

Reply to Peer Reviewers

Reviewer 1

Is the authors searched the Cochrane Database of Systematic Reviews for the novelty of their systemic reviews and why there is no registration number from databases dealing with systematic reviews as PRSPERO.

REPLY: Thank you for the comment. With the addition of a new study investigator, we have now searched the Cochrane Database of Systematic Reviews and cross-checked our search results, however, this did not find new studies or alter our study findings. Unfortunately, there is no registration number as we did not register our protocol prospectively. We apologise for this.

In addition, where is PICO statement, it should be written clearly to be obvious in the manuscript? Also, the research question should be included in the Methods section and finally what is the bias tool used in evaluating the selected studies.

REPLY: Thank you for the comment. The PICO statement is not applicable in this case as this is a meta-analysis of observational studies (i.e. there is no active intervention being compared), “the primary outcome measure of interest was the proportion of *H. pylori* infection in patients with IBS compared to a control group.” We have only analysed for publication bias using the funnel plot and Egger test. Unfortunately, there is no suitable or widely accepted tool to evaluate the bias of individual studies as these are mainly case-control or cross-sectional studies. The Cochrane risk-of-bias tool applies for randomized controlled trials.

Reviewer 2

In this meta-analysis the Authors aimed to study the putative association between *H pylori* infection and IBS. Overall, the manuscript is well written and sufficiently novel. The title is appropriate, although I wonder if it's a possible “disease association” or a matter of cause effect relationship between *H pylori* and IBS! I have the following comments/suggestions for the Authors:

1. In the abstract, the methods and results should be expanded and detailed.

REPLY: Thank you for the positive comments. We, the investigators, have enjoyed the process of researching this area of study. It is difficult to prove causation using only cross-section or case control studies hence we have decided to title it as a possible “disease association” rather than a matter of cause-effect relationship. We have expanded and added additional details to the abstract, methods and results sections. Hope all is in order.

2. There is some concern that the small number of studies included in the meta-analysis and there remarkable heterogeneity may make it difficult to infer valid conclusions.

REPLY: We agree with the reviewer and acknowledged in our discussion of study limitations that the small number of studies included in the meta-analysis and heterogeneity may make it difficult to infer valid conclusions.

3. In the discussion section, the Authors should expand on the putative effect of *H pylori* eradication therapy of IBS symptoms.

REPLY: Thank you for the comment. We added that, “Studies that investigated the effect of *H. pylori* eradication therapy on IBS symptoms also found no significant differences at follow-up [11,

21]" and "The role of *H. pylori* eradication therapy is also unclear as it does not appear to improve IBS symptoms in the limited studies available."

Reviewer 3

The topic of meta-analysis is quite relevant for two reasons. First, the extragastric manifestations of *Helicobacter pylori* infection are being actively investigated. Secondly, recently, the study of the microbiome in IBS patients and the probable association of *Helicobacter pylori* infection with gastrointestinal microbiomes has been rapidly progressing. Authors of the publication would be useful to briefly discuss these aspects of the problem. This can be done in the Discussion section. It also seems appropriate in the Introduction section to present a specific possible pathogenetic model of the association of *Helicobacter pylori* infection with irritable bowel syndrome. After these these minor improvements, the article can be published.

REPLY: Thank you for the positive feedback. We have added in the introduction that, "*H. pylori*, especially strains that produce CagA (cytotoxin-associated gene A) protein, causes chronic inflammation in the stomach and duodenum, microbial dysbiosis [7] as well as elevated systemic inflammation [8]." Hope all is in order.

Reviewer 4

1. In the literature, the association between *H. pylori* infection and functional dyspepsia (FD) had been widely investigated and discussed but no consistent information was achieved so far. Two studies included in this meta-analysis article [references 13 & 14] evaluating patients with *H. pylori* infection in patients with dyspepsia with or without IBS which seems to have deviated from the theme of this manuscript.

REPLY: The two studies (references 13 and 14) did examine specific subsamples, those who have dyspepsia vs those with IBS vs those symptomless. The ROME criteria classifies IBS as a distinctly separate functional bowel disorder from dyspepsia. IBS is characterized by lower abdominal pain or discomfort in association with disordered defecation. Dyspepsia presents as recurrent upper abdominal pain or discomfort associated with symptoms of early satiety, fullness, bloating, and nausea. Because upper GI function regularly affects lower GI tract function (i.e., the gastro-colic reflex), and lower GI function routinely affects upper GI function (i.e., constipation slows gastric emptying), it should not be surprising that these 2 areas are intimately linked. However, this is outside of the scope of the current paper.

2. Following the first question, the putative role of *H. pylori* infection in patients with 'overlap syndrome with IBS and FD' deserves further address in the manuscript.

REPLY: This has been briefly addressed in the manuscript, "*H. pylori* infection has been linked to several conditions, including dyspepsia". Patients with 'overlap syndrome with IBS and FD' is outside of the scope of the current research.

3. There are many studies investigating gut microbiota and its association with IBS and loads of evidence indicates dysbiosis might be a hallmark of IBS, and *H. pylori* infection affect mainly the upper gastrointestinal (GI) tract instead of lower GI tract, the authors need to address their hypothesis in linking *H. pylori* infection and IBS robustly.

REPLY: Thank you for the comment. “In considering the possible pathogenic mechanisms of *H. pylori* in relation to IBS, *H. pylori* infection has been associated with elevated inflammatory markers [8], increased mast cell activation [28] and gastric mucosal and neural remodeling [29]. Vacuolating cytotoxin A (VacA) [30] and the neutrophil-activating protein (HP-NAP) [28] of *H. pylori* are both potent mast cell stimulators. Although a definite and reproducible pattern of immune response has yet to be recognized in patients with IBS, increased mast cell activation and immune activity in the gut may correlate with symptoms of visceral hypersensitivity [31].”

We agree with the reviewer that the hypothesis linking *H. pylori* infection and IBS remains controversial. We have also added the comment in our discussion section that, “Last but not least, the influence of *H. pylori* on the composition of distal gut microbiota is also an important area that deserves further study. Microbial dysbiosis is a known hallmark of IBS [41]. However, it is unclear how *H. pylori*, which is thought to affect mainly the upper GI tract, may affect the lower GI tract [42].”