

ANSWERING REVIEWERS

Reviewer: Masoud Amiri

Number ID: 00717554

Dear reviewer,

First of all, we are grateful for the very important comments, which were crucial for the improvement of the manuscript. The changes made according to your advice are described below:

- 1- Unfortunately, we did not find any article that shows the effect of refrigerator in the declining of *H. pylori* prevalence. Instead, the articles demonstrate that the availability of food refrigeration declined the prevalence of gastric cancer mainly due to the reduction of salt ingestion.
- 2- The “socioeconomic level”, written in the article, was a typo. We removed that.
- 3- We added, in the manuscript, that the increasing of the socioeconomic status would be the most important method for declining *H. pylori* prevalence, as you suggested.

Best Regards,

Fabício Freire de Melo,

Professor, PhD

Reviewer: Anonymous

Number ID: 00717554

Dear Reviewer,

Thank you very much for the comments you made on our manuscript. They were extremely important in improving the quality of the manuscript. The changes are described below:

- 1- We changed the text title (“From pathogenesis to clinical management: an overview about *Helicobacter pylori* gastric infection”) for a better one: “Pathogenesis and clinical management of *Helicobacter pylori* gastric infection”.
- 2- We added more specific keywords: *Helicobacter pylori*, Virulence Factors, Immune Response, Antibiotics, Vaccines
- 3- We reviewed the English errors, including “respectives” in the abstract, which was substituted by “related”.
- 4- We uniformed the color of the text.
- 5- We adjusted the referencing according journal style.
- 6- The English errors were corrected.
- 7- We added a reference to the text excerpt: “In this way, proper clinical management with a well-made diagnosis followed by effective treatment are important steps in the improvement of the patients’ clinical outcome.” - Abadi AT, Kusters JG. Management of *Helicobacter pylori* infections. BMC Gastroenterol. 2016 Aug 12;16(1):94. doi: 10.1186/s12876-016-0496-2.
- 8- We added more details to the following part of the text, as you suggested:

In addition, some transition metals are essential for living organisms, as they are present as cofactors of enzymatic reactions and some physiological processes, being involved in enzymes that carry out the genetic material replication and transcription, attenuation of oxidative stress, and cellular energy production. In bacteria, these metals are crucial for survival and successful infection^[33]. Nickel is an indispensable metal for *H. pylori*, since it is the cofactor for two important enzymes: urease and hydrogenase. These enzymes have a strong role in the infection process^[10]. The activity of *H. pylori* urease contributes to the colonization of the microorganism, once this enzyme catalyzes the hydrolysis of urea to carbon dioxide and ammonia which are buffer substances, attenuating the acidity of stomach environment^[34]. In turn, hydrogenase is part of a signaling cascade that induces an alternative airway, allowing *H. pylori* to use molecular hydrogen as a source of energy for its metabolism^[35].

9- We draw a table with the known adhesions contributing for the colonization:

Table 1. *Helicobacter pylori* adhesion molecules

<u>Adhesin</u>	<u>Functions</u>	<u>References</u>
<u>BabA</u>	Specific binding to the b and H-1 Lewis antigens from the surface of the gastric epithelial cells.	[106]
<u>SabA</u>	Binding to Le ^x , which is upregulated in gastric epithelial cells by <i>H. pylori</i> after initial colonization mediated by <u>BabA</u> . Also allows the adherence of the bacterium to laminin, an extracellular matrix protein.	[107, 108]
<u>AlpA</u> and <u>AlpB</u>	Mediation of adherence to gastric mucosal cells and promotion of inflammatory intracellular signaling cascades (might induce IL-8 and IL-6).	
<u>OipA</u>	Adhesion to the gastric mucosa cells and promotion of <u>proinflammatory</u> environment (associated with IL-8 increase, mucosal damage and duodenal ulcer).	[110]
<u>HopQ</u>	Interaction with CEACAM family proteins of gastric mucosal cells, allowing <u>CagA</u> translocation. Might inhibits the activity of Natural Killer (NK) cells and T Cells.	[111]
<u>HopZ</u>	Interaction with undetermined receptors, promoting adhesion to gastric cells.	[112]

10- Finally, in the following section, we added more details from recent published papers. A table showing what has claimed as prophylactic vaccine and what are both prophylactic and therapeutic vaccines was drawn.

3.3 Vaccines

The development of vaccines is a promising alternative that aims the prophylaxis and/or the treatment of the infection (Table 2)^[101]. Recently, studies have focused on the development of reverse vaccines with the help of bioinformatics, and five antigenic epitopes have been prioritized as potential vaccine candidates: babA, sabA, fecA, vacA and omp16.^[100] However, their development has been a major challenge in *H. pylori* field, since many studies have not been successful in experimental models. In contrast, in China, a randomized phase 3 study with children has been conducted, being efficacious and safe in providing oral vaccine with recombinant B urease against *H.*

pylori^[102], but a more accurate evaluation of its long-term effect is required. In another study by Wang and colleagues (2014)^[103], intramuscular administration was compared with oral administration of the multi-epitope vaccine, evidencing a better protection rate by oral administration. The development of nanovaccines is being explored as well, and presents a nice potential to become an excellent alternative in triggering an effective immunological response against *H. pylori* infection^[90].

Table 2. Preliminary effects of developing vaccines against *Helicobacter pylori* infection

Vaccine	Prophylactic	Therapeutic
<u>EpiVax</u> / <i>H. pylori</i> vaccine	Yes	Yes
<u>Helicovaxor</u> ®	Yes	No
<u>Imevax</u> /IMX101	Yes	No
Wuhu <u>Kangwei</u> Biological Technology	Yes	No

Best regards,

Fabício Freire de Melo,

Professor, PhD

Reviewer: Anonymous

Number ID: 00004011

Dear reviewer,

Your comments were very important for the manuscript improvement. We are grateful for them. We described below the changes made according your suggestions:

- 1- We added other pathologies related to HP infection:
“In addition, recent studies have associated *H. pylori* infection with a wide range of diseases. The infection was linked with the pathophysiology of neurological, dermatological, hematologic, cardiovascular, ocular, metabolic, hepatobiliary and allergic diseases^[81].”
- 2- We added a discussion about antibiotic resistance and hybrid therapy, as you suggested:
“In addition, the use of hybrid therapy have been suggested as an alternative to the standard approaches in some countries. This therapeutic scheme consists of administering proton pump inhibitor (PPI) and amoxicillin for 14 days, adding clarithromycin and a nitroimidazole as a quadruple therapy for the final 7 days^[94].”

Best regards,

Fabício Freire de Melo

Professor, PhD

Reviewer: Anonymous

Number ID: 00503587

Dear reviewer,

The comments you made on our manuscript were crucial for its improvement. An English/grammar review was performed and the errors were corrected. The title of the manuscript has also been changed to "Pathogenesis and clinical management of Helicobacter pylori gastric infection".

Best regards,

Fabício Freire de Melo

Professor, PhD

Reviewer: Enzo Ierardi

Number ID: 02535507

Dear Reviewer,

Your comments have been extremely helpful in improving this manuscript. We added more information to the text in order to make it more informative. We added new tables and a figure to provide more information in a less confused way. The except of the manuscript with the wrong information about the quadruple therapy with bismuth (a first line therapy) was corrected. A discussion about tailored therapy was also added, as suggested.

Best Regards,

Fabício Freire de Melo

Professor, PhD