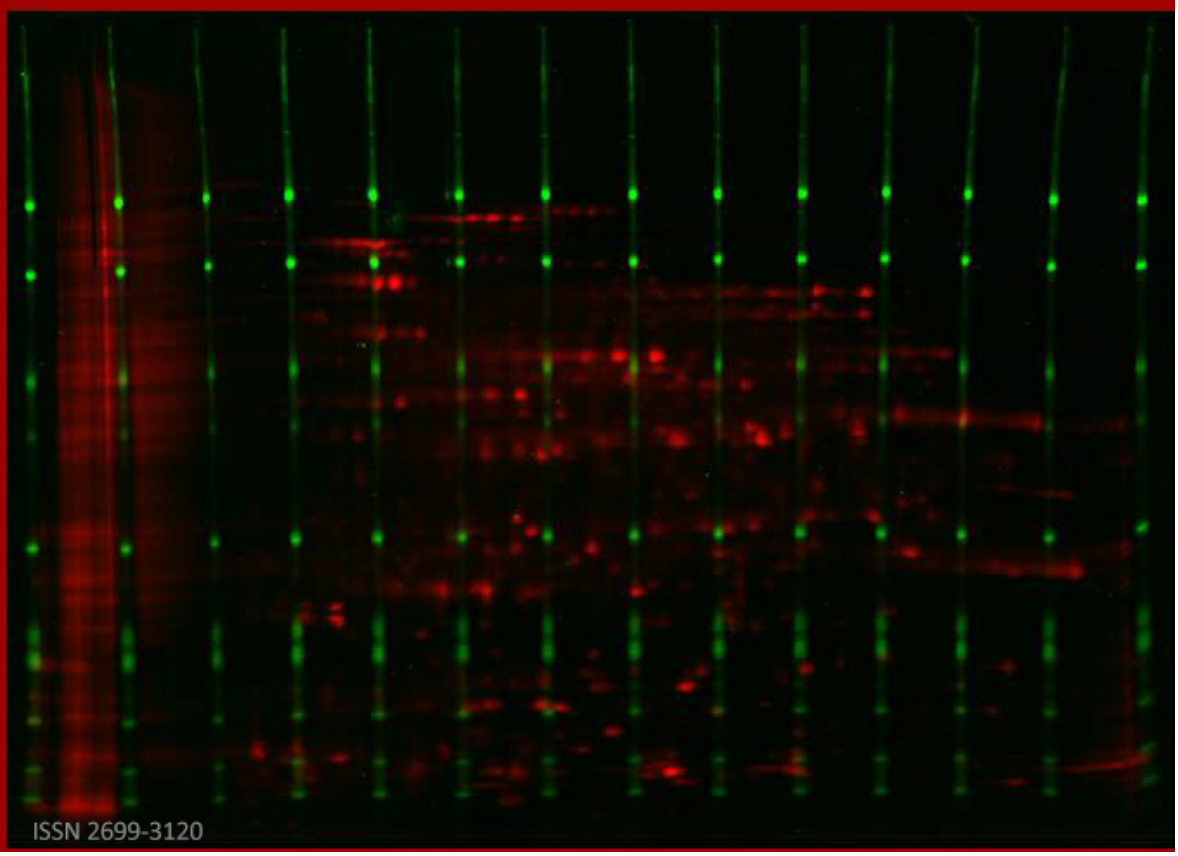
Volume 6 · Number 2 · October 2022

MERCATOR Journal Biomolecular Analysis

Laboratory Protocols

Mass spectrometry & Proteomics

Editor Simone König



MERCATOR Journal Biomolecular Analysis Vol 6 · No 2 · October 2022 · pp 1-9

ISSN 2699-3120

Digital laboratory notes of the Core Unit Proteomics CUP
Interdisciplinary Center for Clinical Research IZKF
Medical Faculty
Westphalian Wilhelms University of Münster
Germany
https://campus.uni-muenster.de/cu-proteomics
Edited by Simone König
CUP/IZKF, Röntgenstr. 21, 48149 Münster
koenigs@uni-muenster.de
Ph. +49-251-8357164

Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Research Article

Update on the eradication of *Helicobacter pylori* infection in adult and pediatric patients from the northern region of Pakistan

Shahid Aziz[1,4#], Mashhood Ali[2], Mulazim Hussain[3], Anum Javed[1,4], Ayesha Fatima[1,5], Tehzeeb Zehra[6], Faizan Rashid[1,4], Muhammad Faraz[1,4], Toufeeq ur Rehman[5], Rani Faryal[4], Rabaab Zahra[4], Tanvir Ahmad[1], Simone König[7], Faisal Rasheed[1]

[1]Patients Diagnostic Lab, Life Science Group, Isotope Application Division, Pakistan Institute of Nuclear Science and Technology, Islamabad, Pakistan, 44000
[2]Department of Gastroenterology, Pakistan Institute of Medical Sciences, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan, 44080
[3]Children Hospital, Pakistan Institute of Medical Sciences, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan, 44080
[4]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan, 45320
[5]Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan, 45320
[6]Shifa Falahee Foundation Clinic, Shifa International Hospital, Islamabad, 44000
[7]Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster, Münster, Germany, 48149
[#]Corresponding author: saziz@bs.qau.edu.pk or sazizhpl@gmail.com

Keywords: Helicobacter, eradication, antibiotics, urea breath test

Abstract

Helicobacter pylori infection is associated with different gastric diseases, notably gastric cancer. The present study is aimed at comparing the effectiveness of various *H. pylori* eradication regimens among adult and pediatric patients. A total of 3178 (396 children) patients were recruited. *H. pylori* infection was diagnosed by nuclear stable isotopic ^{13}C urea breath test (UBT) and positive patients were randomly allocated first-line eradication regimens. The second or third-line regimens were assigned to those who had failed first-line treatment. A negative UBT at least 4 weeks after the completion of treatment indicated a successful eradication of *H. pylori*. Eradication rates (ER) by per-protocol (PP) and intention-to-treat (ITT) analysis were recorded. The overall prevalence of *H. pylori* infection was 61.2% and slightly higher in males as compared to females. The modified concomitant therapy showed higher PP (77.8%) and ITT (72.7%) ER as first-line treatment. Among second-line therapies, PP/ITT ER of 63.6% / 55.3% were observed for quadruple therapy in adults. Standard triple therapy with probiotic and modified concomitant therapy as first-line and quadruple therapy as a second line thus is the choice for *H. pylori* eradication in adult gastric disease patients. In pediatric patients, standard triple therapy was more effective as a first-line with about 68.8% ER.

Introduction

Helicobacter pylori infection affects billions of people worldwide [1]. The bacteria persist and colonize the gastric mucosa, which causes gastroduodenal pathologies including gastritis, ulcer, and most importantly gastric cancer [2, 3]. Therefore, eradication of *H. pylori* is an important aspect of managing gastroduodenal pathologies [4, 2]. Antimicrobial eradication has proved effective in 50-80% cases of *H. pylori* infection. In Pakistan, antibiotic resistance in *H. pylori* is however increasing against commonly used antibiotics for various reasons (prescription of anti-bacterial drugs without susceptibility testing, indiscriminate use of anti-bacterial drugs by patients for various infections and self-medication) together with the lack of resistance surveillance at a national level [3, 5, 6]. Moreover, in Pakistan, the data related to eradication therapies (ET) against *H. pylori* infection is very limited. Therefore, this study aimed to explore the effectiveness of *H. pylori* ET in gastric disease patients (adult and pediatric) and to determine the eradication rates (ER).

Experimental

Patients

A total of 3178 (age 3-85 years, 2782 adults, 396 children age 3-18) gastric disease symptomatic patients having complaints of acid reflux, abdominal pain, heartburn, vomiting and bloating, attending Children Hospital and Department of Gastroenterology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Shifa Falahee Foundation Clinic, Islamabad and Holy Family Hospital, Rawalpindi, were enrolled in this study. Patients with a history of using proton pump inhibitors, histamine antagonists, or antibiotics during the last 4 weeks, pregnant women or breast feeding mothers, patients having had previous gastric surgery or suffering from gastric cancer or systemic illness such as liver cirrhosis or chronic renal failure, were excluded from this study. Ethical approvals (F.1-1/2015/ERB/SZABMU/223) were obtained from hospitals. Patients and guardians in case of children were verbally informed regarding the study and their consent was granted prior to enrollment. Patient personal information or identity was preserved in this work.

In the intention-to-treat (ITT) group every patient randomized to the clinical study was entered in the primary analysis. Accordingly, also patients who dropped out prematurely and those who were non-compliant to the study treatment or took the wrong study treatment were included in this analysis. Consequently, in an ITT analysis, the original randomization and the number of patients in the treatment groups remained unchanged, the analysis population was as complete as possible, and a potential bias due to exclusion of patients was avoided. In addition, per-protocol (PP) analysis was performed to identify a treatment effect which would occur under optimal conditions with fully compliant patients. Therefore, some patients from the full analysis set had to be excluded.

Nuclear stable isotopic ^{13}C urea breath test

Nuclear stable isotopic ^{13}C urea breath test (UBT) was used to determine the status of *H. pylori* infection in the study patients. It was performed using 75 mg ^{13}C urea (Cambridge Isotope Laboratories, Andover, MA, USA) comprising of urea with 99.9% ^{13}C (stable and nonradioactive isotope) as previously described [5]. The $^{13}CO_2/^{12}CO_2$ ratio was analzyed on BreathMAT Mass Spectrometer (Thermo, Germany) at Patients Diagnostic Lab, Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad. A change in the UBT value over baseline of more than 3‰ was considered a positive result [5]. To categorise the

H. pylori load, patients with UBT positive status were divided into three categories: UBT values 3-15 were considered as mild, 16-30 as moderate and ≥31 as marked. Pre-treatment and post-treatment UBT scores were assessed.

H. pylori eradication therapies

Patients with positive UBT were subjected to currently recommended first-line ET. Those patients, who had failed first-line ET, were further treated with established second- and third-line medication (Table 1). Proton pump inhibitor was recommended 30 min before breakfast and dinner, whilst antibiotics were to be taken following these meals. The protocol involved proper guidance of patients and a special follow-up chart for all therapies was given to the patients to keep the record of each and every dose of medicine. To support compliance, patients were carefully instructed to adhere to the drug regimen and were advised of the possible side effects. Compliance and incidence of side effects were evaluated by direct interview at the end of therapy. Good compliance was defined as >90% adherence to the prescribed drugs. The eradication of *H. pylori* infection was assessed 4 weeks after the end of therapy by performing UBT.

Statistical analysis

Descriptive data analysis was carried out using SPSS version 21.0. ER were calculated for ITT and PP. The mean (SD) was computed for continuous variables while percentage or frequency was calculated for categorical variables. Statistical significance was established at p value < 0.05 using Chi square test where appropriate.

Results

The prevalence of *H. pylori* infection was 61.2% (1944/3178) among gastric disease symptomatic patients as determined by UBT. The five common symptoms for *H. pylori* infection (vomiting, acid reflux, heartburn, bloating, abdominal pain) were observed nearly in all these patients. *H. pylori* prevalence was found highest in those having vomiting (78.3%) followed by acid reflux (77.9%), heartburn (77.3%), bloating (76.6%), and abdominal pain (76.4%). The prevalence of *H. pylori* positive patients among males was 61.3% (987/1609) and 61.0% (957/1569) in females with non-significant association (p = 0.840). These patients were categorized into three age groups and the prevalence of *H. pylori* infection was 61.9% (245/396) among patients aged between 3-18 years, 62.6% (1262/2037) in 19-44 years and 58.7% (437/745) in 45+ years with non-significant difference (p = 0.274).

Infected patients (1944) were subjected to first-line ET but 1436 (73.9%) patients did not participate in the follow up (Figure 1). In adult patients, standard triple therapy with probiotic had comparatively high ER (PP 71.0% (44/62), ITT 67.9% (53/78), Table 2) compared to standard triple therapy (PP 63.8% (74/116), ITT 61.6% (93/151)). Levofloxacin-based triple therapy (PP 66.1% (39/59), ITT 58.2% (46/79)) achieved higher eradication than levofloxacin-based triple therapy with probiotic (PP 64.3% (18/28), ITT 61.9% (26/42)). Sequential therapy had PP 69.4% ((25/36), ITT 66.7% (34/51)), azithromycin-based triple therapy PP 41.7% ((10/24), ITT 50.0% (22/44)) and metronidazole-based triple therapy PP 45.5% ((5/11), ITT 43.8% (7/16)) ER. Modified concomitant therapy had the highest ER (PP 77.8% (7/9), ITT (72.7%, 8/11)) among all the study therapies.

Table 1: Recommended regimens for the treatment of *H. pylori* infection

Treatment	Regimens
Adults	
First-line therapies	
Standard triple therapy	omeprazole (40 mg, *bid*), clarithromycin (500 mg, *bid*) and amoxycillin (1000 mg, *bid*) for 7 days
Standard therapy with probiotic	esomeprazole (40 mg, *bid*), PPI, clarithromycin (500 mg, *bid*), amoxycillin (1000 mg, *bid*), Ecotec (>1b CFU, *bid*) for 7 days
Sequential therapy	omeprazole (40 mg, *bid*), amoxycillin (1000 mg, *bid*) for 5 days followed by omeprazole (40 mg, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) for next 5 days
Levofloxacin-based triple therapy	esomeprazole (40 mg, *bid*), levofloxacin (250 mg, *bid*) and amoxycillin (1000 mg, *bid*) for 7 days
Levofloxacin-based triple therapy with probiotic	esomeprazole (40 mg, *bid*), levofloxacin (250 mg, *bid*) and amoxycillin (1000 mg, *bid*), Ecotec (>1b CFU, *bid*) for 7 days
Azithromycin-based triple therapy	omeprazole (40 mg, *bid*), azithromycin (250 mg, *bid*), amoxycillin (1000 mg, *bid*) 7 days
Metronidazole-based triple therapy	omeprazole (20 mg, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) for 7 days
Modified concomitant therapy	rabeparazole (20 mg, *bid*), clarithromycin (500 mg, *bid*), amoxycillin (1000 mg, *bid*) and metronidazole (800 mg, *bid*) for 7 days
Second-line therapies	
Quadruple therapy	omeprazole (40 mg, *bid*), bismuth subsalicylate (530 mg, *qid*), tetracycline (500 mg, *qid*) and metronidazole (500 mg, *tid*) for 14 days
Levofloxacin-based triple therapy	omeprazole (40 mg, *bid*), levofloxacin (250 mg, *bid*) and amoxycillin (1000 mg, *bid*) for 7 days
Moxifloxacin-based triple therapy	omeprazole (40 mg, *bid*), moxifloxacin (500 mg, *bid*) and amoxycillin (1000 mg, *bid*) for 7 days
Third-line therapy	
LOAD therapy	omeprazole (40 mg, *od*), levofloxacin (250 mg, *od*), doxycycline (100 mg, *od*) and nitazoxanide (500 mg, *bid*) for 14 days
Pediatric patients	
First-line therapies	
Standard triple therapy	omeprazole (1 mg/kg, *bid*), amoxycillin (50 mg/kg, *bid*) and clarithromycin (15 mg/kg, *bid*) for 14 days
Standard therapy with probiotic	omeprazole (1 mg/kg, *bid*), amoxycillin (50 mg/kg, *bid*), clarithromycin (15 mg/kg, *bid*) and BC2BIO (*od*) for 14 days
Second-line therapy	
Levofloxacin-based triple therapy	omeprazole (1 mg/kg, *bid*), amoxycillin (50 mg/kg, *bid*) and levofloxacin (20-30 mg/kg, *bid*) for 14 days

*mg: milligrams, *od*: once a day, *bid*: twice a day, *tid*: thrice a day, *qid*: four times a day

Table 2: Distribution of *H. pylori* ER in PP and ITT groups

	Overall ER	PP, UBT positive	PP, UBT negative	ITT, UBT positive	ITT, UBT negative
Adults					
First-line therapies					
Standard triple therapy	61.6% (93/151)	36.2% (42/116)	63.8% (74/116)	45.7% (16/35)	54.3% (19/35)
Standard therapy with probiotic	67.9% (53/78)	29.0% (18/62)	71.0% (44/62)	43.7% (7/16)	56.3% (9/16)
Sequential therapy	66.7% (34/51)	30.6% (11/36)	69.4% (25/36)	40.0% (6/15)	60.0% (9/15)
Levofloxacin-based triple therapy	58.2% (46/79)	33.9% (20/59)	66.1% (39/59)	65.0% (13/20)	35.0% (7/20)
Levofloxacin-based triple therapy with probiotic	61.9% (26/42)	35.7% (10/28)	64.3% (18/28)	42.9% (6/14)	57.1% (8/14)
Azithromycin-based triple therapy	50.0% (22/44)	58.3% (14/24)	41.7% (10/24)	40.0% (8/20)	60.0% (12/20)
Metronidazole-based triple therapy	43.8% (7/16)	54.5% (6/11)	45.5% (5/11)	60.0% (3/5)	40.0% (2/5)
Modified concomitant therapy	72.7% (8/11)	22.2% (2/9)	77.8% (7/9)	50.0% (1/2)	50.0% (1/2)
Second-line therapies					
Quadruple therapy	55.3% (26/47)	36.4% (12/33)	63.6% (21/33)	64.3% (9/14)	35.7% (5/14)
Levofloxacin-based triple therapy	36.4% (4/11)	60.0% (3/5)	40.0% (2/5)	66.7% (4/6)	33.3% (2/6)
Moxifloxacin-based triple therapy	14.3% (1/7)	80.0% (4/5)	20.0% (1/5)	100% (2/2)	-
Third-line therapy					
LOAD therapy	20.0% (1/5)	75.0% (3/4)	25.0% (1/4)	100% (1/1)	-
Children					
First-line therapies					
Standard triple therapy	68.8% (11/16)	31.3% (5/16)	68.8% (11/16)	-	-
Standard therapy with probiotics	45.0% (9/20)	50.0% (7/14)	50.0% (7/14)	66.7% (4/6)	33.3% (2/6)
Second-line therapy					
Levofloxacin-based triple therapy	0.0% (0/10)	100% (8/8)	-	100% (2/2)	-

In pediatric patients, ER with standard triple therapy were higher (PP/ITT 68.8% (11/16)) as compared to standard triple therapy with probiotic (PP 50.0% (7/14), ITT 45.0% (9/20)). Patients, who failed first-line therapies (n=199) were subjected to second-line therapies and

only 75 (37.7%) participated in the follow up. Low ER of second-line therapies were observed in adult patients (levofloxacin-based triple therapy: PP 40.0% (2/5), ITT 36.4% (4/11); moxifloxacin-based triple therapy: PP 20.0% (1/5), ITT 14.3% (1/7)). Quadruple therapy was more successful (PP 63.6% (21/33), ITT 55.3% (26/47)) as a second line in adult patients. Levofloxacin-based triple therapy as a second line was not able to eradicate *H. pylori* infection (PP/ITT 0%, 0/10)) in pediatric patients. LOAD therapy as a third line was not useful (PP 25.0% (1/4), ITT 20.0% (1/5)) among adult patients. Additionally, there was a significant impact of *H. pylori* load as ER in patients with a mild UBT score were higher than for patients with moderate and marked scores (Table 3).

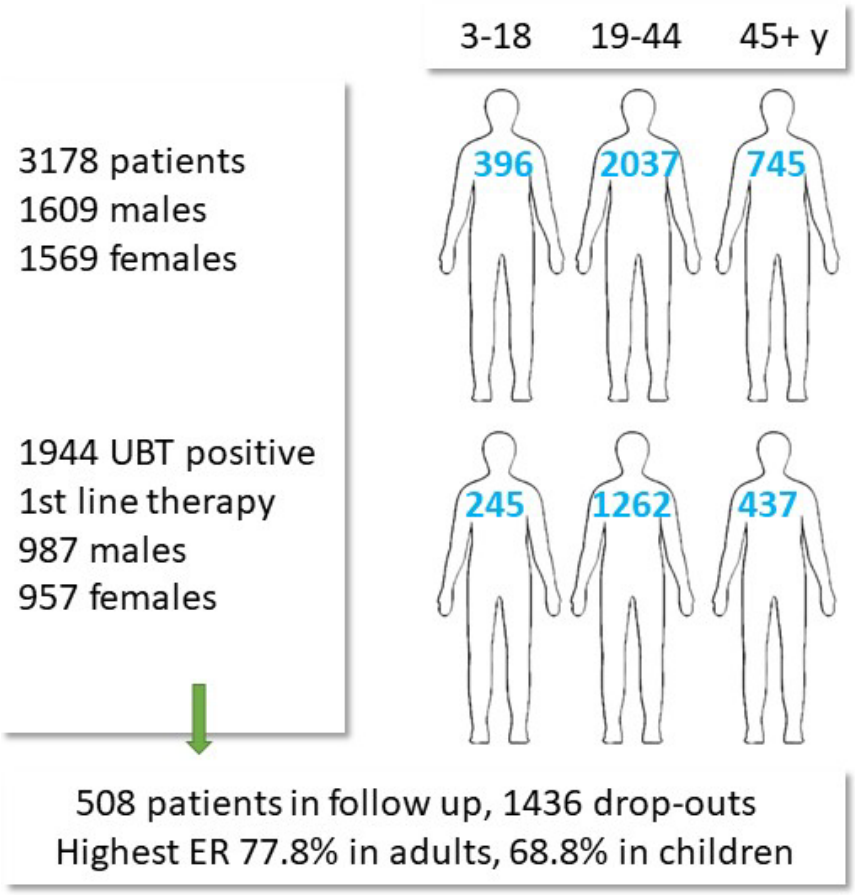


Figure 1: Patient cohort and ER after first-line therapy

Discussion

The prevalence of *H. pylori* infection in populations of developing and developed countries has been reported as 70-90% and 10-30%, respectively [7]. Our previous study found more than 70% prevalence of *H. pylori* infection in the north region of Pakistan [6]. Recently, we have observed a frequency of gastric cancer of about 6.5% among symptomatic patients of gastric diseases in this region of Pakistan [5]. Gastric cancer is the second most common cause of death among all types of cancers in the world.

Standard triple therapy has been accepted universally and is being commonly used to eradicate *H. pylori* infection [9]. In a 2019 research study, high *H. pylori* ER among the patients treated with standard triple therapy (PP 89.6%, ITT 85.2%) and with probiotic (PP 89.2%, ITT 81.9%) were reported [10]. In our present study, values were considerably lower (PP 63.8%,

ITT 61.6%; with probiotic PP 71.0%, ITT 67.9%). In the year 2000 in Pakistan, better ER using standard triple therapy were seen (PP 72%, ITT 67%) [11]. These results are in agreement with the study showing that probiotic-based therapies increase the ER of *H. pylori* infection [12]. The supplementation with specific strains of probiotic such as Ecotec (mixture of four different microbial strains: *Lactobacillus acidophilus,* LA-5*, Bifidobacterium,* BB-12*, Streptococcus thermophiles,* STY-31, *Lactobacillus delbrueckii ssp*. *Bulgaricus,* LBY-27) may be helpful in increasing ER, particularly, when antibiotic therapies are relatively ineffective [13]. In a study conducted in South India, ER for sequential therapy were higher (PP 81.5%, ITT 73.3%) as compared to our report (PP 69.4%, ITT 66.7%) [14]. Levofloxacin-based triple therapy showed promising results (PP 92.4%, ITT 89.3%) [15]. Another study suggests that it could be used as an empirical therapy as well as a rescue therapy in patients after multiple eradication failure [16]. In contrast to these reports, the ER of levofloxacin-based triple therapy in our study were not substantial (PP 66.1%, ITT 58.2%). Additionally, levofloxacin-based triple therapy with probiotic (PP 64.3%, ITT 61.9%) had nearly the same ER when compared with therapy without probiotic. ER using azithromycin-based triple therapy (PP 77.1%, ITT 79.5%) were higher than the ER observed in our study (PP 41.7%, ITT: 50.0%) [8].

ER (PP 45.5%, ITT 43.8%) measured in the current study using metronidazole-based triple therapy were also lower than the rates observed by others (PP 94.7%, ITT 91.3%) [17]. Modified concomitant therapy as a first line showed satisfactory results in the present investigation with ER (PP 77.8%, ITT 72.7%) being higher than for other therapies, but it did not reach those of another study (PP 93.1%, ITT 79.4%) [18].

Table 3: Impact of *H. pylori* load using UBT values on ER (p = 0.000)

Pre-treatment UBT values	***H. pylori* ER**
Mild (3-15)	69.7% (161/231)
Moderate (15.1-30)	56.7% (102/180)
Marked (>30.1)	49.0% (49/100)
Total	61.6% (312/511)

In a study conducted recently, quadruple and levofloxacin-based triple therapies used as second-line therapies to treat *H. pylori* infected patients showed overall ER of 66.7% and 55.6%, respectively [19]. In the present investigation among adult patients, quadruple therapy (PP 63.6%) and levofloxacin-based triple therapy (PP 40.0%) showed comparatively low ER when used as second line. Moxifloxacin-based triple therapy as second line showed good ER (PP 73.8%) in the previous study [20]. In contrast to our report, ER (PP 20.0%) using moxifloxacin-based triple therapy as a second line was not as satisfactory as expected, and this therapy should not be used as a second-line therapy to treat *H. pylori* infection. LOAD therapy as a third line was also unsuccessful.

Among *H. pylori* infected pediatric patients, ER after standard triple therapy and with probiotic according to PP analysis were 64.5% and 81.8%, respectively [21]. The results observed in our study among children were different (PP 68.8% and 50.0%), possibly due to strain difference (*Bacillus clausii)* in probiotic. Levofloxacin-based triple therapy failed in pediatric patients when used as a second line. There is a need for more research to find better options. All the ET used in this study showed less ER when compared with the rates reported in other studies from different regions of the world, which might be due to increased resistance in *H. pylori* isolates against commonly used antibiotics [5]. In order to reduce the antibiotic

resistance in *H. pylori* and other bacterial strains, test and treat strategies should be implemented. Additionally, the *H. pylori* antibiotic susceptibility profiling guided therapy may prove highly valuable even with multiple prior treatment failures for *H. pylori* infection. This avoids the use of unnecessary anti-bacterial drugs by identifying patients expected to benefit from ET to achieve very high success rates and good adherence [22].

Conclusion

There are reports of high antibiotics resistance among isolates of *H. pylori* in Pakistan [5]. A variety of factors are responsible for *H. pylori* eradication failure including poor diet and patient compliance, high bacterial load, internalization of bacteria, stomach acidity, nucleotide polymorphisms, antibiotic washout, and most significantly antibiotic resistance. A suitable *H. pylori* ET is of great concern including the need for determining ER in local settings. Standard triple therapy with probiotic and modified concomitant therapy as first-line and quadruple therapy as a second line showed best *H. pylori* ER in adult gastric disease patients. In pediatric patients, standard triple therapy was more effective as a first-line. Eradication of *H. pylori* is an important aspect of managing gastroduodenal pathologies.

Acknowledgements

We all remember our beloved PIMS Gastroenterology colleague, Dr. Rizwan Ali (late) for his sincere efforts of this *H. pylori* eradication study. We also appreciate the contribution of our PIMS colleagues Dr. Haseeb Noor, Dr. Saqib Hussain and Dr. Furqan Tahir and staff member of PINSCTECH, Kashif Siddique, in helping us for collection of data and guidance of patients.

Conflicts of Interest

The Authors declared no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Gisbert JP, McNicholl AG. Optimization strategies aimed to increase the efficacy of H. pylori eradication therapies. *Helicobacter*. 2017, 22(4):e12392.

[2] Smelt HJ, Smulders JF, Gilissen LP, et al. Influence of *Helicobacter pylori* infection on gastrointestinal symptoms and complications in bariatric surgery patients: a review and meta-analysis. *Surgery for Obesity and Related Diseases*. 2018, 14(10):1645-1657.

[3] Camcioglu Y, Okur DS, Aksaray N, et al. Factors affecting physicians' perception of the overuse of antibiotics. *Médecine et Maladies Infectieuses*. 2020, 50(8):652-657.

[4] Ram M R, Teh X, Rajakumar T, et al. Polymorphisms in the host CYP2C19 gene and antibiotic-resistance attributes of *Helicobacter pylori* isolates influence the outcome of triple therapy. *Journal of Antimicrobial Chemotherapy*. 2019, 74(1):11-16.

[5] Rasheed F, Campbell BJ, Alfizah H, et al. Analysis of clinical isolates of *Helicobacter pylori* in Pakistan reveals high degrees of pathogenicity and high frequencies of antibiotic resistance. *Helicobacter*. 2014, 19(5):387-399.
[6] Rasheed F, Yameen A, Ahmad T, et al. Rate of active *Helicobacter pylori* infection among symptomatic patients of Pakistan. *Malaysian Journal of Pathology*. 2017, 39(1):69-72.
[7] Wang W, Jiang W, Zhu S, et al. Assessment of prevalence and risk factors of *Helicobacter pylori* infection in an oilfield Community in Hebei, China. *BMC Gastroenterology*. 2019, 19(1):1-8.
[8] Khoshnood A, Hakimi P, Salman-Roghani H, et al. Replacement of clarithromycin with azithromycin in triple therapy regimens for the eradication of *Helicobacter pylori*: A randomized clinical trial. *Journal of Medicine and Life*. 2014, 7(2):254.
[9] Malfertheiner P, Megraud F, O'morain CA, et al. Management of *Helicobacter pylori* infection—the Maastricht V/Florence consensus report. *Gut*. 2017, 66(1):6-30.
[10] Chang YW, Park YM, Oh CH, et al. Effects of probiotics or broccoli supplementation on *Helicobacter pylori* eradication with standard clarithromycin-based triple therapy. *The Korean Journal of Internal Medicine*. 2020, 35(3):574.
[11] Wong BC, Chang FY, Abid S, et al. Triple therapy with clarithromycin, omeprazole, and amoxicillin for eradication of *Helicobacter pylori* in duodenal ulcer patients in Asia and Africa. *Alimentary pharmacology & therapeutics*. 2000, 14(11):1529-1535.
[12] Fallone CA, Moss SF, Malfertheiner P. Reconciliation of recent *Helicobacter pylori* treatment guidelines in a time of increasing resistance to antibiotics. *Gastroenterology*. 2019, 157(1):44-53.
[13] Dang Y, Reinhardt JD, Zhou X, et al. The effect of probiotics supplementation on *Helicobacter pylori* eradication rates and side effects during eradication therapy: a meta-analysis. *PloS one*. 2014, 9(11):e111030.
[14] Ashokkumar S, Agrawal S, Mandal J, et al. Hybrid therapy versus sequential therapy for eradication of *Helicobacter pylori*: a randomized controlled trial. *Journal of Pharmacology and Pharmacotherapeutics*. 2017, 8(2):62-67.
[15] Latif S, Akther N, Amjed S, et al. Efficacy of standard triple therapy versus levofloxacin based alternate therapy against *Helicobacter pylori* infection. *The Journal of Pakistan Medical Association*. 2018, 68:1295.
[16] Lim JH, Kim SG, Song JH, et al. Efficacy of levofloxacin-based third-line therapy for the eradication of *Helicobacter pylori* in peptic ulcer disease. *Gut and Liver*. 2017, 11(2):226.
[17] Adachi T, Matsui S, Watanabe T, et al. Comparative study of clarithromycin-versus metronidazole-based triple therapy as first-line eradication for *Helicobacter pylori*. *Oncology*. 2017, 93(Suppl. 1):15-9.
[18] Kim SJ, Chung JW, Woo HS, et al. Two-week bismuth-containing quadruple therapy and concomitant therapy are effective first-line treatments for *Helicobacter pylori* eradication: a prospective open-label randomized trial. *World Journal of Gastroenterology*. 2019, 25(46):6790.
[19] Shoosanglertwijit R, Kamrat N, Werawatganon D, et al. Real-world data of *Helicobacter pylori* prevalence, eradication regimens, and antibiotic resistance in Thailand, 2013–2018. *Journal Of Gastroenterology And Hepatology Open.* 2020, 4(1):49-53.
[20] Kim SE, Roh JH, Park MI, et al. Effect of 7-day bismuth quadruple therapy versus 14-day moxifloxacin triple therapy for second-line *Helicobacter pylori* eradication therapy. *The Korean Journal of Gastroenterology.* 2019, 73(1):26-34.
[21] Ustundag GH, Altuntas H, Soysal YD, et al. The effects of synbiotic “Bifidobacterium lactis B94 plus Inulin” addition on standard triple therapy of *Helicobacter pylori* eradication in children. *Canadian Journal of Gastroenterology and Hepatology*. 2017, 2017:8130596.
[22] Yu L, Luo L, Long X, et al. Susceptibility-guided therapy for *Helicobacter pylori* infection treatment failures. *Therapeutic Advances in Gastroenterology*. 2019, 1756284819874922.