Reviewer's report

Title: Distribution of Helicobacter pylori virulence markers in patients with gastroduodenal diseases in Pakistan

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Reviewer: Peter Malfertheiner

Reviewer's report:

The manuscript describes the distribution of H. pylori-derived virulence factors in Pakistani patients with dyspeptic symptoms. The topic is interesting and extends the current knowledge to a country that has not been extensively studied so far, but there are major and minor points that need to be addressed before the authors should resubmit the manuscript or submit somewhere else.

Major issues:

· The manuscript illustrates numerous combinations of virulence factors studied without highlighting any of their findings. The authors need to emphasize on 1-2 main issues instead of uncritically listing all data without main structure. This is in particular important for the abstract.

· Throughout the manuscript numerous associations (e.g. inflammation and presence of virulence factors) are reported without differentiating significance levels. Only significant associations should be presented, all other should not be demonstrated. If there is a trend (e.g. p<0.1), the trend should be clearly separated from significant results.

· Histological assessment was performed by the updated Sydney classification, but no scores are presented for the different diseases. Table 3 remains unclear. What are the definitions for inflammation? Since all patients were H. pylori-positive, a positive histology with respect the activity, chronicity should be present in all of them, for correlations towards virulence factors, the histological data need to be presented as scores and evaluated in their diseases groups (NUD, GC, GU, and DU).

· Identify always statistical tests applied (in tables)

· Omit any redundancy in the result section CagA data are presented three-times, two times in the text and once in table 2. Refer in the text only to relevant findings in broader sense, without repeating all numbers presented in tables anyhow.

· The only publication dealing with a similar topic (H. pylori, CagA, VacA-genotypes in Pakistani dyspeptic patients (Ahmad et al. 2008) is not included. Data of the manuscript need to be discussed in context to this study. Furthermore, different genotypes of VacA should be analyzed similarly to allow direct comparison.

· VacA-related data should be completely demonstrated as whole genotype like vacAs1am1, omit the presentation of frequencies of single VacA parts like
VacAm1 or VacAm2 etc.

- As mentioned by the authors, data of the sequence analysis of limited value and confirm only the correctness of PCR primers. These data should be stated shortly without further discussion of the mutations identified, since the low number of sequences does not allow any conclusion.

Minor issues:
- Table 1 contains primer S2, but no related data are demonstrated in the manuscript.
- The associated VacA alleles vacAs1am1 and VacAs1bm1 (discussion line 5) represent only 43% of all subjects. Which allelic types make up the other 57%?
- Correct format issues in table 2 (missing parenthesis, different letter size)

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests.