82041_Auto_Edited.docx

WORD COUNT 4093
TIME SUBMITTED 03-APR-2023 02:10PM
PAPER ID 98294134

10
Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 82041

Manuscript Type: ORIGINAL ARTICLE

Basic Study

Susceptibility patterns and virulence genotypes of *Helicobacter pylori* effecting eradication therapy outcome among Egyptian patients with gastroduodenal diseases

Ahmed Morad Asaad, Gasser El-Azab, Eman Abdelsameea, Osama Elbahr, Ahmed Kamal, Mohamed Abdel-Samiee, Ahmed Abdelfattah, Heba Abdallah, Doha Maher, Ahmed El-Refaie, Samar Ebrahim Ghanem, Shamshul Ansari, Samah Mohammed Awad

Abstract

BACKGROUND

Helicobacter pylori is considered a significant human pathogen that is responsible for variety of illnesses, including mucosa-associated lymphoid tissue lymphoma, gastric cancer, peptic ulcers, and gastritis.

AIM

To investigate the frequency of *H. pylori* infection and its resistance patterns among Egyptian patients andto determine the influence of *H. pylori* virulence genetic determinants on the eradication success of 14-day triple therapy regimen.

METHODS

H. pylori infections were investigated in 72 patients with gastroduodenal complications suggestive of *H. pylori* infection. The *cagA* and *vacA* genotypes of cultured strains were

studied using polymerase chain reaction (PCR). The patients underwent 14 days of triple-therapy treatment. The treatment response was examined using histology and a rapid urease testsix weeks after therapy discontinuation.

RESULTS

The intention-to-treat eradication rate was 59.2% (95% confidence interval CI: 48.2%–70.3%). Rates of *H. pylori* resistance to clarithromycin, amoxicillin, andmetronidazolewere 52.8%, 81.9%, and 100%, respectively.Successful eradication of *H. Pylori* was more significantly associated with *vacA* s1-positive strains (adjusted odds ratio [aOR] = 0.507, 95%CI: 0.175 – 0.822). A significant association was found between failed eradication rate and *H. pylori* strains resistant to clarithromycin (aOR = 0.204, 95%CI: –0.005–0.412) and amoxicillin (aOR = 0.223, 95%CI: 0.026–0.537).

CONCLUSION

This study's low *H. pylori* eradication rate following 14-day triple therapy is concerning and worrying. *H. pylori* pan-resistance to metronidazole followed by the high resistance to ciprofloxacin, amoxicillin, and clarithromycin in this research is challenging and of great concern.

Key Words: *H. pylori*; eradication therapy; virulence; clarithromycin resistance

Asaad AM, El-Azab G, Abdelsameea E, Elbahr O, Kamal A, Abdel-Samiee M, Abdelfattah A, Abdallah H, Maher D, El-Refaie A, Ghanem SE, Ansari S, Awad SM. Susceptibility patterns and virulence genotypes of Helicobacter pylori effecting eradication therapy outcome among Egyptian patients with gastroduodenal diseases.. *World J Gastroenterol* 2023; In press

Core Tip: In this research, 72 patients with *H. pylori* infections were investigated. Half of the *H. pylori* strains had the *cagA* gene, and more than half of the strains were resistant

to antibiotics except tetracycline and clarithromycin. However, clarithromycin and tetracycline were effective at higher doses to achieve effective eradication by the clarithromycin-based therapy. Most importantly, this study pointed out that an alternative therapeutic regimen should be adopted to achieve effective infection eradication in Egypt.

INTRODUCTION

Helicobacter pylori is a prominent human pathogen and is responsible for a variety of diseases, such as mucosa-associated lymphoid tissue (MALT) lymphoma, duodenal or peptic ulcer, gastritis, and gastric cancer[1,2]. As a result of its causative relationship to gastric adenocarcinoma, the World Health Organization (WHO) has identified this pathogen as a carcinogen of Class I[3]. According to the global estimate, *H. pylori* infects roughly 4.4 billion people with prevalence rates ranging from 20% to 90% in developed and underdeveloped nations, accordingly[2].

Pathologically, the existence of gastric mucosa inflammatory changes in areas with abundant *H. pylori* organisms together with the pathognomonic existence of either lymphoid aggregates and/or follicles with germinal centers and neutrophilic infiltration constitutes the definition of chronic *H. pylori* gastritis[2, 3].

Previous epidemiological research has produced a long list of microbial virulence factors that have a crucial role in *H. pylori* colonization, persistence, serotype/genotype diversity, host immune responses, pathogenicity, and disease severity. These factors involve the outer inflammatory protein (*oip*A) gene, the cytotoxin-associated gene (*cag*A), the vacuolating cytotoxin gene (*vac*A), the *bab*A2adhesin gene, the epithelium gene A (*ice*A), and the duodenal ulcer-promoting gene (*dup*A)[4–7].

The variability of *H. pylori* strains is believed to be related to the genetic structural diversity with associated polymorphic arrangements of different virulence determinant genes[4]. For example, the vacA gene, which encodes a vacuolating toxin, is found in the vast majority of *H. pylori* strains and is an important virulence factor. Because of sequence variability in the middle region (m), m1 and m^2 alleles, signal region (s), s1 or

s2 alleles, and the intermediate region I subtypes 1 or 2,notable diversity in the vacuolating activity of different strains is found.iceA1 and iceA2 are two major alleles of the iceA gene, which is another example of microbial genetic variation[8].

Triple therapy for 14 days with proton pump inhibitors and a mixture of two antibiotics, clarithromycin (CLA) and amoxicillin (AMX) or metronidazole (MZ) is the standard treatment for *H. pylori* infections[9, 10]. CLA is the preferred antibiotic in areas in which resistance to this antibiotic is < 15%[10]. However, the continuous surge in antimicrobial resistance, including CLA-resistance, has been accompanied by a failure to eradicate *H. pylori* infections in a significant proportion of cases worldwide. The prevalence of *H. pylori* resistance to CLA ranges from 11.1% in Europe to 92.3% in Africa, reaching 18.9% in Asia and 29.3% in America as described in clinical reports[11–13].

Only a few studies addressing *H. pylori* infections, pathogenicity, and epidemiology among Egyptian patients are available. Besides, data regarding *H. pylori* resistance to CLA is scarce. Therefore, this research aimed to determine the *H. pylori* infection frequency and its resistance patterns among Egyptian patients and to determine the influence of *H. pylori* virulence genetic determinants on the eradication success of a 14-day triple therapy regimen.

MATERIALS AND METHODS

This cross-sectional observational study was done from August 2021 to June 2022 at the National Liver Institute (NLI), a 760-bed tertiary care hospital in Shebin El-Kom, Egypt. The research adhered to the Helsinki Declaration principles and received ethical approval from the ethics NLI research committee: IRB number 00308/2022.Written consent was obtained from all participants. The research followed the international principles of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[14].During the research period, 86 adult cases with different dyspepsia symptoms (vomiting, epigastric, and/or abdominal pain, and/or heartburn) and/or

other symptoms indicative of *H. pylori* infection and likely to require *H. pylori* eradication therapy were enrolled in this research.

All cases provided a medical history and underwent a physical examination, a quick urease test, and inspection of the esophagus, stomach, and duodenum using an upper endoscopy.

Endoscopy

Under topical lignocaine anesthesia, each patient underwent an upper gastrointestinal endoscopy (Olympus X Q40, Olympus Optical, Tokyo, Japan). Each patient's antrum and stomach corpus were biopsied during the endoscopy to obtain two sets of biopsy samples. The first set of biopsies was utilized for a rapid urease test utilizing a rapid urease test kit (CLO test; Kimberly-Clark Ltd., Draper, Utah, USA). Three hours later, the second set was transferred on ice to the laboratory for bacteriological culture after being packed in 300 mL of sterile normal saline. For histopathological analysis, the third specimen was immediately fixed in 10% formalin. The fourth section was added to a buffered solution (10 mmol/LTris, pH 8, 10 mmol/L ethylenediaminetetraacetic acid[EDTA], and 0.5 percent sodium dodecyl sulfate[SDS]) and then frozen at –8 °C for DNA extraction and polymerase chain reaction (PCR) assays. *H. pylori* was diagnosed using three techniques: *(1) H. pylori* culture, (2) histopathology using hematoxylin and eosin (H&E) and Gemisa staining, and (3) rapid urease test. At baseline, a patient was considered *H. pylori*-positive if he had a positive culture or rapid urease test that was validated by histological features (foveolar-neutrophilic infiltration, lymphoid follicles and/or aggregates, and verified positive Gemisa stained I rods). Six weeks after the triple therapy cessation, a second gastrointestinal endoscopy was done to determine and confirm the presence of *H. pylori* and whether or not the duodenal ulcer had been successfully cured. Eradication was defined as the absence of histological evidence and rapid urease test negative results.

***H. pylori* culture and antimicrobial susceptibility testing**

Using a tissue grinder, the biopsy specimen was homogenized and plated onto brain-heart infusion (BHI) agar plates (Difco, Detroit, MI, USA) that were supplemented with vancomycin (6 mg/mL), amphotericin B (8 mg/mL), trimethoprim (5 mg/mL), and 10% glycerol. Under microaerophilic conditions (85% N_2,5% O_2, and 10% CO_2) in a humid atmosphere, the plates were incubated at 37 °C for 3 to 5 days. *H. pylori* was recognized based on Gram staining, helical shape, and biochemical assays that were positive for oxidase, catalase, and urease[15].

As suggested by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the E-test minimum inhibitory concentration technique was used to test for antimicrobial susceptibility. Tests were done on Mueller–Hinton agar plates enriched with 7% horse blood (bioMérieux) using E-test strips (AB Biodisk, Slona, Sweden)[16]. Tetracycline (TET), clarithromycin (CLA), amoxycillin (AMX), rifampicin (RIF), ciprofloxacin (CIP) were among the antibiotics that were evaluated. The isolate was considered resistant to AMX, CLA, and MZ if minimal inhibitory concentrations (MICs) were > 0.125 mg/L, > 0.5 mg/L, and > 8 mg/L, respectively. Besides, the isolate was considered resistant to CIP, RIF, and TET if MICs were > 1 mg/L[16].

DNA extraction

Each *H. pylori* isolate was sub-cultured and incubated for 72 h after which7–10 colonies were pooled together.DNA was extracted according to the manufacturer"s instructions using the QIAamp DNA micro kit (Qiagen, Germany) and then was eluted in 200 µl of 1 TE buffer (pH 8.0; 10 mmol/LTris-HCl, 1 mmol/L EDTA) and stored at –20°C until PCR amplification.

Molecular identification of *cag*A and *vac*A genotypes of *H. pylori*

As previously disclosed, all isolates were subject to multiplex PCR to determine *cagA* and *vacA* genotypes[17–19]. Table 1 contains a list of all primers utilized for this

research. Multiplex PCR was carried out in a thermocycler (Cyclogene,Techne, UK)in a reaction mixture volume of 50ul. Each reaction contained 25ul of 2x multiplex PCR Master mix (Hot start DNA polymerase, multiplex buffer, dNTP mix Mgcl2; Thermoscientific, Vilinus, Lithuania), 2ul of each primer (40 pmol), and 200 ng of template DNA (6µl) according to DNA concentration in yield. The reaction volume was brought to to 50µl with the addition of nuclease free water. PCR grade water and DNA from *H. pylori* strain ATCC 43504 were used as negative and positive controls, respectively. Multiplex PCR was carried out by the simultaneous addition of primers in the same reaction mixture after test of each primer pair separately: (1) 35 cycles of 95°C for 1 min, (2) annealing at 54°C for 1.5 min, (3) extension at72°C for 1 min, and (4) a final extension at 72 °C for 10 min. The amplified PCR products were electrophoresed on 1.5% agarose gels using 1xTBE after which the gel was stained with ethidium bromide using a 100bp ladder as the molecular weight standard, visualized under an ultraviolet light source, and photographed using a BioRad Gel Doc device.

H. pylori eradication therapy and follow-up

Patients were given omeprazole (20 mg) and two antibiotics, AMX (1 g) and CLA (500 mg), twice daily for 14 days[9]. Clinical follow-up, including side-effect monitoring, was performed until the medication was completed. Using a rapid urease test, the therapeutic response was examined six weeks following the termination of therapy. Successful eradication of *H. pylori* was indicated by a negative result from the rapid urease test, and microbiological cultures and by the absence of neutrophilic infiltration with a decrease in lymphoid inflammatory changes in the biopsy tissue following treatment[9, 20].

Statistical analysis:

Coding, validating, and analyzing the data required the use of SPSS version 22 (SPSS Inc., Chicago, IL, USA). Data were shown using average, median, and frequencies (%). A chi-squared test or Fisher's exact test was used to compare categorical data&, a Student's t-test was used for numerical data. P values of ≤ 0.05 based on a two-tail test

were considered to be significant. Potential risk factors were identified using a binary logistic regression analysis that included an antecedent 95% confidence intervals (CI) and adjusted odds ratio (aOR).

RESULTS

Patients characteristics and H. pylori infection

Table 2 shows the demographic and clinical characteristics of the patients. The age of the patients ranged from 19 to 59 years (median, 39.5 years), and 57 (79.2%) were males. Based on endoscopic examination, more than half of patients (54.2%) had gastritis, while 33.3% and 12.5% of patients had gastric ulcer and duodenitis, respectively. Based on histopathological examination of the tissue biopsy, features of chronic gastritis and rods of *H. plyori* were seen based on Giemsa stain (figure 1A, B).

Thirty-six (50%) strains were *cagA*-positive among 72 *H. pylori* isolates in this research, and in 50 (69.4%) of the *H. pylori* strains, *vacA* gene was detected. For the *vacA* gene s and m region sub-typing, 42 and 18 strains were positive for s1 and s2, respectively, while 27 and 33 isolates were positive for m1 and m^2, respectively (figure 2 A, B).

Antimicrobial susceptibility test outcomes

During the research period, a total of 86 patients were recruited. Among them, 10 patients were negative for *H. pylori* infection, and four patients failed to complete treatment, leaving 72 patients eligible for the study protocol. Among the 72 *H. pylori* isolates, the resistance rates to MNZ, AMX, RIF, CLA, CIP, and TET were 100%, 81.9%, 62.5%, 52.8%, 41.7%, and 37.5%, respectively.

Triple therapy outcomes

The eligible patients underwent 14-day triple therapy with two antibiotics: (1) AMX (1 g), (2) CLA (500 mg), and (3) omeprazole (20 mg). In 45 individuals, the 14-day triple therapy was successful in completely eliminating *H. pylori*; however, in the other 27 patients, the infection persisted despite treatment. *H. pylori* eradication rates were

59.2% (95%CI: 48.2–70.3%) for intention-to-treat (ITT) and 62.5% (95%CI: 51.3%–73.7%) for per protocol treatment.

In patients with *vac*A s1 (P = 0.02)-, s2 (P = 0.03)-, or m1 (P = 0.01)-positive strains, *H. pylori* eradication occurred more frequently. It was not surprising that the rates of resistance to AMX (P = 0.012) and CLR (P = 0.005) were higher in *H. pylori* isolates from patients who experienced unsuccessful eradication. However, no significant association with eradication therapy and resistance rates to CIP, TET, and RIF was found (Table 3).

With 95%CI) and aOR the multiple logistic regression analysis revealed possible risk factors associated with *H. pylori* eradication therapy (Table 4). Successful eradication of *H. pylori* was more significantly associated with strains harboring *vac*A s1 genotype (aOR = 0.507, 95%CI: 0.175–0.822). In contrast, failed eradication rates were significantly associated with *H. pylori* strains resistant to AMX (aOR = 0.223, 95%CI: 0.026–0.537) and CLA (aOR = 0.204, 95%CI: –0.005–0.036).

DISCUSSION

Eradication therapy of *H. pylori* infections has been deemed beneficial for cases with gastroduodenal disorders, such as gastric MALT lymphoma, peptic ulcer disease history, gastric cancer, dyspepsia, atrophic gastritis, and hyperplastic polyps and additionally, for cases with certain extra-gastrointestinal disorders, such as unexplained iron-deficiency anemia, chronic idiopathic urticaria, and idiopathic thrombocytopenic purpura[1, 21].

Despite establishment of multiple *H. pylori* eradication treatment regimens in different worldwide regions, the usual 14-day triple therapy (piperacillin[PIP]/AMX//CLA[PAC]) produced adequate eradication rates for both adults and children in Egypt[21–23]. However, cure rates in this study were found to be unsatisfactory and disappointing. Our findings show that the14-day triple therapy efficacy of for *H. pylori* eradication (59%) is lower than that reported from previous Egyptian studies (ITT range: 72%–83%)[22, 23]. A previous meta-analysis report investigated the global trend in eradication rates of two different first line therapeutic

regimens (PAC and PIP/amoxicillin/metronidazole[PAM]) for 8061 patients infected with *H. pylori* from 30 countries[24]. In this report, the cure rate of PAC (77.1%, 95%CI = 75%–79%) was significantly higher than that (70%, 95%CI = 67.7%–72.3%) of PAM (OR = 0.70, 95%CI = 0.56–0.88; P < 0.002). Previous clinical studies worldwide showed an unacceptable and continuous decrease in *H. pylori* triple eradication therapy-associated cure rates[13, 25]. It is noteworthy that the overall global cure rates of these protocols are < 80%, which have recently been considered regimens with disappointing efficacy.

Inadequate treatment duration, antimicrobial resistance, inadequate stomach acid suppression, poor adherence to eradication regimens, and quick metabolism of PPI have all been implicated in the failure of the traditional triple therapy to eradicate a pathogen according to previous ecological research[26, 27].CLA resistance has been recognized as the primary cause of routine triple treatment failure. In a recent meta-analysis report investigating 66,142 patients from 65 countries, failure to achieve eradication was 7-fold higher in patients with CAL-resistant *H. pylori* infections (OR: 6.97; 95%CI: 5.23–9.01; *P* = 0.001) when treated with a CLA-containing regimen than patients with susceptible strains.[13] Therefore, in nations with a high prevalence of CLA resistance (> 15%–20%), bismuth quadruple treatment is recommended. Pooled data from 25 randomized trial studies including 3990 patients showed that the ITT eradication rate of standard triple therapy (65.7%) was significantly lower than bismuth- containing regimens (74.9%; OR: 1.60; 95%CI: 1.07–2.39). Besides, in the PP study, the pooled eradication rate for bismuth-containing regimens was 86.7% versus33.3% for the usual triple regimen (OR: 10.64; 95%CI: 2.96–39.53)[28]. It is noteworthy that all isolates in this study were MZ-resistant, and more than half of isolates were resistant to AMX and CLR. These findings are not surprising as MZ has been abused by the public without prescription for various gastrointestinal infections and diarrhea, while both AMX and CLR have been included in empiric therapies for respiratory infections or non-tuberculous mycobacterial infections in our region. Therefore, continuous monitoring of susceptibility patterns of *H. pylori* to various

antimicrobials seems crucial as multi-drug-resistant *H. pylori* strains undoubtedly induce failure of *H. pylori* eradication therapy.

H. pylori *cag*A and *vac*A genotypes are considered among the most important factors implicated not only in pathogenesis of gastroduodenal diseases but also in influencing the sequelae of treatment protocols. The association of *cag*A-positive strains with the *H. pylori* eradication therapy outcomes were demonstrated by former clinical and ecological investigations[4–9]. However, the results from these studies were inconsistent and controversial. In this study, strains with or without *cag*A had no effect on eradication rates, a finding that is similar to previous reports. In a previous meta-analysis report including 25 studies, the influence of the virulence factors, *vac*A and *cag*A, on *H. pylori* eradication therapy in 2693 cases was investigated by Wang *et al*[29]. In their report, the pooled *H. pylori* eradication rate was 77% (95%CI: 70%–83%) for *cag*A-negative patients and 85% (95%CI: 81%–89%) for *cag*A-positive with an 8% higher eradication rate among *cag*A-positive strains. In addition, using subgroup analyses based on clinical presentations, eradication detection method, location, and therapeutic regimen types, the authors conclude that *cag*A-negative strains responded to successful *H. pylori* therapy eradication rates than *cag*A-positive strains with pooled risk ratios (RR) of 1.118 (95%CI: 1.051–1.189; P < 0.001) for Asia and1.138 (95%CI: 1.000–1.295; *P* = 0.049) for Europe. In South America, *cagA*-positive strains and-negative strains exhibited comparable *H. pylori* treatment rates (RR: 1.104, 95%CI: 0.953–1.279; *P* = 0.186).

The *vac*As1-positive *H. pylori* strains are typically more virulent and more closely linked to progressive gastroduodenal disorders as reported in many previous studies[29]. An increase in blood flow to the site of infection and stronger inflammatory responses were stimulated by more virulent strains as reported by clinical-and epidemiological-based evidence. Besides, the more virulent strains are usually more susceptible to antimicrobials because of faster replication. *H. pylori* strains containing *vac*A s1 were substantially related to a greater *H. pylori* eradication rate in the current investigation.

This result is consistent with earlier findings in which *vac*A s1-positive strains was found to pose a significant risk for the development of gastric illnesses with easier eradication in diseased people. Wang *et al* discovered that the pooled *H. pylori* eradication rate was 73% (95%CI: 61%–85%) for *vac*A s2and 83% (95%CI: 75%–91%) for *vac*A s1 with a 10% improvement in eradication rates in the *vac*A s1 group compared to the *vac*A s2 group (95%CI: 1.040–1.303; $P = 0.008$). In their meta-analysis report, *vac*A s1 status was associated with better eradication rates in the triple therapy subgroup according to an examination of subgroups based on different worldwide locations (RR: 1.175, 95%CI: 1.012–1.360)[29]. Brennan *et al* reported that the incidence of the more virulent s1genotype was substantially lower among previously treated individuals than among those who had never received therapy (58.3% *vs* 74.3%). Besides, a significantly increase in the frequency of the least pathogenic s2/m^2 genotype was seen in previously treated individuals (36.7% *vs* 21%)[7]. Our findings and results of other studies clearly demonstrate that *vac*A s1 genotype would useful for predicting successful outcomes of *H. pylori* eradication therapy.

This study has some limitations. First, this research may have been limited in its capability to investigate other virulence marker-associated pathogenic roles due to the lack of molecular analysis beyond PCR and genome sequencing. Second, this was research within a single center. Our findings cannot therefore be applied to other situations. To further understand the phenotypic and genotypic links between *H. pylori* virulence, antibiotic resistance, and the efficacy of eradication therapy, further molecular-based epidemiological multi-center investigations with longer monitoring durations are required.

CONCLUSION

This study's low *H. pylori* eradication rate following 14-day triple therapy is concerning and worrying. The pan-resistance of *H. pylori* to MZ followed by the high resistance to AMX, CLA, and CIP in this research is challenging and of great concern. These findings draw attention to the urgent need of performing *H. pylori* antimicrobial susceptibility

testing before starting eradication therapy in addition to continuous surveillance of *H. pylori* resistance patterns in our region to provide data that can guide empirical treatment. In addition, 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.

ARTICLE HIGHLIGHTS

Research background

+ADw-html+AD4APA-p+AD4-H. pylori has been implicated in the development of gastric cancer and gastric MALT lymphoma. However, the bacterial eradication reduces the risk of theses gastric complications. The therapeutic regimens currently in use and the duration of therapy differ in different countries affecting the therapy outcomes. The therapeutic outcomes have been also found to be affected by the virulence characteristics of the infecting strains. The strains with more virulent characteristics possessing vacA s1 and m1 are eradicated more efficiently than the strains harboring less virulent characteristics.+ADw-/p+AD4APA-/html+AD4-

Research motivation

To demonstrate that the infecting strains possessing more virulent characteristics are eradicated efficiently with a 14-days triple therapy. 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

Research objectives

To evaluate the *H. pylori* infection frequency and its resistance patterns among Egyptian patients, and to determine the *H. pylori* virulence characteristics influencing the eradication success of 14-day triple therapy regimen.

Research methods

The patients suggestive of *H. pylori* nfections were subjected for the endocscopy based biopsy specimens collection. The collected biopsy specimens were used to evaluate the *H. pylori* infection by a combination of diagnostic tests that included urease test, bacterial culture, and histopathological investigation. The extracted DNA was subjected for the PCR based *cagA* and *vacA* genotypes investigation. The *H. pylori* infected patients were suggested for triple-therapy for 14 days. After 6 wk of completing the therapy, the treatment response was examined utilizing histology & rapid urease test.

Research results

Among the 86 recruited patients the infection was found in 76 individuals. All of the strains were resistant to metronidazole, while 52.8% and 81.9% of the isolates were resistant to clarithromycin and amoxicillin, respectively. *H. pylori* successful eradication was more significantly associated with *vacA* s1-positive strains (aOR=0.507, 95%CI: 0.175 – 0.822). *H. pylori* strains resistant to clarithromycin (aOR=0.204, 95%CI: -0.005 – 0.412) & amoxicillin (aOR=0.223, 95%CI: 0.026 – 0.537) were significantly associated with failed eradication rate.

Research conclusions

The low eradication rate of 14-days triple therapy in this study is worrisome that indicates finding of alternative therapy to achieve effective eradication. The findings of complete failure of metronidazole and reduced efficacy of amoxicillin, clarithromycin and ciprofloxacin draw attention to the urgent need of antimicrobial susceptibility testing guided eradication therapy. In addition, the strains with virulent properties of *vac*A s1 are easier to eradicate and could be used as an indicator to predict the successful outcome of eradication therapy.

Research perspectives

Clarithromycin, ampicillin, and metronidazole based 14-days eradication therapy is ineffective and discouraged in these populations. Nationwide extensive studies should

be considered to document the efficacy and to find the alternative therapeutic regimens in respect to the duration. Furthermore, antimicrobial susceptibility testing based therapy should be encouraged to help reduce the development of antimicrobial resistance.

ACKNOWLEDGEMENTS

The authors would like to thank the colleagues in the department of laboratory and microbiology and endoscopy unit and the patients in National Liver Institute who aided in this research.

82041_Auto_Edited.docx

ORIGINALITY REPORT

6%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	66 words — 2%
2	journals.plos.org Internet	44 words — 1%
3	malariajournal.biomedcentral.com Internet	29 words — 1%
4	S.T. Kaplan, D. Hemsinli, S. Kaplan, A. Arslan. "Amputation predictors in diabetic foot ulcers treated with hyperbaric oxygen", Journal of Wound Care, 2017 Crossref	22 words — 1%
5	pinnacle.allenpress.com Internet	20 words — < 1%
6	mdpi-res.com Internet	17 words — < 1%
7	Ling Deng, Xiao-Yi He, Bin Tang, Yang Xiang, Juan-Juan Yue. "An improved quantitative real-time polymerase chain reaction technology for Helicobacter pylori detection in stomach tissue and its application value in clinical precision testing", BMC Biotechnology, 2020 Crossref	15 words — < 1%

8	Xing Yu, Chengdang Wang, Mi Wang, Yinchen Wu, Linlin Zhang, Qinyu Yang, Long Chen. "Cronkhite-Canada syndrome: a retrospective analysis of four cases at a single medical center", Scandinavian Journal of Gastroenterology, 2022 Crossref	15 words — < 1%
9	Yu-jie Li, Xue-hong Bai, Xi Tang, Zhi-yong Yang et al. "Hepatopulmonary syndrome delays postoperative recovery and increases pulmonary complications after hepatectomy", European Journal of Gastroenterology & Hepatology, 2021 Crossref	15 words — < 1%
10	id.nii.ac.jp Internet	15 words — < 1%
11	www.researchsquare.com Internet	12 words — < 1%
12	www.tandfonline.com Internet	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS