**Dear Science Editor Ma,**

We would like to submit our revised manuscript for consideration of publication in the World Journal of Gastroenterology. Enclosed please find our revised manuscript entitled "Second-line rescue treatment of *Helicobacter pylori* infection: Where are we now?". We highly appreciate the valuable comments from the reviewers and have made an itemized, point-by-point response to the comments, referring to page numbers where possible.

We appreciate your consideration of this manuscript for publication in the World Journal of Gastroenterology. We hope it will now meet with your approval.

Sincerely Yours,

Dr. Ping-I Hsu

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***Editor´ s comment:***

1. **Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).**

**Reply to the comment:** According to the Editor’s comment, we have provided language certificate letter by professional English language editing companies (Please see attached file).

2. **Audio core tip: In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information. The accepted formats are mp3 or wma.**

**Reply to the comment:** According to the comment, we have made an Audio core tip (recorded as .mp3 file) and have uploaded it via the F6Publishing system.

3. **Your manuscript should be prepared with word-processing software, using 12 pt Book Antiqua font and 1.5 line spacing with ample margins.**

**Reply to the comment:** According to the comment, we have prepared our manuscript with Word-processing Software, using 12 pt Book Antiqua font and 1.5 line spacing with ample margins.

4. **Please revise and perfect your manuscript according to peer-reviewers’ comments. Please upload the required files on the system.**

**Reply to the comment:** We have revised our manuscript according to peer-reviewers’ comments, and have uploaded the required files on the system.

**Reply to Reviewer 1 (Code: 02536252)**

**Comment 1. The authors need to discuss the present status in antibiotic resistance as well as resistance mechanisms for *H. pylori* eradication therapy in the discussion sections by citing some of followings. 1: Mori H, Suzuki H, Matsuzaki J, Masaoka T, Kanai T. Acquisition of double mutation in gyrA caused high resistance to sitafloxacin in Helicobacter pylori after unsuccessful eradication with sitafloxacin-containing regimens. United European Gastroenterol J. 2018 Apr;6(3):391-397. doi: 10.1177/2050640617737215. Epub 2017 Oct 8. PubMed PMID: 29774152; PubMed Central PMCID: PMC5949976. 2: Suzuki H, Mori H. World trends for H. pylori eradication therapy and gastric cancer prevention strategy by H. pylori test-and-treat. J Gastroenterol. 2018 Mar;53(3):354-361. doi: 10.1007/s00535-017-1407-1. Epub 2017 Nov 14. Review. PubMed PMID: 29138921; PubMed Central PMCID: PMC5847180. 3: Mori H, Suzuki H, Matsuzaki J, Masaoka T, Kanai T. Antibiotic resistance and gyrA mutation affect the efficacy of 10-day sitafloxacin-metronidazole-esomeprazole therapy for Helicobacter pylori in penicillin allergic patients. United European Gastroenterol J. 2017 Oct;5(6):796-804. doi: 10.1177/2050640616688995. Epub 2017 Jan 19. PubMed PMID: 29026593; PubMed Central PMCID: PMC5625875. 4: Mori H, Suzuki H, Matsuzaki J, Tsugawa H, Fukuhara S, Miyoshi S, Hirata K, Seino T, Matsushita M, Nishizawa T, Masaoka T, Kanai T. Rifabutin-based 10-day and 14-day triple therapy as a third-line and fourth-line regimen for Helicobacter pylori eradication: A pilot study. United European Gastroenterol J. 2016 Jun;4(3):380-7. doi: 10.1177/2050640615618043. Epub 2015 Nov 13. PubMed PMID: 27403304; PubMed Central PMCID: PMC4924440. 5: Nishizawa T, Maekawa T, Watanabe N, Harada N, Hosoda Y, Yoshinaga M, Yoshio T, Ohta H, Inoue S, Toyokawa T, Yamashita H, Saito H, Kuwai T, Katayama S, Masuda E, Miyabayashi H, Kimura T, Nishizawa Y, Takahashi M, Suzuki H. Clarithromycin Versus Metronidazole as First-line Helicobacter pylori Eradication: A Multicenter, Prospective, Randomized Controlled Study in Japan. J Clin Gastroenterol. 2015 Jul;49(6):468-71. doi: 10.1097/MCG.0000000000000165. PubMed PMID: 24921211. 6: Nishizawa T, Suzuki H. Mechanisms of Helicobacter pylori antibiotic resistance and molecular testing. Front Mol Biosci. 2014 Oct 24;1:19. doi: 10.3389/fmolb.2014.00019. eCollection 2014. Review. PubMed PMID: 25988160; PubMed Central PMCID: PMC4428472. 7: Asaoka D, Nagahara A, Matsuhisa T, Takahashi S, Tokunaga K, Kawai T, Kawakami K, Suzuki H, Suzuki M, Nishizawa T, Kurihara N, Ito M, Sasaki H, Omata F, Mizuno S, Torii A, Ohkusa T, Mine T, Sakaki N. Trends of second-line eradication therapy for Helicobacter pylori in Japan: a multicenter study in the Tokyo metropolitan area. Helicobacter. 2013 Dec;18(6):468-72. doi: 10.1111/hel.12063. Epub 2013 Jun 18. PubMed PMID: 23773231. 8: Nishizawa T, Suzuki H, Matsuzaki J, Muraoka H, Tsugawa H, Hirata K, Hibi T. Helicobacter pylori resistance to rifabutin in the last 7 years. Antimicrob Agents Chemother. 2011 Nov;55(11):5374-5. doi: 10.1128/AAC.05437-11. Epub 2011 Sep 6. PubMed PMID: 21896915; PubMed Central PMCID: PMC3195021. 9: Nishizawa T, Suzuki H, Tsugawa H, Muraoka H, Matsuzaki J, Hirata K, Ikeda F, Takahashi M, Hibi T. Enhancement of amoxicillin resistance after unsuccessful Helicobacter pylori eradication. Antimicrob Agents Chemother. 2011 Jun;55(6):3012-4. doi: 10.1128/AAC.00188-11. Epub 2011 Apr 12. Erratum in: Antimicrob Agents Chemother. 2013 Feb;57(2):1106. PubMed PMID: 21486961; PubMed Central PMCID: PMC3101459. 10: Tsugawa H, Suzuki H, Muraoka H, Ikeda F, Hirata K, Matsuzaki J, Saito Y, Hibi T. Enhanced bacterial efflux system is the first step to the development of metronidazole resistance in Helicobacter pylori. Biochem Biophys Res Commun. 2011 Jan 14;404(2):656-60. doi: 10.1016/j.bbrc.2010.12.034. Epub 2010 Dec 11. PubMed PMID: 21147064. 11: Hirata K, Suzuki H, Nishizawa T, Tsugawa H, Muraoka H, Saito Y, Matsuzaki J, Hibi T. Contribution of efflux pumps to clarithromycin resistance in Helicobacter pylori. J Gastroenterol Hepatol. 2010 May;25 Suppl 1:S75-9. doi: 10.1111/j.1440-1746.2009.06220.x. PubMed PMID: 20586871. 12: Suzuki H, Nishizawa T, Hibi T. Helicobacter pylori eradication therapy. Future Microbiol. 2010 Apr;5(4):639-48. doi: 10.2217/fmb.10.25. Review. PubMed PMID: 20353303.**

**Reply to the comment:** According to the reviewer’s valuable comments, we have discussed the present status in antibiotic resistance in thre revised manuscript (P4, line 19: *Primary resistance to amoxicillin is either null or <1% in most countries[9]. In contrast, the rate of primary clarithromycin-resistance ranges from 49% (Spain) to 1% (the Netherlands) worldwide[10]. High primary resistance to clarithromycin and low resistance to metronidazole have been observed in Japan; moderate resistance to clarithromycin and high resistance to metronidazole were reported in Korea; and high primary resistance to both clarithromycin and metronidazole was observed in China[11]. High primary resistance to both clarithromycin and metronidazole has also been reported in some other countries, such as Italy, Spain, Mexico and Vietnam. Low clarithromycin resistance is generally observed in northern Europe, including the Netherlands, Sweden and Ireland[10,11].*

*In patients who experience eradication failure following standard triple therapy, the rates of drug resistance to clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline are 65-75%, 30-56%, 26-37%, 0-6.1% and 0-10%, respectively[12-16]. Whereas for patients who experience failure of non-bismuth quadruple therapy, the rates of drug resistance to clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline are 75%, 75%, 25%, 0%, and 0%, respectively[17,18]. This data implies that amoxicillin, tetracycline and levofloxacin are good choices of antibiotics for rescue treatment of H. pylori infection.*

*Point mutations play a primary role in the antimicrobial resistance of H. pylori, and different mutations involving the rdxA gene have been identified in metronidazole resistant strains[19]. Resistance to clarithromycin in H. pylori is commonly caused by point mutations in the rrl gene encoding two 23S rRNA nucleotides, namely 2142 and 2143[20]. Another mechanism associated with the development of clarithromycin resistance is the efflux pump system[21,22]. Fluoroquinolone acts on the site of the type A DNA gyrase enzyme, which is encoded by the gyrA gene, to inhibit DNA cleavage and rejoining[23]. Gene mutations in gyrA are associated with fluoroquinolone resistance. In particular double mutations at both N87 and D91 in gyrA have been reported to increase fluoroquinolone resistance[24].*).

 **We thank the reviewer’s valuable and constructive comments!**

**Reply to Reviewer 2 (Code: 00001114)**

**Comment 1: This entitles “Second-line rescue treatment of *Helicobacter pylori* infection: Where are we now? " is well-written and comprehensive review about this subject. I have following one comment – I would request to add strategy for choice of second line regimen. The Maastricht V/Florence Consensus Report said that after a first failure, if an endoscopy is carried out, culture and standard antimicrobial susceptibility testing (AST) are recommended to tailor the treatment. What do the authors think if test-and-treat strategy is necessary before selecting second-line regimen? In Japan, PPI-containing triple therapy with metronidazole (MTZ) and amoxicillin is a standard second line regimen. Because this triple therapy is only covered under Japan’s national health insurance. We clinically did not check AST but we can achieve eradication rate of around 90% because MTZ resistance rate is relatively low in Japan. I think choice of second line regimen depends on its regional factors. So I would ask the authors to give comments to readers when they select second line regimen.).**

**Reply to the comment:** According to the reviewer’s valuable comments, we have given comments concerning antimicrobial susceptibility testing to readers when they select second-line regimen (P9, line 11: *After a first failure of H. pylori treatment, if an endoscopy is arranged, the Maastricht V/Florence Consensus Report recommends antimicrobial susceptibility testing (AST)[31] to enable tailoring of the rescue eradication therapy. However, AST is not routinely performed in clinical practice due to the invasiveness of the endoscopy procedure, the availablity of laboratory culture facilities and cost considerations. If AST data are not available, 10-day TL quadruple therapy can be used as a rescue treatment since it achieves an eradication rate of >90% following failure of standard triple, concomitant and bismuth quadruple therapies. In addition, the novel 10-day TL quadruple regimen can maintain a high eradication rate (>90%) for H. pylori strains with levofloxacin resistance[35]. However, the choice of second line rescue regimen also depends on regional factors. In Japan, PPI-containing triple therapy with metronidazole and amoxicillin is the standard second line regimen and is covered under Japan’s national health insurance. This second-line therapy can also achieve an eradication rate of around 90% because metronidazole resistance rate is relatively low in Japan.*).

**We thank the reviewer’s valuable and constructive comments!**

5. **Please provide the decomposable figure of Figures, whose parts are movable and editable. So you can put the original pictures in PPT and submit it in the system.**

**Reply to the comment:** According to the comments, we have provided the decomposable figure of Figures, whose parts are movable and editable, and have also submitted it via the F6Publishing system.

6. **Please check the repeated parts with “Ping-I Hsu, Feng-Woei Tsai, Sung-Shuo Kao, Wen-Hung Hsu et al. "Ten-Day Quadruple Therapy Comprising Proton Pump Inhibitor, Bismuth, Tetracycline, and Levofloxacin is More Effective than Standard Levofloxacin Triple Therapy in the Second-Line Treatment of Helicobacter pylori Infection: A Randomized Controlled Trial", The American Journal of Gastroenterology, 2017” And please revise the manuscript!**

**Reply to the comment:** According to the comments, we have checked the repeated parts of the manuscript with the paper entitled of "Ten-Day Quadruple Therapy Comprising Proton Pump Inhibitor, Bismuth, Tetracycline, and Levofloxacin is More Effective than Standard Levofloxacin Triple Therapy in the Second-Line Treatment of Helicobacter pylori Infection ", and have revised the manuscript (P7, line 10: *An important drawback of levofloxacin-amoxicillin triple therapy is poor eradication efficacy in the presence of fluoroquinolone resistance. Bismuth salts have a synergistic effect on antibiotics and have been used to increase eradication rates[32].*; P8, line 6: *A randomized control study showed that as a second-line anti-H. pylori treatment, 10-days of TL quadruple therapy achieved a much higher eradication rate compared with 10-days levofloxacin triple therapy containing esomeprazole, amoxicillin and levofloxacin (98% vs. 68%, respectively)[35]*; P8, line 12: *There were only 7 patients recruited into the study with eradication failure by bismuth quadruple therapy as a first-line treatment, and both TL quadruple and levofloxacin-amoxicillin triple therapies had a 100% eradication rate in this subgroup of patients. The data suggests that 10-day TL quadruple therapy is a good option for second-line treatment after failure of standard triple, concomitant and bismuth quadruple therapies.*).

7. **Please include postcode here!**

**Reply to the comment:** According to the comments, we have added postcode 813 in the address (P1 line 10: *National Yang-Ming University, Kaohsiung 813*).

8. **You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by...".**

**Reply to the comment:** We would like to delte the part of "Supported by...".

9. **Abbreviations and acronyms are often defined the first time they are used within the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention.**

**Reply to the comment:** We have re-checked the manuscript and adhere to this convetion. Many thanks!

10. **Please correct all cited references number like [number], then keep them superscript.**

**Reply to the comment:** We corrected all cited references number in the format [number] with superscript position in the revised manuscript.

11. **Please distinguish between the title of the article series. Three levels of subtitles are allowed: (1) First subtitle: All in bold and capital; (2) Second subtitle: All in bold and italic; and (3) Third subtitle: All in bold.**

**Reply to the comment:** We revised all the subtitles according to the regulations.

12. **Please check and confirm that there are no repeated references!**

**Reply to the comment:** We haved checked and confirmed that there are no repeated references.

13. **Please don’t include abbreviations in the name of the figure.**

**Reply to the comment:** We have revised the name of the figure according to the comment.