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!