overall findings addressed the issues of using 23S rRNA mutations in clinical diagnosis.

Materials and methods

Study samples

The present work was designed as a prospective randomised clinical study across 13 hospitals (Children’s Hospital 2, Children’s Hospital 1, Trieu An Hospital, Tam Nhat Clinic, Dai Phuc Clinic, Hoan My Hospital, DHY Ducou Hospital, Phap Viet Hospital, Yersin International Clinic, Dong Nai International Hospital, Nguyen Tri Phuong Hospital, Van Hanh General Hospital, Gia Dinh People’s Hospital) in Ho Chi Minh City, Vietnam, from July 2015 to January 2016 (Data availability). The study was approved by Nam Khoa Biotek Diagnostic Ethics Committee (ID: NCKH 04/02-15/NK). Written informed consent was obtained from each patient or the patient’s parents for the use of this study. Biopsy specimens of the gastric mucosa were obtained from 193 patients, including 136 children (3–15 years of age) and 57 adults (19–69 years of age). These patients showed indication of endoscopy for the examination of dyspeptic symptoms (i.e. gastric ulcer).

Helicobacter pylori culture and antimicrobial susceptibility testing

The H. pylori culture and susceptibility testing were performed as described in previous studies. Briefly, biopsy specimens were homogenised in 500 μL transport medium (20% glycerol; 0.9% NaCl in Milli-Q water), and were subsequently inoculated onto H. pylori selective agar plates at 37°C in a microaerophilic atmosphere. Biochemical identification of H. pylori was performed using Gram stain (Gram negative), oxidase test (oxidase positive), catalase test (catalase positive) and urease test (urease positive). Susceptibility testing was performed on Muller-Hinton agar plates supplemented with 10% lysed horse blood for the following antibiotics: amoxicillin (0.25 μg/mL), clarithromycin (0.75 μg/mL), levofloxacin (1 μg/mL), metronidazole (8 μg/mL), and tetracycline (2 μg/mL). The MIC values were obtained by the epilometer test (E-test; bioMérieux, Marcy l’Étoile, France) for clarithromycin in accordance with the manufacturer’s protocol using 10% lysed horse blood supplemented in Mueller-Hinton Z agar (bioMérieux). Bacterial suspensions were prepared in Mueller-Hinton broth and adjusted to a McFarland turbidity of three. Resistance criteria for clarithromycin was defined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST); susceptible (MIC ≤0.25 μg/mL) and resistance (MIC >0.5 μg/mL).

PCR amplification and sequence detection of 23S rRNA mutation

The PCR mixture (20-μL final volume) contained HotStar Taq master mix (Qiagen, Hilden, Germany) and 10 pmol of forward DP1 (5'-GTAAAAACGACGGCCAGTACCAGCGGCGGTAACTATA-3') and reverse ZGE23 (5'-TATTATTGGTGACATAGACAGGCCAGTACGTTACA-3') primers. These primers contain sequences (written in bold-faced type) that are specific for SP6 (DP1) and M13 (ZGE23), and underlined sequences indicate 23S rRNA amplicon of 308 bp comprising of 2142, 2143 and 2182 positions. H. pylori colonies on selective medium was added to 1x TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 7.6) and heated up to 100°C for 5 min, followed by centrifugation at 8000 rpm. 1 μL of supernatant was added to the PCR mix to amplify 23S rRNA gene. The PCR cycling conditions were 95°C for 15 min to activate HotStar Taq DNA polymerase, followed by 40 cycles of 94°C for 15 sec, 57°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 5 min. The PCR products were purified prior to sequencing by Illustra ExoStar 1-Step (GE Healthcare Life Sciences, Buckinghamshire, United Kingdom) according to manufacturer’s instructions, and followed by Big-Dye (Perkin-Elmer Applied Biosystems, Foster City, USA) amplification using SP6 and M13 primers. Sequencing was then performed using ABI 3130XL sequencer. In total, 193 sequences were obtained and analysed using MEGA version 5.0 against wild-type 23S rRNA.
Antimicrobial resistance of \textit{Helicobacter pylori} isolates

To assess the antimicrobial resistance of \textit{H. pylori} in Vietnam, susceptibility testing was performed and the resistance rate of each antimicrobial is listed in Table 1. The prevalence of antimicrobial resistance was detected in the following order, from highest to lowest: clarithromycin, metronidazole, levofloxacin, tetracycline and amoxicillin. Of all the antimicrobial agents, the majority of isolates were resistant to clarithromycin as shown in 85.5% of all patients (84.6% in children and 87.7% in adults). The occurrence of metronidazole resistance was lower than clarithromycin (overall 37.8% vs. 85.5%) in this study, as compared to the other published reports\textsuperscript{16-20}. Antimicrobial resistance in adults is predominately higher than children and 5.3% of adults without statistical significance. A statistically significant difference was observed in the resistance rate of levofloxacin ($p = 0.0103$) between children and adults in Figure 1.

Minimum inhibitory concentration values of clarithromycin-resistant isolates predominately range from 2 μg/ml to 16 μg/ml. To validate the clarithromycin resistant isolates, MIC values were obtained from a total of 193 clinical isolates using an E-test. Based on EUCAST proposed breakpoints, the respective occurrence of clarithromycin susceptible and resistant isolates was 24 (12.4%) and 165 (85.5%) of the total number of isolates used in this study. The distribution of MICs showed that the majority of clinical isolates resistant to clarithromycin (135 of 165 isolates, 81.8%, including 97 children and 38 adults) ranged from 2 μg/mL to 16 μg/mL (Figure 2). Of all isolates, only five subjects (including four children and one adult) showed a MIC of 24 μg/mL, and one adult subject had a MIC >256 μg/mL. (Figure 2).

Mutations of 23S rRNA gene in \textit{Helicobacter pylori} isolates

To investigate the point mutations in the 23S rRNA gene of clarithromycin-resistant isolates, mutations at position 2142 (A2142G or A2142C), 2143 (A2143G), and 2182 (T2182C) were analysed in this study. Sequence analyses showed the point mutations in the 23S rRNA gene were detected not only in clarithromycin-resistant isolates, but also in clarithromycin-susceptible isolates. In Table 2, both A2143G and T2182C mutations were predominantly detected in 91.7% ($n = 177$) of the clarithromycin-resistant and –susceptible isolates. Only two clarithromycin-resistant isolates in adults had the A2142G and T2182C mutations with a respective MIC value of 8 μg/mL and >256 μg/mL. In addition, a total of 10 clarithromycin-resistant and –susceptible isolates had no mutations in the 23S rRNA gene. The present study also identified four isolates with both A2143G and T2182C mutations at MIC values ranging from 0.38 to 0.5 μg/mL, which are considered to be intermediate resistance strains\textsuperscript{34}.

Discussion

Antimicrobial resistance in \textit{H. pylori} has become a global health problem because the prevalence of infection and incidence is increasing worldwide\textsuperscript{17-29}. The increasing \textit{H. pylori} resistance to antimicrobial agents, such as clarithromycin, is considered the main
factor for reduced treatment success in several countries, including Vietnam and Japan\(^{17,18,40-42}\). Therefore, the understanding of geographical region specific prevalence is crucial for treatment of *H. pylori* infection.

Vietnam is categorised as a region with a high prevalence of *H. pylori* infection and an intermediate risk of gastric cancer\(^{43,44}\). In Vietnam (Ho Chi Minh City and Hanoi), clarithromycin and metronidazole are recommended as a first-line therapy regimen\(^1\). Our present study showed that the overall resistance rate for clarithromycin and metronidazole was 85.5% and 37.8%, respectively. The high incidence of *H. pylori* strains resistant to clarithromycin and metronidazole in Vietnam might be attributed to the following: (i) unregulated or widespread over-the-counter use of antibiotics, (ii) clarithromycin is prescribed frequently for treatment due to its high bactericidal effect, and (iii) antibiotics are often used to treat *H. pylori* infection and other infections including respiratory tract infections (clarithromycin) and intestinal parasites (metronidazole)\(^{13,15,46}\). Of note, this study highlighted that clarithromycin resistance was the highest among the 193 *H. pylori* clinical isolates collected in 2015–2016, as compared to the other studies in which metronidazole has the highest resistance rate (69.9%–76.1%).
in Vietnam\textsuperscript{4-20}. The observation of high clarithromycin resistance rate from our data suggested the increasing occurrence of resistant strains among other antimicrobial agents. Therefore, constant surveillance for antimicrobial resistance rates is necessary to gain insights into effective eradication therapy of \textit{H. pylori} infection.

Another interest of this study was to assess the variations of MIC values obtained from the clarithromycin-resistant strains. Our representative clinical isolates obtained from the gastric mucosa revealed that the majority of strains resistant to clarithromycin conferred MIC values ranging from 2 \(\mu\)g/mL to 16 \(\mu\)g/mL. There is also a degree of variation on the MIC range between studies\textsuperscript{19,23,45-49}. The variability of MIC values for resistant isolates might be attributed to different gastric sites. The evidence is supported by Borody \textit{et al.} who demonstrated that the bimodal distribution of clarithromycin resistance of isolates cultured from 4 gastric sites (i.e. antrum, distal body, proximal body and fundus) ranged from <0.016 \(\mu\)g/mL to 256 \(\mu\)g/mL\textsuperscript{20}. The recent studies also demonstrated that MIC values for clarithromycin resistance vary at different gastric sites\textsuperscript{47-49}. Therefore, the present results confirm previous studies that multiple gastric biopsies from different sites of the stomach are crucial for accurate diagnosis of \textit{H. pylori} infection.

Furthermore, antimicrobial susceptibility testing using MIC values is often used to determine the appropriate dosage of antimicrobial for a patient’s prescription. However, the respective antimicrobial resistance rate is based on the defined MIC breakpoints, which are much lower than the achievable tissue concentrations of antimicrobial agents such as clarithromycin (ranging from 5.2 \(\mu\)g/mL to 22.2 \(\mu\)g/mL\textsuperscript{31}). Only a few reports have studied the eradication rate of \textit{H. pylori} infection with high MIC values (e.g. >24 \(\mu\)g/mL), highlighting that the significant eradication rate of 50\%–80\% on MIC-defined resistant strains can be achieved by administering PPI with precise antibiotic dosage and appropriate treatment duration\textsuperscript{30,32,33,35}. Hence, further longitudinal studies on treatment efficacy and treatment guidelines are necessary for successful treatment.

Point mutations at positions 2142, 2143 and 2182 on the 23S rRNA gene were commonly reported\textsuperscript{25-27}. Yet it remains unclear whether or not these point mutations could be a strong predictor of clarithromycin resistance\textsuperscript{25,29-33}. In some studies, only the A2142G mutation was found to be associated with high MIC values\textsuperscript{19,45}. While other studies showed that mutations at positions 2142 and/or 2143 were associated with clarithromycin resistance\textsuperscript{19,23,34,46-48}. In addition, mutation T2182C was only reported in one study\textsuperscript{46}. Here, we reported that \textit{H. pylori} strains with mutations in A2143G and T2182C exhibited not only in clarithromycin-resistant strains, but also in susceptible strains as observed in Table 2. Similar to Phan \textit{et al.}’s study\textsuperscript{47}, none of the clarithromycin-resistant strains portrayed A2142C mutation in our study. It is important to note that the association of MIC values and point mutations was not identified in our work. Additionally, a proportion of all isolates had no point mutations in the 23S rRNA gene (Table 2). Further investigation on other nucleotide positions of the 23S rRNA region should be performed on these resistant strains\textsuperscript{38,39}. Additionally, we suggested that a proportion of these resistant strains, which are not related to the 23S rRNA gene sequence, could be potentially related to other mechanisms such as the presence of an efflux pump (e.g. outer membrane protein \textit{hefA}) or polymorphisms in the \textit{CYP2C19} gene\textsuperscript{30-42}.

**Conclusions**

In conclusion, our present results confirm that MIC values are critical for accurate identification of antimicrobial resistant strains. Susceptibility tests prior to treatment are necessary to select the optimal \textit{H. pylori} therapy regimens in Vietnam. Further studies on other resistance mechanisms, particularly the mutations of the host genes, will provide additional insights into the development of diagnostic biomarkers and therapeutic drugs.

**Consent**

Written informed consent for publication of their clinical details was obtained from the parents of the patients.

**Data availability**

\textit{F1000Research}: Dataset 1. A summary of patient information, antimicrobial susceptibility and clarithromycin resistance patterns, 10.5256/f1000research.8239.d118249\textsuperscript{63}

**Author contributions**

C.Q. performed data analysis, interpreted the data, constructed and drafted the manuscript, and coordinated the analysis aspect of the study. S.T.P. participated in data interpretation, drafted the manuscript and provided critical revision of the manuscript. K.T.T. performed the experiments and responsible for data collection. B.T.P designed and coordinated the microbiological experiments. L.V.H. supervised and assisted the design of clinical study. N.B.L.L and T.K.T. assisted the microbiological experiments. K.Q. participated in result discussion and provided critical revision of the manuscript. V.H.P. supervised the clinical study, interpreted the data and provided critical revision of the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Grant information**

This work was supported by the Nam Khoa-Biotek and Nguyen Tri Phuong hospital.
45. Katelaris PH: *Helicobacter pylori*: antibiotic resistance and treatment options. J Gastroenterol Hepatol. 2009; 24(7): 1155–1157. PubMed Abstract | Publisher Full Text

46. Gonzales R, Bartlett JG, Besner RE, et al.: Principles of appropriate antibiotic use for treatment of acute respiratory tract infections in adults: background, specific aims, and methods. Ann Intern Med. 2001; 134(6): 479-486. PubMed Abstract | Publisher Full Text

47. Selgrad M, Tammer I, Langner C, et al.: Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. World J Gastroenterol. 2014; 20(43): 16245–16251. PubMed Abstract | Publisher Full Text | Free Full Text

48. Ayala G, Galvan-Portillo M, Chihu L, et al.: Resistance to antibiotics and characterization of Helicobacter pylori strains isolated from antrum and body from adults in Mexico. Microb Drug Resist. 2011; 17(2): 149–155. PubMed Abstract | Publisher Full Text

49. Rimbara E, Noguchi N, Tanabe M, et al.: Susceptibilities to clarithromycin, amoxicillin and metronidazole of *Helicobacter pylori* isolates from the antrum and corpus in Tokyo, Japan, 1995-2001. Clin Microbiol Infect. 2005; 11(4): 307–311. PubMed Abstract | Publisher Full Text

50. Borody TJ, Clancy R, Warren EF, et al.: Antibiotic sensitivities of *Helicobacter pylori* vary at different gastric mucosal sites. Dordrecht, London, Kluwer Academic; 2003; 373–381. Publisher Full Text

51. Gustavsson LE, Kaiser JF, Edmonds AL, et al.: Effect of omeprazole on concentrations of clarithromycin in plasma and gastric tissue at steady state. Antimicrob Agents Chemother. 1995; 39(9): 2078–2083. PubMed Abstract | Publisher Full Text | Free Full Text

52. Kim JM, Kim JS, Jung HC, et al.: Distribution of antibiotic MICs for *Helicobacter pylori* strains over a 16-year period in patients from Seoul, South Korea. Antimicrob Agents Chemother. 2004; 48(12): 4843–4847. PubMed Abstract | Publisher Full Text | Free Full Text

53. De Francesco V, Margiotta M, Zullo A, et al.: Clarithromycin-resistant genotypes and eradication of *Helicobacter pylori*. Ann Intern Med. 2006; 144(2): 94–100. PubMed Abstract | Publisher Full Text

54. van Doorn LJ, Glupczynski Y, Kusters JG, et al.: Accurate prediction of macrolide resistance in *Helicobacter pylori* by a PCR line probe assay for detection of mutations in the 23S rRNA gene: multicenter validation study. Antimicrob Agents Chemother. 2001; 45(5): 1500–1504. PubMed Abstract | Publisher Full Text | Free Full Text

55. Owen RJ: Molecular testing for antibiotic resistance in *Helicobacter pylori*. Gut. 2002; 50(3): 285–289. PubMed Abstract | Publisher Full Text | Free Full Text

56. Hulten K, Gibreel A, Skold O, et al.: Macrolide resistance in *Helicobacter pylori*: mechanism and stability in strains from clarithromycin-treated patients. Antimicrob Agents Chemother. 1997; 41(11): 2550–2553. PubMed Abstract | Free Full Text

57. Occhialini A, Urdaci M, Doucel-Populaire F, et al.: Macrolide resistance in *Helicobacter pylori*: rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob Agents Chemother. 1997; 41(12): 2724–2728. PubMed Abstract | Free Full Text

58. Brin TT, Shioya S, Suzuki R, et al.: Discovery of novel mutations for clarithromycin resistance in *Helicobacter pylori* by using next-generation sequencing. J Antimicrob Chemother. 2014; 69(7): 1796–1803. PubMed Abstract | Publisher Full Text | Free Full Text

59. Rimbara E, Noguchi N, Kawai T, et al.: Novel mutation in 23S rRNA that confers low-level resistance to clarithromycin in *Helicobacter pylori*. Antimicrob Agents Chemother. 2008; 52(9): 3465–3466. PubMed Abstract | Publisher Full Text | Free Full Text

60. Liu ZQ, Zheng PY, Yang PC: Efflux pump gene *hefA* of *Helicobacter pylori* plays an important role in multidrug resistance. World J Gastroenterol. 2008; 14(33): 5217–5222. PubMed Abstract | Publisher Full Text | Free Full Text

61. High antibiotic resistance rate: A difficult issue for *Helicobacter pylori* eradication treatment. World J Gastroenterol. 2015; 21(48): 13432–13437. PubMed Abstract | Publisher Full Text | Free Full Text

62. Quoc C, Pham ST, Tran KT, et al.: Dataset 1 in: Antimicrobial susceptibility and clarithromycin resistance patterns of *Helicobacter pylori* clinical isolates in Vietnam. F1000Research. 2016. Data Source
Open Peer Review

Current Referee Status:  

Version 1

Referee Report 02 August 2016

doi:10.5256/f1000research.8861.r13392

Simon Cutting
School of Biological Sciences, Royal Holloway, University of London, Egham, UK

In general this is an interesting paper reporting on resistance of the antibiotic clarithromycin in Vietnam and the relevance of this resistance to Helicobacter pylori infection. The main outcome of this work is that it demonstrates the need for susceptibility testing prior to treatment. It is encouraging that this work is led by Vietnamese scientists and the work appears of a high standard.

It would have been useful to have a figure showing the mutational hotspots within the 23S rRNA gene.

General

Bacterial species names should be written in italics

The MICs stated on page 3 (3rd para) show considerable variation with a large range, i.e., >256 mg/ml and >0.5 mg/ml. What is considered significant?

MIC should be used as an abbreviation in Figures and Tables as well as text, e.g. Fig 2 and Table 2

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 31 May 2016

doi:10.5256/f1000research.8861.r14071

Duc Trong Quach
Department of Internal Medicine, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam

This is one among the informative studies about the antimicrobial susceptibility and clarithromycin resistance patterns of Helicobacter pylori clinical isolates in Vietnam. The title is appropriate for the content. The methods and analysis of the results are well-described and appropriate.
However, there are several minor revisions that the authors should be considered to make results of the paper more clinically meaningful:

1. The design of this study is not prospective randomized one. It is a cross-sectional study.

2. The clinical information of the patients recruited in the study should be clarified: are they naïve patients or not. This information is essential to understand the true situation of antimicrobial susceptibility in Vietnam. As a result, the conclusion “Susceptibility tests prior to treatment are necessary to select the optimal \textit{H. pylori} therapy regimens in Vietnam” may be not appropriate without this information.

3. The authors should also addressed the weak points of the studies. Although this is a multi-center study, all of the medical centers locates in southern Vietnam. The picture of antimicrobial susceptibility and clarithromycin resistance patterns of Helicobacter pylori has been shown to be somewhat different in Central and Northern Vietnam. Therefore, the author should change the title from “in Vietnam” to “in southern Vietnam”, or they can keep the title as it was but add a sentence which mentions this weak point of the study.

\textbf{I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.}

\textbf{Competing Interests:} No competing interests were disclosed.