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Helicobacter pylori virulence genes

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Response to the Editor

Ad1. Comment: Language certificate: For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies.

Ad1. Authors' response: Language certificate by professional English language editor has been provided as requested.

Ad2. Comment: Please offer the audio core tip.

Ad2. Authors' response: Audio core tip has been recorded and added.

Ad3. Comment: 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.

Ad3. Authors' response: The manuscript has been thoroughly checked and acronyms have been updated/corrected.

Ad5. Comment: 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.

Ad5. Authors' response: The titles of the article series have been corrected accordingly.

Ad6. Comment: Please check and confirm that there are no repeated references!

Ad6. Authors' response: All references have been thoroughly checked to confirm there are no repeated references.

Ad7. Comment: Please add PubMed citation numbers (PMID NOT PMCID) and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

Ad7. Authors' response: DOI citations were added where applicable. However, we were unable to identify DOI numbers for references 70, 73 and 77. Moreover, because we cited two additional studies, we updated the reference list accordingly. All references were checked to include PMID and not PMCID numbers. All references have all authors listed.

Response to reviewers

Reviewer#1

Ad1. Comment: The study is a excellent summary of various H. Pylori virulence genes and their role in pathogenesis. The text structure is very good and easy read. The limit of this work is the few population date available to evaluate the complex relationships among host genetics, the environment factors and the presence, as well as combinations, of various H. pylori virulence genes. The future directions are extend the investigations which could have a high impact clinical practice.

Ad1. Authors' response: We would like to thank the reviewer for the kind comment. The authors agree that the importance of *H. pylori* virulence genes and their intricate relationships should be studied in more detail with more patients included to provide better understanding of the pathogenesis which could eventually impact clinical practice.

Reviewer#2

Ad1. Comment: It might be better to divided into more subtitles such as: bacterial adhesins and toxins, children and adults, with their own prevalence, clinical significance, and potential relationships.

Ad1. Authors' response: In accordance with the reviewer's comment we added additional subtitles: Adults, Children, Associations with other virulence genes, and Comment. We were unable to include more subtitles as the journal's instructions for authors allow three levels of subtitles only.

Ad2. Comment: What is the difference of other genes with the previous groups? It should be explained.

Ad2. Authors' response: *dupA* is presented separately as it cannot be categorized as either outer membrane protein or toxin, because its exact functions have not yet been fully explained. The title of the paragraph has been changed into "*Virulence genes with other functions"* to avoid confusion.

Ad3. Comment: It is better to summarize the characteristics of genes of each group in a table for children and adults, separately.

Ad3. Authors' response: Although we agree with the reviewer that presenting the data on children and adults separately might be more transparent, unfortunately some important studies included both populations which were not clearly separated (e.g. Kang et al evaluated 260 children and adults and the prevalence of *homB* genes was estimated for both populations jointly). Thus data would be difficult to present otherwise, resulting in duplication of the studies listed in the two tables.

Ad4. Comment: Were the combinations include all available combinations?

Ad4. Authors' response: The authors reviewed a significant amount of available research data on combinations of *H. pylori* virulence genes; however, we were not able to include all possible combinations in our manuscript due to the journal's prescribed word limit. Consequently we provided a general overview and highlighted the most interesting and clinically perspective *H. pylori* virulence gene combinations. We agree with the reviewer that this topic is of special interest and should be summarized in a review article that would focus only on these relationships. Following reviewer's comment, the following statement was added to the text: *"Here, we briefly summarize some of the most intriguing combinations of H. pylori virulence genes."* (Page 22, Combinations of virulence genes, first paragraph)

Reviewer#3

Ad1. Comment: The manuscript meets the criteria of the new review article in the field of Helicobacter pylori virulence genes. However, some issues should be extended on the basis of the latest literature. Jones MD, Li Y, Zamble DB. Acid-responsive activity of the Helicobacter pylori metalloregulator NikR. Proc Natl Acad Sci USA. 2018, 115:8966-8971.

Ad1. Authors' response: We appreciate the reviewer's suggestion to include the above mentioned article; however, although HpNikR appears to be crucial for homeostasis of nickel and is also involved in regulation of gene expression in direct response to acidic pH, we (and others) believe that HpNikR does not meet criteria as virulence gene yet. Hence, we decided not to include it in our review (similar to *ureA*).

Ad2. Comment: Sallas ML, Dos Santos MB, Orcini WA, David EB, Peruquetti RL, Payao SLM, Rasmussen LT. Status 9on/off) of oipA gene: their association with gastritis and gastric cancer and geographic origins. Arch Microbiol 2019, 201:93-97.

Ad2. Authors' response: We appreciate the reviewer's suggestion to include the above mentioned article in revised manuscript. The article has been thoroughly read by the authors, however, we unfortunately cannot include it in revised manuscript due to the journal's prescribed word limit, insufficient description of study population and very limited number of gastric cancer cases evaluated.

Ad3. Comment: Keel M et al., Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from southern region, Saudi Arabia: distribution and association with clinical outcomes and histopatological changes. BMC Gastroenterol 2019, 19: 16.

Ad3. Authors' response: In accordance with the reviewer's suggestion, the following sentences have been added in revised manuscript: "*In the Middle East, cagA is detected in nearly half of the strains[61].*" (Page 14, second paragraph) and "*Interestingly, mixed vacA s1a/s1b/m2 was found to be the most common genotype in Saudi Arabia[61].*" (Page 18, second paragraph)

Ad4. Comment: Sarma A, Saikia L, Gogoi M, Yadav K. Molecular characterization of virulent gene vacA in Helicobacter pylori clinical isolates from patients with gastroduodenal disease in Assam, India. Indian J Med Microbiol 2018, 36:178-185.

Ad4. Authors' response: We appreciate the reviewer's suggestion to include the above mentioned article. The article has been thoroughly read by the authors, however, we unfortunately cannot include it in revised manuscript due to the journal's prescribed word limit and insufficient number of *H. pylori* isolates analyzed.

Ad5. Comment: Abdi E, Latifi-Navid S, Zahri S, Yazdanbod A, Safaralizadeh R. 2017. Helicobacter pylori genotypes determine risk of non-cardia gastric cancer and intestinal- or diffuse-type GC in Ardabil: A very high risk area in Northwestern Iran. Microb Pathog 2017, 107: 287-292.

Ad5. Authors' response: In accordance with the reviewer's suggestion, the following sentences have been added to the text: "*Interestingly, vacA i1 and d1 were shown to be significantly associated with non-cardia GC (OR = 37.52, 95% CI, 3.04-462.17 and OR = 7.17, 95%CI, 1.43-35.94, respectively), but not with cardia GC. The presence of these alleles may also predict the risk according to the GC type, as vacA i1 was linked to intestinal-type adenocarcinoma (OR = 14.04; 95% CI, 2.15-91.77) and vacA d1 to diffuse-type adenocarcinoma (OR = 7.71; 95% CI, 1.13-52.28)[84].*" (Page 18, third paragraph)