

Zurich, 25th of June 2019

Response to Reviewer comments

Reviewer 1

In recent years, an alarming increase in antimicrobial resistance and subsequently failing empiric H. pylori eradication therapies have been noted worldwide, therefore, rapid and accurate determination of H. pylori's antibiotic susceptibility prior to the administration of eradication regimens becomes ever more important. The costs for next-generation sequencing have consistently decreased over the last years with technology development. Considered next generation sequencing technologies may enable the implementation of genotypic DST prior to the administration of antimicrobial therapy for H.pylori eradication. I think this article titled "Advances in H. pylori diagnostics in the era of next-generation sequencing" is interesting and useful, but I think it is more perfect if the author spend more attention on studies using next generation sequencing for the characterization of H. pylori and treatment.

Thank you for your review of our manuscript. We have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

Reviewer 2

This work by Drs. Keller et al., provide a manuscript focusing on the advance in H. pylori diagnostics in the era of next generation sequencing, involving current treatment strategy, diagnostic, and future perspective. Authors searched related literature and try to present latest advance this area. Numerous efforts have been made to provide patients with fast, reliable, accurate diagnostics on Hp infection and post-treatment surveillance, therefore further efforts in these areas will be a welcome. This work is therefore interesting with merit. The manuscript appears well wrote, but is also not in line with its title/main focus on diagnostics, instead, there are a lot of discussion on treatment strategy and sequencing for the characterization of Hp strains. It is advised that authors narrow down or reframe the manuscript to focus on the main purpose and present advances and conclusions to guild the area moving forward in diagnostic, especially those promising future methods. Minor typos and lengthy sentences are found throughout the text, which deserve authors' attention. Many of the references are old, newer one might be more attractive. The work may require revision and summarize research advance in Hp diagnostics to guide future efforts in the field. Authors are encouraged to revise and make manuscript more concise and up-to-date.

Thank you for your review of our manuscript. We have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

Reviewer 3

First, what are the original findings of this manuscript? What are the new hypotheses that this study proposed? What are the new phenomena that were found through experiments in this study? What are the hypotheses that were confirmed through experiments in this study? Second, what are the quality and importance of this manuscript? What are the new findings of this study? What are the new concepts that this study proposes? What are the new methods that this study proposed? Do the conclusions appropriately summarize the data that this study provided? What are the unique insights that this study presented? What are the key problems in this field that this study has solved? Third, what are the limitations of the study and its findings? What are the future directions of the topic described in this manuscript? What are the questions/issues that remain to be solved? What are the questions that this study prompts for the authors to do next? How might this publication impact basic science and/or clinical practice?

Dear Reviewer- This is not an original paper, but a review.

Reviewer 4

- "In general, treatment is recommended in case of detection of H. pylori infection. Among others, the most recent German S2k guideline proposes treatment even in patients with asymptomatic H. pylori gastritis [17]" Cite a paper that refers not only to German, like a Megraud paper: "A 2016 panorama of Helicobacter pylori infection: key messages for clinicians." Panminerva Med. 2016 Dec;58(4):304-317.

This reference was added accordingly

Line 64 to 67: Consequently, it is a challenge for physicians to decide who should be tested for H. pylori infection and who should be treated. In general, treatment is recommended in case of detection of H. pylori infection, even in patients with asymptomatic H. pylori gastritis^[17, 18].

- Figure 1 is copied by Maastricht V consensus: did you have obtained the permission?

Figure 1 was removed as treatment regimens are explained in the text.

- "associated costs for antibiotic therapy are listed in Table 2 and Table 3) Give more details about the sources of the costs

Costs are approximate drug prices from Germany in Euro

This information was added in the review:

Line 238 to 241: Choice of third-line therapy should be guided by phenotypic drug susceptibility testing (DST) or genotypic determination of drug resistance (associated costs for antibiotic therapy are listed in Table 2 and Table 3; approximate drug prices from Germany).

Line 397 to 403: When considering the current costs for H. pylori eradication regimens (Table 2 and Table 3; approximate drug prices from Germany), depending on local resistance rates, initial molecular determination of H. pylori drug susceptibility may be cost efficient, especially, when considering that costs for real-time PCR assays (<20 EUR) and WGS (<100 EUR) have consistently decreased over the last years.

- "Advances in H. pylori diagnostics in the era of next generation sequencing" The title refers to only a part of the review, choose a more comprehensive title

The title was changed based on your and other reviewer comments, and we hope that you find it improved.

Review of current diagnostic methods and advances in H. pylori diagnostics in the era of next generation sequencing

- "The "gold standard method" for H. pylori detection is considered endoscopy in combination with histology and/or culture from the gastric biopsy." 13CUBT is the most accurate tool to detect H. pylori infection and is more cost-effective than endoscopy ("A 2016 panorama of Helicobacter pylori infection: key messages for clinicians." Panminerva Med. 2016 Dec;58(4):304-317.)

We adapted this sentence.

Line 139 to 140: For H. pylori detection, endoscopy is employed in combination with histology and/or culture from the gastric biopsy specimen.

- "However, it is not recommended to do a full phenotypic DST before administration of first-line treatment as i) an invasive endoscopy is required to obtain gastric biopsy specimens from the patient, ii) it is time consuming and costly, iii) less invasive, molecular based methods are also able to detect clarithromycin resistance that is momentarily the main cause of empiric treatment failure." Add the lack of availability - The next generation sequencing for the characterization of H. pylori should be the focus of the paper, instead there is a very long part dedicated to other aspects and few lines about next generation sequencing for the characterization of H. pylori: change the title and extend in the text the analysis of the papers searched on PubMed in this regard.

We have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

Reviewer 5

I would like to mention the following comments:

1- Running title is missing.

Running title was added accordingly.

Running title: Review of advances in H. pylori diagnostics

2- It might be better to rewrite text: First Current: epidemiology information and resistance, diagnosis methods, treatments, and their advantages/disadvantages then available alternative methods and comparison with current methods. differentiation of treatment and chemical prophylaxis, Future: projection of future rates, diagnosis, treatments and their advantages/disadvantages.

We have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

The new subheadings are:

HELICOBACTER PYLORI PREVALENCE, EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE

Current Helicobacter pylori prevalence and epidemiology

Management of Helicobacter pylori infections

Antibiotic resistance in Helicobacter pylori

CURRENT HELICOBACTER PYLORI DIAGNOSTICS

HELICOBACTER PYLORI TREATMENT AND PROPHYLAXIS

NEXT GENERATION SEQUENCING AND HELICOBACTER PYLORI

CONCLUSION AND POTENTIAL FUTURE DIRECTIONS

3- Starting from line 306: What is [tw]?

This abbreviation was deleted according to comments from reviewer 6.

4- Tables 2 and 3: PPI needs to be defined.

PPI was defined accordingly in Table 2 and Table 3.

5- Table 3: four x daily: x means times?

For clarity, we changed "x" to "times" in Table 2 and Table 3.

6- Inclusion and exclusion criteria for search are missing.

We added the inclusion and exclusion criteria accordingly.

Line 293 to 297: Inclusion criteria: i) original research manuscripts ii) characterization of clinical human H. pylori isolates iii) use of second and/or third generation sequencing technologies. Exclusion criteria: i) Reviews, case reports, comments, letters, ii) characterization of non-human H. pylori isolates iii) original research manuscripts that that did not use second or third generation sequencing technology.

7- Why only PubMed has been used and no other databases?

We included a literature search in MEDLINE and EMBASE and added the information in Table 4, Table 5 and Table 6. Moreover, we have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

Reviewer 6

To Authors, The article titled "Advances in H. pylori diagnostics in the era of next-generation sequencing" was reviewed. There are some minor mistakes needed correction in the text. Except these corrections, this article has been written clearly about the importance of H.pylori and eradication. After corrections, this article deserves to be published.

1. In the 172. Line = "rapport" should be corrected as "REPORT"

This was changed accordingly.

2. Patoprazol (PPI) = It should be corrected as " PANTOPRAZOL " in Table 3.

This was changed accordingly.

3. [tw] abbreviations should be canceled in whole text and Tables 4,5 and 6.

This abbreviation was deleted.

Reviewer 7

In this article: Advances in H. pylori diagnostics in the era of next generation sequencing the authors reviewed the gaps between current diagnostic practice (histology, rapid urease test, H. pylori culture, PCR and line probe assays) and new sequencing technologies and their potential implementation in routine clinical laboratory settings in order to complement the currently recommended H. pylori management guidelines and subsequently improve public health. In the first part they reviewed currently available diagnostic methods for detection of H. pylori and its drug resistance and their implementation in H. pylori management. In the second part they focused on the use of next generation sequencing in H. pylori research.

Comments:

1. This article is very well conceived, designed and executed. It provides the state-of-the-art information.

Thank you, we tried to give an overview of currently available diagnostic methods for H. pylori detection and identification of antibiotic resistance. Moreover, we aimed to show which methods that are currently used in basic research could be potentially applied in H. pylori diagnostics in the future.

2. Figures are very informative and clearly state objectives and main findings.

Thank you for the kind comment. We aimed to provide a concise summary of research studies using next generation sequencing for the characterization of H. pylori.

We have deleted Figure 1 based on comments from Reviewer 4 and added information in Table 4, Table 5 and Table 6 based on comments from reviewer 5.

3. Cor tips are missing

Core tip was uploaded in the WJG submission portal. It is pasted below:

With worldwide increasing antibiotic resistance in Helicobacter pylori, drug resistance phenotypes should be determined prior to the administration of antibiotic eradication regimens. Our literature search yielded studies that focused on the prediction of drug resistance phenotypes in H. pylori based on the presence of certain point mutations in the bacterium's genome using next generation sequencing (NGS) technology. Thus, NGS technology may enable the implementation of rapid and accurate genotypic drug susceptibility testing prior to the administration of antimicrobial therapy. This may increase H. pylori eradication rates and ultimately improve patient management.

4. The paper may benefit from adding 3 references listed below 1

1. Shao Y et al. Antibiotic resistance of Helicobacter pylori to 16 antibiotics in clinical patients. J Clin Lab Anal. 2018 May;32(4):e22339.
2. Fallone CA, Moss SF, Malfertheiner P. Reconciliation of Recent Helicobacter pylori Treatment Guidelines in a Time of Increasing Resistance to Antibiotics. Gastroenterology 2019, Apr 15. pii: S0016-5085(19)35704-X.
3. Zanotti G, Cendron L. Structural Aspects of Helicobacter pylori Antibiotic Resistance. Adv Exp Med Biol. 2019 Apr 24. doi: 10.1007/5584_2019_368.

The three references were added accordingly.

Reviewer 8

To the authors, I read with interest your review, although as clinician I wonder when the third generation sequencing technologies for characterization of H pylori will become available in clinical practice! I have only a few comments:

1. Please, follow WJG instructions and complete the submission:

-key words are lacking

Key words were added accordingly.

Keywords: Helicobacter pylori; advances in diagnostics; next generation sequencing; whole genome sequencing; clinical management

-Core-tip is lacking

Core tip was uploaded in the WJG submission portal. It is pasted below:

With worldwide increasing antibiotic resistance in Helicobacter pylori, drug resistance phenotypes should be determined prior to the administration of antibiotic eradication regimens. Our literature search yielded studies that focused on the prediction of drug resistance phenotypes in H. pylori based on the presence of certain point mutations in the bacterium's genome using next generation sequencing (NGS) technology. Thus, NGS technology may enable the implementation of rapid and accurate genotypic drug susceptibility testing prior to the administration of antimicrobial therapy. This may increase H. pylori eradication rates and ultimately improve patient management.

-take care of style of journal references.

References were manually edited to meet the criteria for the WJG reference style.

3. Please, consider altering the title to reflect that it is a review.

*The title was changed based on your and other reviewer comments, and we hope that you find it improved.
Review of current diagnostic methods and advances in H. pylori diagnostics in the era of next generation sequencing*

4. There are too many sub-headings, which is not typically for most review article. Please, consider changing that to a more conventional style.

We have rewritten large parts of the paper based on your and other reviewer comments and hope you find it improved.

The new subheadings are:

HELICOBACTER PYLORI PREVALENCE, EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE

Current Helicobacter pylori prevalence and epidemiology

Management of Helicobacter pylori infections

Antibiotic resistance in Helicobacter pylori

CURRENT HELICOBACTER PYLORI DIAGNOSTICS

HELICOBACTER PYLORI TREATMENT AND PROPHYLAXIS

NEXT GENERATION SEQUENCING AND HELICOBACTER PYLORI

CONCLUSION AND POTENTIAL FUTURE DIRECTIONS