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Basic Study

A combined antrum and corpus biopsy protocol improves *Helicobacter pylori* culture success.

Brennan DE *et al.* Improving *H. pylori* culture success

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RESPONSE TO REVIEWERS

Dear Editor-in-Chief Lian-Sheng Ma,

Many thanks for your kind invitation to submit our revised manuscript to the *World Journal of Gastrointestinal Pathophysiology*. We thank the reviewers and editors for their time and attention in reviewing our manuscript and for their valuable feedback. Please find below our responses. The corresponding changes in the revised manuscript are highlighted in red.

REVIEWER #1:

Reviewer 1, Point 1

The authors tried to explore the success rate of combined gastric antrum and corpus biopsy protocol biopsy to improve *H. pylori* culture. They inoculated gastric antrum and corpus biopsies to the same one Columbia blood AGAR plate and compared them with a single antrum biopsy. It is interesting and important study. However, I have major reservations in recommending it for publishing in the present form.

The experiment was divided into two groups, the gastric antrum and corpus combined biopsy group and the single antrum biopsy group. But the two groups did not do it simultaneously. The former was a prospective study, while the latter was a retrospective analysis. Such a design may result in bias due to the heterogeneity of samples, cultures, conditions, etc. and the credibility of the results is greatly reduced.

Response to Reviewer 1, Point 1

We thank Reviewer 1 for their time and dedication in reviewing our manuscript and we are pleased that they consider the study "interesting and important".

The authors acknowledge the Reviewer's point that the patients from the 2 study groups were not recruited simultaneously. Initially at our centre, single antrum biopsy samples were collected for *H. pylori* culture. The resulting low culture success rate led to a change in practice to determine whether using the combined corpus and antrum biopsy protocol would improve success rates. As a result, the comparison involves analysis of combined samples that were collected prospectively with single biopsy samples that were collected retrospectively. This limitation is now included in the "Discussion" of the revised manuscript. However, it should be noted that for the entire duration of the patient recruitment and sample collection phases of the study, we followed the standardized culture protocols of the European *Helicobacter pylori* Antimicrobial Susceptibility Testing Working Group, of which we have been members since 2008 (1, 2). Therefore, the sample transport protocols, microbiological media and culture conditions and methods were consistent throughout the entirety of the study, thereby limiting heterogeneity in this regard.

Reviewer 1, Point 2

The colonization of *H. pylori* in gastric mucosa epithelium is mostly focal distribution, and the success rate of *H. pylori* culture between one biopsy and two biopsies must

be different. Therefore, if the author wants to prove that the co-culture of gastric antrum and corpus biopsies were superior to the single culture of gastric antrum biopsy, the third group should be set, that is, the co-culture group of any two gastric antrum biopsies. Otherwise, the results are hardly convincing.

Response to Reviewer 1, Point 2

We agree with Reviewer 1 that it is not surprising that the collection of 2 biopsies rather than one yielded a higher culture success rate (mentioned in the "Discussion") and this was our study hypothesis. The reason we chose a combined corpus and antrum approach for the dual biopsy sampling protocol (and not 2 antrum biopsies) was because others have shown differences in the antimicrobial resistance profiles of *H. pylori* isolated from the corpus and antrum of the same patients (3, 4). Additionally, collecting biopsies from both the antrum and the corpus takes into account patchy distribution of *H. pylori* in the stomach, which can occur with proton pump inhibitor use (5-7). These points are included in the "Discussion" of our revised manuscript.

Reviewer 1, Point 3

In the last row of Table 1, there is no data for 24 cases of gastric disease, which is not suitable for prospective study or retrospective case analysis. Generally, in clinical studies, patients with incomplete data should not be enrolled in order to avoid statistical bias.

Response to Reviewer 1, Point 3

The primary outcome of the study was culture success rates in *H. pylori*-infected patients. All patients included in the study were diagnosed with *H. pylori* infection by the rapid urease test (please see the inclusion criteria in the Methods section). As such, we consider the statistical analysis between the 2 groups valid and to increase

our sample size for the culture success analysis, have included samples from the 24 *H. pylori*-positive patients whose endoscopy findings were unknown.

REVIEWER #2:

***Reviewer 2, point** 1*

The claimed MS novelty is that combined antrum and corpus biopsy improves *Helicobacter pylori* cultivation rate and could be useful for the determination of antibiotic resistance. They used urease positive biopsy samples from the patient with different digestion problems for the cultivation of *H. pylori* and found out that combined corpus and antrum biopsy sampling protocol improves *H. pylori* culture success. I am sorry, but I am not able to find any novelty. Sampling from different sites is routinely used to determine resistance in order to increase cultivation success.

Response to Reviewer 2

We thank Reviewer 2 for their time in reviewing the manuscript and for their feedback. Owing to the fastidious nature of *H. pylori*, culture is difficult to perform and not routinely performed at the majority of hospitals as indicated in references (8-10). Moreover, the recent clinical guidelines on the management of *H. pylori* (8, 10-13) do not include recommendations on the specific biopsy sampling protocols for *H. pylori* culture. As such, it is highly likely that biopsy sampling among gastroenterologists and endoscopists, specifically for *H. pylori* culture, is heterogenous. While it has been suggested that the more biopsy specimens used for culture, the higher the chance of recovering *H. pylori* (6), data directly evaluating sampling protocols to improve culture success are indeed lacking. We found one paper from 1989 (Bayerdorffer *et al*. 1989 (14)), which reported that 5 of 10 stomach biopsy samples taken were necessary to detect *H. pylori* with a 95% confidence using culture. We have now included this reference in our revised paper.

Reviewer 2, point 2

It would recommend checking the current state of art in the future before writing the article. "However, studies directly evaluating culture success when different numbers of biopsy samples have been collected are lacking." See e.g. Selgrad M, Tammer I, Langner C, et al. Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of Helicobacter pylori infection. World J Gastroenterol 2014; 20: 16245–51. Kim JJ, Kim JG, Kwon DH. Mixed-infection of antibiotic susceptible and resistant Helicobacter pylori isolates in a single patient and underestimation of antimicrobial susceptibility testing. Helicobacter 2003;8:202–6. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev 2007;20:280e322. In addition, the MS mixes peas, and carrots as the results of the primary identification are interpreted as the state after treatment with antibiotics. I propose to reject MS. I can't even imagine how to improve this paper. Perhaps they can try to publish it as a regional study. Such pitfalls should be identified by the primary editors.

Response to Reviewer 2, point 2.

The 3 papers listed by Reviewer 2 were included in the Discussion of the original manuscript. Below and in the revised discussion, we provide a more detailed description of the data presented in these papers to highlight our point that studies directly evaluating *H. pylori* culture success rates when a single antrum biopsy vs a combined corpus and antrum approach are indeed lacking.

In the paper by Selgrad *et al*. 2014 (3), antimicrobial susceptibility testing was performed on strains isolated from antrum and corpus biopsies from 66 patients. Discordant antibiotic susceptibility between the antrum and corpus isolates was seen in 15.2% (10/66) of the patients. The authors did not evaluate culture success rates in their study or compare culture success when a single antrum vs both antrum and corpus biopsy samples were used.

In the paper by Kim *et al*. 2003 (4), the authors analysed 220 pairs of *H. pylori* isolates that had been obtained from both the antrum and the corpus of each patient. 50% (109/220) of patients harboured antibiotic-resistant *H. pylori,* of which hetero-resistance among the 2 biopsy sites from each patient was present in 38% of cases (41/109). The authors conclude that neither single site can be considered representative for reliable antimicrobial susceptibility testing. The authors did not evaluate culture success rates in their study or compare culture success when a single antrum vs both antrum and corpus biopsy samples were used.

In the paper by Megraud *et al.* 2007 (6), the authors discuss the patchy distribution of *H. pylori* and suggest that the more biopsy specimens analysed, the greater the chance of *H. pylori* detection based on the study by Bayerdorffer *et al*. 1989 (14) (mentioned in Response to Reviewer 2, point 1 above). The authors did not evaluate culture success rates in their paper or compare culture success when a single antrum vs both antrum and corpus biopsy samples were used.

In summary, our data supports the finding that additional biopsies increase culture success and presents data directly evaluating culture success rates when a dual corpus antrum sampling method is used compared to a single antrum biopsy protocol.

SCIENCE EDITOR:

According to reviewer suggestion the manuscript is not appropriate for publication in World Journal of Gastroenterology.
Language Quality: Grade A (Priority publishing)
Scientific Quality: Grade D (Fair)

Response to Science Editor

Based on the recommendation of the Company editor-in-chief, we have revised the manuscript for submission to the *World Journal of Gastrointestinal Pathophysiology*.

COMPANY EDITOR-IN-CHIEF

I recommend the manuscript to be published in the *World Journal of Gastrointestinal Pathophysiology*.

Response to Company editor-in-chief

We thank the editor-in-chief for recommending publication of our article in the *World Journal of Gastrointestinal Pathophysiology*. Based on this recommendation, we have revised the manuscript for submission to the *World Journal of Gastrointestinal Pathophysiology*.

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