Dear esteemed Editor and Reviewers

First, we would like to thank The Journal's Editor for his kind care and The Reviewers for their valuable comments which help us improve the quality of our manuscript.

We have revised the manuscript and made some changes in response to some comments of the reviewers (changes are highlighted in yellow color in the revised manuscript). However, we have our rebuttal to certain comments.

Authors' Response

To Reviewer 1:

- **Comment #1:**
  The title and abstract are appropriate for the content of the text. Furthermore, the article is well constructed, the experiments were well conducted, and analysis was well performed. Similarity is very high. Similarity is very high. It should be less than 20 %. If you paraphrase it again, it is appropriate to publish.

Response: Thanks to this comment. Considering similarity index, we have edited the manuscript and rephrase many paragraphs to reduce similarity to an accepted level. Using iThenticate program, the similarity index was reduced to 17% throughout the edited manuscript.

To Reviewer 2:

- **Comment #1:**
  1- Please add the antibiotic in start of the title

Response: We think that adding the antibiotics to the title would make the title to be very long. We believe that any title should be concise, informative and attractive. This study included susceptibility testing of *H. pylori* to a battery of antibiotics including: AMX, CLA, ciprofloxacin CIP, RIF and TET. Susceptibility testing of this pathogen to individual antibiotics seems crucial to better understand the resistance trends and traits among any pathogen and to check for MDR, XDR and PDR.

- **Comment #2 and 3:**
  2- The English is fine and I think it is durable. 3- Novelty of the data is fine and I think the clinicians in the Egypt can earn from it to have better susceptibility profile of anti-hp treatment.

Response: Thanks for these motivating comments.

- **Comment #4:**

  4- The main purpose of this study should be clearly stated in the introduction.

Response: The main purpose of the study is clearly written at the end of introduction; p: 5, lines: 107-110, besides, this aim was justified as in lines: 105-107.

- **Comment #5:**

  Reference 3 "Global Initiative for Cancer Registry Development. Lyon: International Agency for Research on Cancer; 2020. Available from: https://gicr.iarc.fr/about-the-gicr/the-value-of-cancer-data/. accessed February 2021" in introduction is quite wrong so I advise to add the proper reference which belongs to the 1994, etc.

Response: This reference was changed by a more relevant reference as in edited manuscript.

- **Comment #6:**

  5- I think there is no need to figure showing h. pylori among the samples from patients with gastritis and neutrophil. The proceeding fig is enough to show even the infiltration, so no need to it. Also please use the arrows to point the important keys in each figure, otherwise we have to dig up the whole picture.

Response: This figure (B) was deleted in the edited manuscript.

- **Comment #7:**

  6- I am not fine with this statement in the result section "In this study, eradication of H. pylori was obtained more frequently in patients with vacA s1 (P= 0.02), s2 (P = 0.03), or m1 (P = 0.01) positive strains." WHY? why should a certain part of patients be more resistant to antibiotic therapy while the profile of virulence genes are quite different? ? What a biologic rationale behind? This should be reflected in the discussion.

Response: This comment is representing the research question in this study. Although in many previous ecological and epidemiological studies, *vac*As1-positive *H. pylori* strains are usually more virulent and more closely associated with progressive gastroduodenal diseases [29]. Besides, the more virulent strains are usually more susceptible to antimicrobials because of faster replication, the findings of these reports remain controversial and inconsistent. This study focused to investigate this research point in our locality with very scarce data in our locality. Briefly, discussing this issue has been presented in discusion; pps: 18-19, lines: 343-364.

- **Comment #8:**

  7- Page 8 line 172, vag????????? something should be wrong please correct it.

Response: It was corrected; *vag*A, instead of *vag* A

- **Comment #9:**

  8- Although the authors reported the susceptibility tests according the E test but I feel they needed to have method as agar dilution as control, at least for certain number of isolates. Their report is a bit high and I afraid to think more about it. I would blame the method for reporting such high rate resistance rate in this research.

Response: We would like to mention that E test is an accurate susceptibility testing method for MIC determination approved by CLSI, EUCAST and other international performance guidelines. So, this method do not require another method as a control. In general, antimicrobial susceptibility testing is performed by detecting growth zone inhibition by disc diffusion method or detecting MIC by agar/tube dilution method, or E test method, or semiautomated equipment as VITEK, MicroScan, etc.. The MIC values determined by E test methods can be interpreted according to performance standard guidelines supported by CLSI, and EUCAST. So, The E test MIC values are approved to be valid in both routine laboratory work and in research studies.

- **Comment #10:**

  9- Authors got 4 biopsies, two antrum and 2 corpus, thereafter they went to conduct biopsy bacterial culture, Giemsa staining and urease test. I can not see an enough consistency among this type of biopsy sampling. They should have taken samples from lonely antrum or corpus. I assume that the authors have a good explanation for this type of sampling.

Response:

We agree with reviewer, regarding describing how biopsies were collected as it might be confusing. We have edited this paragraph regarding 2 sets of biopsies were collected. Regarding biopsies from antrum and corpus, we think that taking biopsies from 2 different anatomical sites seems significant to avoid mis-isolation of the pathogen in culture according to the international performance standards in microbiology laboratories.

- **Comment #11:**
  10- 100% resistance report for the hp isolates? Why? How? Also 52% for clarithromycin? I am wondering about the method to measure the susceptibility profile. Authors need to explain the methods and also a good explanation in their discussion to convince the readers finely.

Response: We agree with the Reviewer regarding the high antimicrobial resistance rates among H. pylori isolates. The continuous and sharp increase in antibiotic resistance all over the world would not be ignored. The world is now facing a global crisis of antimicrobial resistance with limited options to treat serious and fatal infections. This issue among H. pylori isolates have been raised in discussion and presented in p: 16-17; lines: 301-324. Briefly, the high resistance rate in our study could be explained by many factors presented in this study. However, the E test method results would not be implicated as it is a valid and accurate method (see response to comment#9).

- **Comment #12:**
  11- Page 10, line 216, "H plyori" please fix the mistake throughout the paper.

Response: Correction has been done throughout the paper.

- **Comment #13:**
  12- Table 1, where is the sequence for this primer? 13- Why authors have re-cultured the bacteria for 72 hours? "Each H. pylori isolate was sub-cultured and incubated for 72 hours" I am unclear about it.

Response: The sequence of all primers used in this study is presented in table 1. Considering culture of H. pylori, it is well known that this pathogen is a slow-grower and needs certain growth conditions for better isolation and identification. Therefore, H. pylori is better to be incubated in culture for 72 hours (instead for 24 hours in other

rapidly growers) as per the standard bacteriological techniques and performance standard guidelines by CLSI.

- **Comment #14:**

  14- There are high rate of resistance reported in this assay, I think the heteroresistance may be occurred since the sampling was a bit strange to me, there is a paper discussing the topic and I would mention them in below, please read and use them in your revised paper while authors are highly recommend to add up a explanation for this results. ** Rizvanov, Albert A., Thomas Haertlé, Lydia Bogomolnaya, and Amin Talebi Bezmin Abadi. "Helicobacter pylori and its antibiotic heteroresistance: A neglected issue in published guidelines." Frontiers in microbiology 10 (2019): 1796. *** Kao, C.Y., Lee, A.Y., Huang, A.H., Song, P.Y., Yang, Y.J., Sheu, S.M., Chang, W.L., Sheu, B.S. and Wu, J.J., 2014. Heteroresistance of Helicobacter pylori from the same patient prior to antibiotic treatment. Infection, Genetics and Evolution, 23, pp.196-202. 15- I think there is an urgent need to talk about application of this result in Egypt. I think there are a lot of people who can use them. This is lacking in the discussion section.

Response: Thanks for this valued comment and interesting information regarding heteroresistance. We would like to clarify that the aim of this study is to investigate the prevalence of *H. pylori* infection and its resistance patterns among Egyptian patients, and to assess the impact of *H. pylori* virulence genetic determinants on the eradication success of 14-day triple therapy regimen. Indeed, heteroresistance is out of the scope of this study. However, further studies could illustrate this interesting point, as in our work, we do interpret the MIC values as S, I, and R. Perhaps in further studies, recording the exact MIC values for all isolates and tracking the MIC creep phenomena would be useful to better understand the heteroresistance in H. pylori.

To Reviewer 3:

- **Comment #1:**

  How many patients have cirrhosis and poor response to treatment? Is there a possibility of a bias in the inclusion of these patients? Please, add comments to the Discussion.

Response:

Thanks for this valued comment. Indeed, as per objectives the study, patients with liver cirrhosis were recorded among a range of comorbid conditions. The aim of this study is to determine the frequency of *H. pylori* infection and its resistance patterns among Egyptian patients, and to assess the impact of *H. pylori* virulence genetic determinants on the eradication success of 14-day triple therapy regimen. We know that this comment is focusing on an interesting research point, and perhaps further study could investigate this particular point in depth.

To Reviewer 4:

- **Comment #1:**

  1. While the aim of this study is mentioned as "to assess the impact of H. pylori virulence genetic determinants on the eradication success" in Aims , nothing was referred about that in Conclusions or Core tip. Title is also out-of-focus.

Response: Thanks for this comment. We would like to clarify that conclusion has highlighted the interpretation of our finding as it is presented [the *vac*A s1-positive *H. pylori* isolates are easier to eradicate and could be used as an indicator to predict the successful outcome of eradication therapy] in p: 20, lines: 378-382. We think that the title is informative and focusing on resistance patterns and eradication therapy failure among patients in our locality. From an epidemiological point of view, it seems important specially with scarce data in our region and many other developing countries.

- **Comment #2:**

  2. The manuscript seems to consist of two stories. One is the relation between the virulence genotype of H. pylori isolate and eradication sensitivity. The other is the influence of anti-microbial sensitivity of H. pylori isolates on eradication efficiency. However, the both stories are not clearly defined, moreover, the relation between both stories remains obscure.

Response: We think that this work is focusing in one major issue which could be summarized in the research question; what are causes of eradication therapy failure among patients in our locality? It is well documented that many virulence factors have been described for H. pylori as colonization, persistence, serotypes/genotypes diversity, host immune response, pathogenicity resistance trends, virulent genotypes, and the pathological-molecular association of these virulent factors. The aim of this study is to to investigate the prevalence of *H. pylori* infection and its resistance patterns among Egyptian patients, and to assess the impact of *H. pylori* virulence

genetic determinants on the eradication success of 14-day triple therapy regimen. This aim was supported by findings and discussion and was summarized in conclusion.

- **Comment #3:**

  3. The most impressive and interesting finding in this study would be that the H. pylori isolates bearing VacA-s1 genotype are significantly more conductive to eradication therapy. Actually, multivariate analysis in Table 4 showed that aOR and pValue of s1 are 0.003 and 0.507, respectively. The figures are impressive. However, this evidence regarding the relation between s1 and eradication sensitivity was already demonstrated in a meta-analysis in a previous report (The association between vacA or cagA status and eradication: A meta analysis. PLOS ONE 2017;12:e0177455). 4. Therefore, some further analysis concerning the s1 and eradication should be added to the manuscript. ……., the following analyses might be fruitful; 1) the difference between the severity of gastric mucosal inflammation (eg, Updated Sydney System Score) and s1-/s2 genotypes 2) the difference in sensitivity to AMX/CLR and s1/s2 genotypes

Response: Thanks for this valued comment. We agree with reviewer that the relation between s1 and eradication sensitivity was already demonstrated in a meta-analysis in a previous report (The association between vacA or cagA status and eradication: A meta analysis. PLOS ONE 2017;12:e0177455. However, we would like to clarify that there are few studies on the epidemiology and pathogenicity of *H. pylori* infection among patients in our locality and data about the virulent genotypes is scarce. So, we think that findings in this study could raise attention of clinicians and researchers to further investigate this issue. Considering further analysis in this study, we would believe that many research points could not be collected in one basket. Perhaps, further research work would build up upon our findings in our locality.

To Reviewer 5:

- **Comment #1:**

4 The manuscript rather adequately described the background, present status and significance of the study. But authors postulated that treatment could be prescribed for 7 to 14 days which is not correct. Current Maastrich VI recommended the only 14 days treatment. By the way, authors cited previous consensus Maastrich V, which also recommended 14-days treatment. Also, the deadline for standard therapy with clarithromycin is 15% resistance rate but not 20% as written in the paper.

Response: Thanks for this valued comment. Updated consensus Maastrich V has been added and the necessary corrections were performed in the edited manuscript.

- **Comment #2:**
  The aim of the study is to investigate the prevalence of H. pylori… The study included 86 patients of one hospital, so authors should use term frequency, but not prevalence.

Response: The necessary corrections were performed in the edited manuscript.

- **Comment #3:**
  5 The manuscript describes methods in adequate. The only RUT for H. pylori primary diagnostics and as a control test after therapy could be criticized because of low sensitivity. 6 The main contribution of the study is the new data regarding high prevalence of resistance of H. pylori in Egypt to most of the antibiotics currently used for eradication therapy and as a result unacceptable eradication rate. 7 The manuscript cited not the latest reference. Authors didn't cite important recently published papers like Maastrich VI consensus report and papers on European registry on management of H. pylori infection.

Response: Updated consensus Maastrich V has been added and the necessary corrections were performed in the edited manuscript.

Best regards