Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Rm 1.46 Department of Clinical Medicine

Trinity Centre

Tallaght Hospital

Dublin 24

Ireland

February 2nd 2018

Manuscript number: 37541

Dear Xue-Jiao Wang,

Many thanks for the opportunity to revise our manuscript entitled:

"Can bacterial virulence factors predict antibiotic resistant Helicobacter pylori infection?"

Please find below our response to the reviewer's and editorial comments.

Please do not hesitate to contact me if any further information is required.

Yours sincerely,

Sinead Smith PhD

Editorial comments

- All of the suggested editorial changes have been made
- The requested ARRIVE guidelines have been uploaded
- The references have been changes as per editors suggestions
- The statistical tests used have been included in the results section

Reviewer number: 01557050

Dr. Brennan and Dr. Smith, et al. reported 'Can bacterial virulence factors predict antibiotic resistant Helicobacter pylori infection? The article is informative and well-presented. The reviewer has some minor comments.

Comments 1. In Table 3, please insert by one line in 'Susceptible to clarithromycin (WT), 'Resistant to clarithromycin, 'Point mutations' each.

And in Table 4, please insert by one line in 'Susceptible to fluoroquinolones (WT), 'Resistant to fluoroquinolones, 'Point mutations' each.

Response:

We thank the reviewer for their comments and are happy that the reviewer thinks that "the article is informative and well presented". We have made the suggested changes to Tables 3 and 4, which are highlighted in red in the attached manuscript file.

Reviewer number: 00504545

It is a very good paper clearly written and described, showing and increased of the antibiotic resistance in less virulent strange in Ireland and this a very interesting finding

Response:

We thank the reviewer for their kind comments. As this reviewer has not suggested any revision, we have not highlighted any changes in the revised manuscript in relation to reviewer number 00504545 comments.

Reviewer number: 03474116

General: In this study, the authors investigated to evaluate the association between virulence factor type of H. pylori and antibiotic resistance in H. pylori-infected patients in Ireland. Primary clarithromycin resistance was significantly lower in cagA-positive strains than in negative strains. Similarly, in patients infected with vacA s1 type, primary clarithromycin resistance was significantly lower than in those infected with vacA s2 type. Authors concluded that less virulent strains of H. pylori are associated with primary clarithromycin resistance.

Response:

We thank the reviewer for their important feedback. Please find below a point-by-point response to their comments:

Q1. Less virulent strains of H. pylori are associated with primary clarithromycin resistance. Why not fluoroquinolone?

Response: One possible explanation for this is that the percentage of patients with primary fluoroquinolone resistance was small compared those with clarithromycin resistance (15.2% (n=16/105) vs 50.5 (n=53/105), respectively; Tables 3 and 4). There was an overall trend towards fluoroquinolone resistance and less virulent strains (Figs 3A, 3C, Figs 4A-C). Perhaps, if a larger cohort was examined, a statistically significant association between less virulent strains of *H. pylori* and fluoroquinolone resistance would be observed.

Q2. Why are less virulent (cagA-negative and vacA s2-containing) strains of H. pylori associated with primary clarithromycin resistance?

Response: Based on hypotheses previously described in the published literature (1, 2), we believe that there are 2 properties associated with less virulent strains of *H. pylori* that may influence the risk of acquiring antibiotic resistance. Firstly, less virulent strains are not dividing as rapidly as more virulent stains. As antibiotics are thought to be more effective on rapidly growing bacteria, bacteria in resting phase are exposed to antibiotics for longer, thereby increasing the chance of resistance-mediating mutations arising. Secondly, less virulent strains are known to induce less gastric inflammation resulting in a lower level of mucosal blood flow and antibiotic diffusion when compared to more virulent strains. The resulting suboptimal antibiotic concentrations could also contribute to the development of antibiotic resistant strains. These points and associated references are highlighted in red in the attached manuscript.

Q3. Most of East Asian population infected with high virulent strains of H. pylori, cagA-positive and vacA s1m1 type. Do you think that eradication rate in East Asian population is higher than that in H. pylori-infected patients in Ireland?

Response: In the absence of a recent meta-analysis evaluating eradication rates in the East Asian population, we are unable to directly and accurately compare our eradication rate with that in the East Asian population.

Q4. Ref 4 showed that the absence of cagA is a risk factor for developing metronidazole resistance. Why did not author check association with metronidazole resistance and virulence of H. pylori?

Response: We did not evaluate the association between metronidazole resistance and *H. pylori* virulence in the current study as the genotypic antibiotic resistance analysis was performed using DNA samples isolated from patient tissue biopsies and the GenoType HelicoDR assay, which detects clarithromycin- and fluoroquinolone-conferring mutations. To the best of our knowledge there are no molecular tests currently available commercially to reliably detect metronidazole resistance**.**

Q5. Fig1: Cag positive stain of H pylori may be difficult to become clarithromycin resistance strain. Is it true?

Response: Our findings suggest that CagA-positive strains are less likely to become clarithromycin resistant. However, we did not perform scientific experiments to specifically demonstrate that CagA-positive strains are less likely to become clarithromycin resistant in the current study. Experiments to screen our CagA-positive strains grown in the presence of clarithromycin and subsequently monitor for clarithromycin resistance would address this hypothesis and will form the basis of our future studies.

Q6. Please add data of history of clarithromycin use, smoking and alcohol in Fig 1.

Response: We thank the reviewer for this suggestion. Unfortunately we did not routinely record information on history of clarithromycin use, smoking or alcohol use from the patients included in the study.

Q7. The data about histological and endoscopic findings in Table 2 is not accurate. Please re-evaluate according to the up-dated Sydney system or OLGA grading system.

Response: The data in Table 2 has been recorded from the patient endoscopy and histology reports available. It is not currently routine practice in the Irish healthcare setting to use the OLGA or up-dated Sydney grading system for histology, as our gastric cancer risk is low (as evidenced in Table 2).

Q8. Please divide data into total, Naïve and Previously in Table 2.

Response: The data in Table 2 has now been sub-grouped into overall, treatment-naïve and previously treated and highlighted in red in the attached manuscript.

Q9. What statistical analysis method did author perform in Table 3 to 6? Because it is expected that the statistical methods will be different, you should check with statisticians.

Response: The Fisher exact test was performed in Tables 3 to 6 by the first author on the paper who has been awarded a post-graduate certificate in statistics from Trinity College Dublin (2015). The statistical test is now indicated in the footnotes for Tables 3-6 and in the results section.

References

1. Sugimoto M, Yamaoka Y. Virulence factor genotypes of Helicobacter pylori affect cure rates of eradication therapy. Archivum immunologiae et therapiae experimentalis. 2009 Jan-Feb;57(1):45-56.
2. Khan A, Farooqui A, Manzoor H, Akhtar SS, Quraishy MS, Kazmi SU. Antibiotic resistance and cagA gene correlation: a looming crisis of Helicobacter pylori. World journal of gastroenterology : WJG. 2012 May 14;18(18):2245-52.