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High-dose dual therapy for *Helicobacter pylori* eradication inducing less impact on the gut microbiota

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Abstract

Background *Helicobacter pylori* (*H. pylori*) eradication regimens may have different effects on the gut microbiota. Few studies have analyzed the safety of high-dose dual therapy (HDDT) from a micro-ecological perspective. This study aimed to compare the impact of *H. pylori* eradication with HDDT and bismuth quadruple therapy (BQT) on gut microbiota.

Patients and methods H. Pylori-infected treatment-naive patients were recruited and screened from September 2023 to April 2024 and randomly assigned to the HDDT group (esomeprazole 20 mg, amoxicillin 750 mg, qid, 14 days) or BQT group (esomeprazole 20 mg, amoxicillin 1000 mg, clarithromycin 500 mg, and bismuth potassium citrate 600 mg, bid, 14 days). Fresh stool specimens were collected and stored before treatment and at week 2 and week 8 after treatment. The diversity and composition of the gut microbiota were compared and analyzed in both groups using 16 S rRNA gene sequencing.

Results Forty-nine *H. pylori* positive patients were enrolled and randomly assigned to either the HDDT ($n=24$) or the BQT group ($n=25$) group. Compared with baseline, alpha and beta diversities significantly changed at week 2 after receiving BQT and did not recover fully at week 8. However, in the HDDT group, the diversities at week 2 changed mildly without statistical significance, compared to baseline. Additionally, a greater number of species had alterations in their abundances in the BQT group compared to the HDDT group at week 2. However, the abundances of these species were restored to their previous levels at week 8 in both the HDDT and BQT groups.

Conclusions Compared to BQT, HDDT exerted less impact on the diversity and composition of the gut microbiota.

Clinical trial registration ChiCTR2100053268.

Keywords *Helicobacter pylori*, Gut microbiota, High-dose dual therapy, Bismuth quadruple therapy

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Introduction

Helicobacter pylori (*H. pylori*) is an important human pathogen that is associated with gastric cancer, mucosa-associated lymphoid tissue (MALT) lymphoma, peptic ulcer disease, and various other conditions [1–3]. Domestic and foreign guidelines have indicated that regardless of symptoms or complications, *H. pylori*-infected patients should receive eradication treatment [4, 5].

The proposal to eliminate *H. pylori* has given rise to concerns about the disruption of the gut microbiota caused by *H. pylori* eradication. A previous study demonstrated that there were substantial differences in the extent and severity of perturbations among different *H. pylori* eradication regimens [6, 7]. The gut microbiota plays a crucial role in human health, and dysbiosis is associated with various diseases [8, 9]. Consequently, when selecting eradication regimens for patients infected with *H. pylori*, it is essential to take into account the impact of these regimens on the gut microbiota.

In recent years, high-dose dual therapy (HDDT), comprising a potent acid suppressant such as esomeprazole or vonoprazan and amoxicillin, has been emerging rapidly. This is due to its benefits of high eradication efficacy and a relatively lower incidence of adverse side effects [10–13]. In the latest Chinese national clinical practice guideline, HDDT has been recommended for initial eradication treatment in individuals with *H. pylori* infection [14]. Nevertheless, at present, there are few studies exploring the impact of HDDT on the gut microbiota. Thus, the aim of this study was to evaluate the impact of HDDT on the gut microbiota, and to compare the alterations in the gut microbiota following *H. pylori* eradication using HDDT and BQT.

Materials and methods

Study design and participants

This research was carried out in Hainan Province, China, spanning from September 2023 to April 2024. The inclusion criteria were established as follows: (1) both genders within the age range of 18 to 70 years; (2) diagnosis of *H. pylori* infection via the carbon-13/14 urea breath test ($^{13}C/^{14}C$-UBT). Patients meeting any of the following exclusion criteria were excluded: (1) patients who had previously undergone *H. pylori* eradication treatment; (2) individuals with a history of gastrointestinal surgery or the presence of severe gastrointestinal conditions, such as peptic ulcers, gastrointestinal bleeding, or malignant tumors; (3) presence of severe systemic diseases; (4) use of antibiotics, acid suppressants, probiotics, or or non-steroidal anti-inflammatory drugs (NSAIDs) within the previous month; (5) allergy to penicillin or other medications employed in this study; (6) pregnant or lactating women; (7) participating in other clinical trials; and (8) patients who were deemed unqualified for enrollment following assessment by the researchers. All patients provided informed consent and the study protocol was approved by the Institutional Ethics Board of Wenchang People's Hospital ([2021]-1) and registered in the China Clinical Trials Registry (ChiCTR2100053268).

Interventions and follow-up

All participants were randomly assigned to either the HDDT or BQT group in a 1:1 ratio using a computer-generated randomization list. The HDDT regimen comprised esomeprazole (Zhengda Tianqing Pharmaceutical Co., Ltd, Jiangsu, China) at a dosage of 20 mg and amoxicillin (Gener-sanyang Pharmaceutical Co., Ltd., Hainan, China) at 750 mg, both to be taken four times daily for 14 days. Subjects were instructed to consume esomeprazole half an hour prior to breakfast, lunch, dinner, and bedtime, while amoxicillin was to be taken after breakfast, lunch, dinner, and bedtime. The BQT regimen consisted of esomeprazole (Zhengda Tianqing Pharmaceutical Co., Ltd, Jiangsu, China) 20 mg, amoxicillin (Gener-sanyang Pharmaceutical Co., Ltd, Hainan, China) 1000 mg, clarithromycin (Shandong Xinhua Pharmaceutical Co., Ltd, Shandong, China) 500 mg, and bismuth potassium citrate (Youcare Pharmaceutical Group Co., Ltd, Beijing, China) 600 mg, all to be taken twice daily for 14 days. Esomeprazole and bismuth potassium citrate were taken half an hour before breakfast and dinner, and amoxicillin and clarithromycin were administered after breakfast and dinner. Throughout the course of this study, with the exception of the above 14 days of medication, the use of other medications such as antibiotics, gastric acid-suppressing agents, or probiotics was prohibited. ^{14}CUBT was used to determine the *H. pylori* status 6 weeks after the completion of treatment.

Fecal sampling

Fresh stool samples were collected at three time points: at the baseline (before treatment), at week 2 (following the completion of 14 days of treatment), and at week 8 (6 weeks after 14 days of treatment). Subjects were instructed to collect their stool using a pre-prepared standard sterile specimen collection receptacle and then required to transport the collected stools to the hospital within a 2-hour time frame. Once at the hospital, the stool specimens were promptly transferred to a -80℃ refrigerator immediately and stored there until the time of DNA extraction.

DNA extraction, PCR amplification, and 16 S rRNA gene sequencing

Microbial DNA from stool samples was extracted using the OMEGA Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions and stored at -20 °C prior to analysis. DNA purity

and concentration were determined using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. PCR amplification of the 16 S rRNA gene V3–V4 hyper-variable regions was conducted using the forward primer 338 F (5'-ACTCCTACGGGAGGCAGCA-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). All amplifications were performed in the PCR component containing 5 μl of buffer (5×), 0.25 μl of Fast pfu DNA polymerase (5 U/μl), 2 μl (2.5 mM) of dNTPs, 1 μl (10 μM) of each forward and reverse primer, 1 μl of DNA template, and 14.75 μl of dd H_2O. The PCR conditions were as follows: 98 °C for 5 min; 25 cycles of 98 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s; and a final extension step at 72 °C for 5 min. PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After purification and quantification, amplicons were standardized to equimolar levels and pair-end sequencing was performed using the Illumina MiSeq platform with the MiSeq Reagent Kit v3 at Bioyi Biotechnology Co., Ltd., Wuhan, China.

Microbiota sequencing data analysis

Microbiota sequencing data were processed by Quantitative Insights into Microbial Ecology (QIIME). Raw sequence data were demultiplexed using the demux plugin following by primers cutting with cutadapt plugin. Sequences were then quality-filtered, denoised, merged, and chimeras removed using the DADA2 plugin [15]. Amplicon sequence variants (ASVs) were aligned with multiple alignment using fast Fourier transform (MAFFT) and used to construct a phylogeny using fasttree2 [16]. The diversity plugin was used to estimate alpha diversity indices and beta diversity metrics. Taxonomy was assigned to the OTUs using the classify-sklearn naïve Bayes taxonomy classifier in the feature-classifier plugin against the SILVA Release 132 database [17].

Bioinformatics and statistical analysis

QIIME and R packages (version 3.2.0) were employed for the analysis of microbiome bioinformatics [18]. Alpha diversity (Chao1, Shannon, and Simpson indices) and beta diversity were calculated using the phyloseq package [19]. The statistical significance of the Chao1, Shannon, and Simpson indices among the groups was assessed using Wilcoxon rank- sum test or Kruskal–Wallis test, and visualized as box plots. Beta diversity was evaluated through principal coordinate analysis (PCoA) based on weighted UniFrac distance metrics and analysis of similarities (ANOSIM). The Wilcoxon rank- sum test and Kruskal–Wallis test were used to compare the differences in the relative abundance of bacterial taxa both within and between groups. The linear discriminant analysis effect size (LEfSe) analysis was adopted to identify the differential taxa between groups. The functional profiles of the gut microbiota were estimated using Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Moreover, Welch's t test was carried out to compare the abundances of metabolic pathways between two groups.

Continuous clinical parameters are expressed as mean ± standard deviation (SD), and categorical variables are shown as frequencies and percentages. For the comparison of continuous variables between the two groups, Student's t-test was utilized. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test.

Results

Baseline demographics and follow-up

A total of 105 patients were initially screened and 56 were excluded from the study. Consequently, 49 patients who tested positive for *H. pylori* were enrolled and then randomly assigned to the HDDT group ($n = 24$) or the BQT ($n = 25$) group. There was no significant difference in the baseline data between the two groups (Table 1). The incidence rates of adverse events were notably higher in the BQT group compared to the HDDT group ($P < 0.001$). The specific details regarding these adverse events are illustrated in Table 2. During the treatment period, one patient in the HDDT group was excluded due to non-compliance with the per-protocol treatment, and one patient in the BQT group ceased taking the medication because of side effects. One patient in the HDDT group experienced treatment failure, while the remaining patients achieved successful eradication of the *H. pylori* infection.

In total, 137 fecal samples were collected. At baseline, 49 fecal samples were gathered, with 25 samples coming from the BQT group and 24 samples from the HDDT group. At week 2, 47 fecal samples were collected. By week 8, after excluding three patients who took antibiotics for respiratory tract infections and three patients who were lost to follow-up, 41 fecal samples were obtained.

The diversity of gut microbiota before and after treatment

After quality-filtered, denoised, merged, and chimeras removed, a total of 7,389,898 clean reads (constituting 2068 ASVs) sequenced from 137 samples were left for further in-depth analysis. The number of sequence reads per sample varied within the range from 33,629 to 81,458. In the BQT group, the number of ASVs obtained at week 0 was 334, at week 2 was 284, and at week 8 was 356. In the HDDT group, the number of obtained ASVs was 372 at week 0. This then changed to 353 at week 2. And at

Table 1 Baseline characteristics of study patients

Baseline factors	HDDT group (*N* = 24)	BQT group (*N* = 25)	*P*-value
Sex (female: male)	15:9	14:11	0.644
Age (years)	41.8 ± 12.3	47.5 ± 13.9	0.131
Body mass index (kg/m^2)	23.5 ± 2.5	24.2 ± 3.4	0.441
Marital status (single or divorced: married)	3:21	4:21	1.000
Place of residence (suburban area: urban area)	16:8	21:4	0.158
Drinking water (tap water: well water)	364:6	365:5	0.761
Style of dining (dining at home: dining out)	20:4	22:3	0.953
Education status (Junior high school and below: high school: college or more)	9:8:7	10:10:5	0.745
Smoking (yes: no)	21:3	19:6	0.503
Drinking (yes: no)	23:1	21:4	0.370
Digestive Symptoms	18(75.0%)	15(60.0%)	0.263
Abdominal pain	10(41.7%)	6(24.0%)	0.187
Heartburn	1(4.2%)	2(8.0%)	1.000
Acid reflux	7(29.2%)	3(12.0%)	0.256
Abdominal distension	5(20.8%)	6(24.0%)	0.791
Belching	3(12.5%)	2(8.0%)	0.962
Endoscopy diagnosis			
Superficial gastritis	8(33.3%)	8(32.0%)	0.921
Erosive/Hemorrhagic gastritis	16(66.7%)	14(56.0%)	0.444
Atrophic gastritis/Intestinal metaplasia	3(12.5%)	4(16.0%)	1.000
Gastric polyp	1(4.2%)	3(12.0%)	0.632
Family history of gastric carcinoma	2(8.3%)	0(0.0%)	0.452
Chronic disease history	3(12.5%)	3(12.0%)	1.000
Hypertension	1(4.2%)	1(4.0%)	1.000
Diabetes mellitus	2(8.3%)	0(0.0%)	0.452
Hyperlipidemia	1(4.2%)	2(8.0%)	1.000
Long-term medication history	2(8.3%)	1(4.0%)	0.971
Antihypertensive drugs	1(4.2%)	1(4.0%)	1.000
Hypoglycemic agents	1(4.2%)	0(0.0%)	0.984
History of antibiotics use in two years	13(54.2%)	11(44.0%)	0.670
Penicillin	12(50.0%)	8(32.0%)	0.200
Cephalosporin	9(37.5%)	6(24.0%)	0.305
Ofloxacin	3(12.5%)	0(0.0%)	0.632
Metronidazole/ tinidazole	1(4.2%)	1(4.0%)	1.000

Table 2 Adverse events

Adverse event rate	HDDT group	BQT group	*P*-value
Patients with adverse events	1(4.2%)	13(52.0%)	< 0.001
Mild	1(4.2%)	8(32.0%)	
Moderate	0(0.0%)	3(12.0%)	
Severe	0(0.0%)	2(8.0%)	
Variety of adverse events			
Bitter taste	1(4.2%)	6(24.0%)	
Diarrhea	0(0.0%)	5(20.0%)	
Nausea	0(0.0%)	4(16.0%)	
Darkened tongue	0(0.0%)	3(12.0%)	
Vomiting	0(0.0%)	2(8.0%)	
Stomachache	0(0.0%)	1(4.0%)	
Weak	0(0.0%)	1(4.0%)	

week 8, the number stood at 369. The rarefaction analysis was conducted for each fecal sequence dataset, resulting in the generation of rarefaction curves. As depicted in Supplementary Figure S1, when the sequencing depth reached approximately 5,000 sequence reads, the rarefaction curves for each individual sample gradually leveled off, reaching a plateau. This finding implies that the read coverage was adequate and sufficient to capture the majority of the bacterial diversity present within each sample, ensuring that the subsequent analyses based on these sequenced data would be able to comprehensively represent the gut microbiota characteristics to a reasonable extent.

As shown in Fig. 1a–c, alpha diversity in the BQT group significantly decreased at week 2 (Chao1, $P<0.0001$; Shannon, $P<0.0001$; Simpson, $P<0.0001$). At week 8, alpha diversity increased compared with week 2, but still did not return to baseline (Chao1 index, $P<0.0001$; Shannon index, $P=0.011$; Simpson index, $P=0.021$). Additionally, there were significant differences in the beta diversity of gut microbiota at week 2 ($R=0.538$, $P=0.001$) and week 8 ($R=0.147$, $P=0.002$) compared to baseline (Fig. 2a). However, in the HDDT group, compared with the baseline, the alpha diversity (Chao1, Simpson and Shannon index) mildly decreased at week 2 and then gradually increased. These changes were not statistically significant ($P>0.05$) (Fig. 1d–f). Compared with baseline,

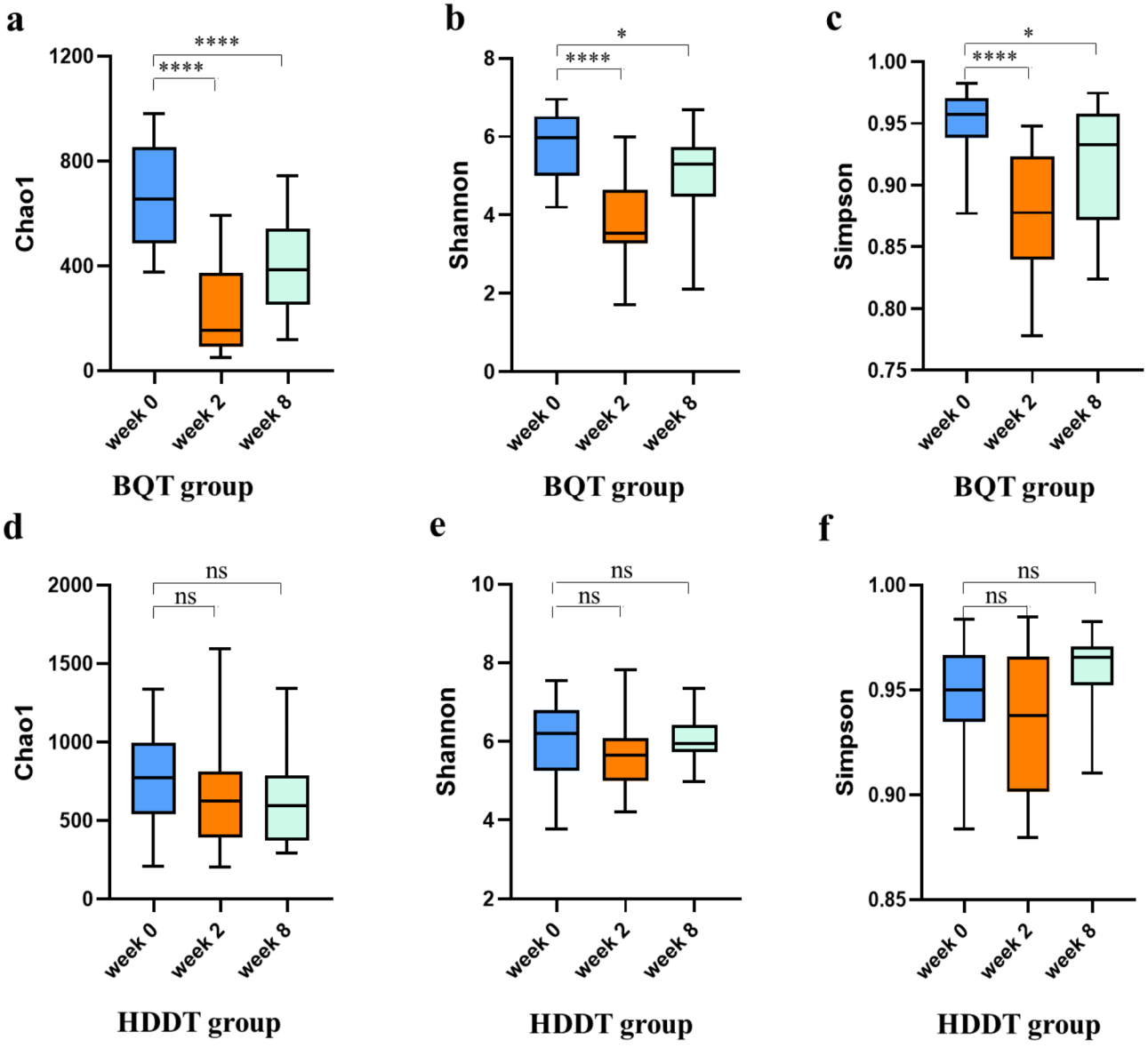

Fig. 1 Changes in the alpha diversity at different time points among HDDT group and BQT group. (**a**-**c**) the BQT group, (**d**-**f**) the HDDT group. *$0.01 \le P<0.05$, ****$P<0.0001$, ns, not significant

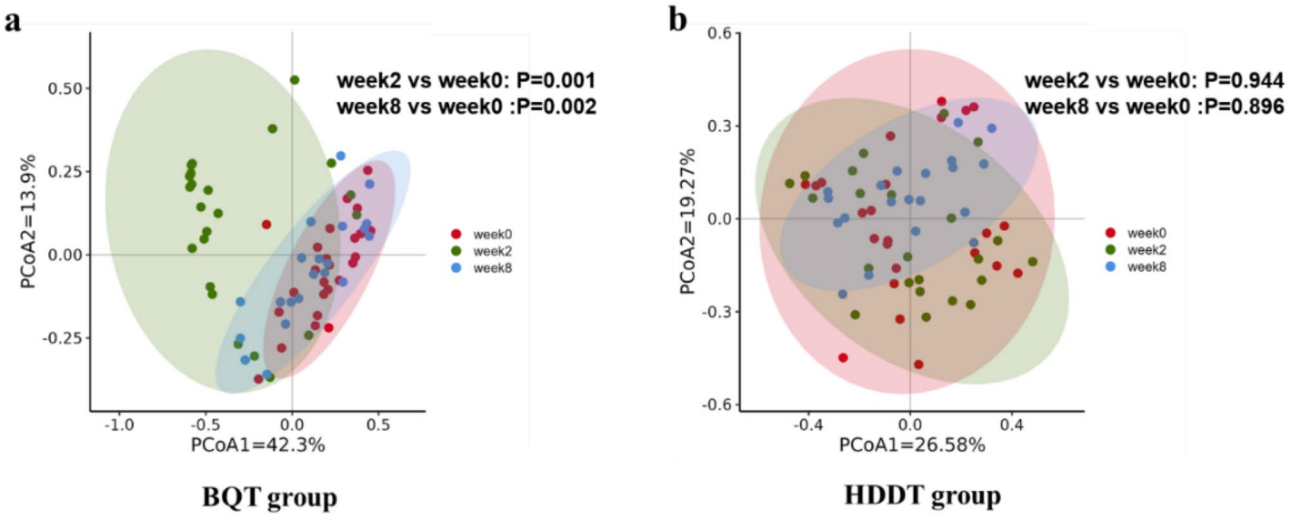

Fig. 2 Changes in the beta diversity at different time points among groups. (**a**) the BQT group, (**b**) the HDDT group

no significant difference in beta diversity was found at week 2 (R = -0.039, P = 0.944) and week 8 (R = -0.034, P = 0.896) in the HDDT group (Fig. 2b).

Analysis of the diversity at the same time point among BQT group and HDDT group

To further compare the differences in the effects of BQT and HDDT, we analyzed the diversity and beta diversity of the gut microbiota at the same time point between the two groups. At week 0, There was no significant differences in the Chao1, Shannon, and Simpson index among the two groups (all $P > 0.05$) (Fig. 3a–c). The alpha diversity of the BQT group at week 2 was significantly lower than that of the HDDT group (all $P < 0.0001$) (Fig. 3d–f), and the differences persisted up to week 8 (Chao1 index, $P = 0.0045$; Shannon index, $P = 0.0006$; Simpson index, $P = 0.0008$) (Fig. 3g–i).

As shown in Fig. 4a, no difference in beta diversity was detected among BQT group and HDDT group at baseline ($R = 0.055$, $P = 0.058$). However, the PCoA revealed distinct clustering, and ANOSIM also confirmed that the differences were significant at week 2 ($R = 0.349$, $P = 0.001$) (Fig. 4b). At week 8, the beta diversity showed recovery, but the difference between groups remained significant ($R = 0.127$, $P = 0.029$) (Fig. 4c).

The composition of gut microbiota before and after treatment

We also analyzed the effect of *H. pylori* eradication treatment on the composition of the gut microbiota at the phylum and genus levels. In the BQT group, Firmicutes (61.13%) and Bacteroidetes (23.11%) were the most abundant phyla at the baseline. At week 2, the relative abundance of Firmicutes and Bacteroidetes in the BQT group decreased substantially, and the relative abundance of Proteobacteria significantly increased. The most abundant phyla in the BQT group became Firmicutes (44.51%) and Proteobacteria (43.86%). At week 8, the relative abundances of Firmicutes (58.79%) and Bacteroidetes (21.39%) were largely restored, and the composition of the phyla was similar to that at baseline (Fig. 5a). However, HDDT group had similar phylum profiles across the time-points, and the relative abundance of Firmicutes and Bacteroidota did not vary by much over time, while Proteobacteria increased at week 2, then restored at week 8 (Fig. 5c).

The composition of the gut microbiota at the genus level among the two groups at different time points are presented in Table S1. The top 10 genera with the highest relative abundances of the two groups are shown in Fig. 5b and d. In the BQT group, the most abundant genera were *Faecalibacterium* (10.52%) and *Phocaeicola_A* (10.12%) at the baseline. However, a significant perturbation of the gut microbiota was observed at week 2. When compared to the baseline, the relative abundances of *Klebsiella* and *Streptococcus* witnessed a notable increase, reaching 36.98% and 14.11% respectively. In contrast, the relative abundances of *Faecalibacterium* and *Phocaeicola_A* decreased to 3.42% and 4.27% respectively at this time point. At week 8, the relative abundance of most genera tended to restore to the baseline, while the relative abundances of *Faecalibacterium*, *Phocaeicola_A*, *Phascolarctobacterium_A* remained lower than those at the baseline, and the relative abundances of *Klebsiella* and *Streptococcus* continued to be higher than their baseline values. However, when compared with the BQT group, the relative abundances of different genera in the HDDT group exhibited relatively minor variations across the different time points.

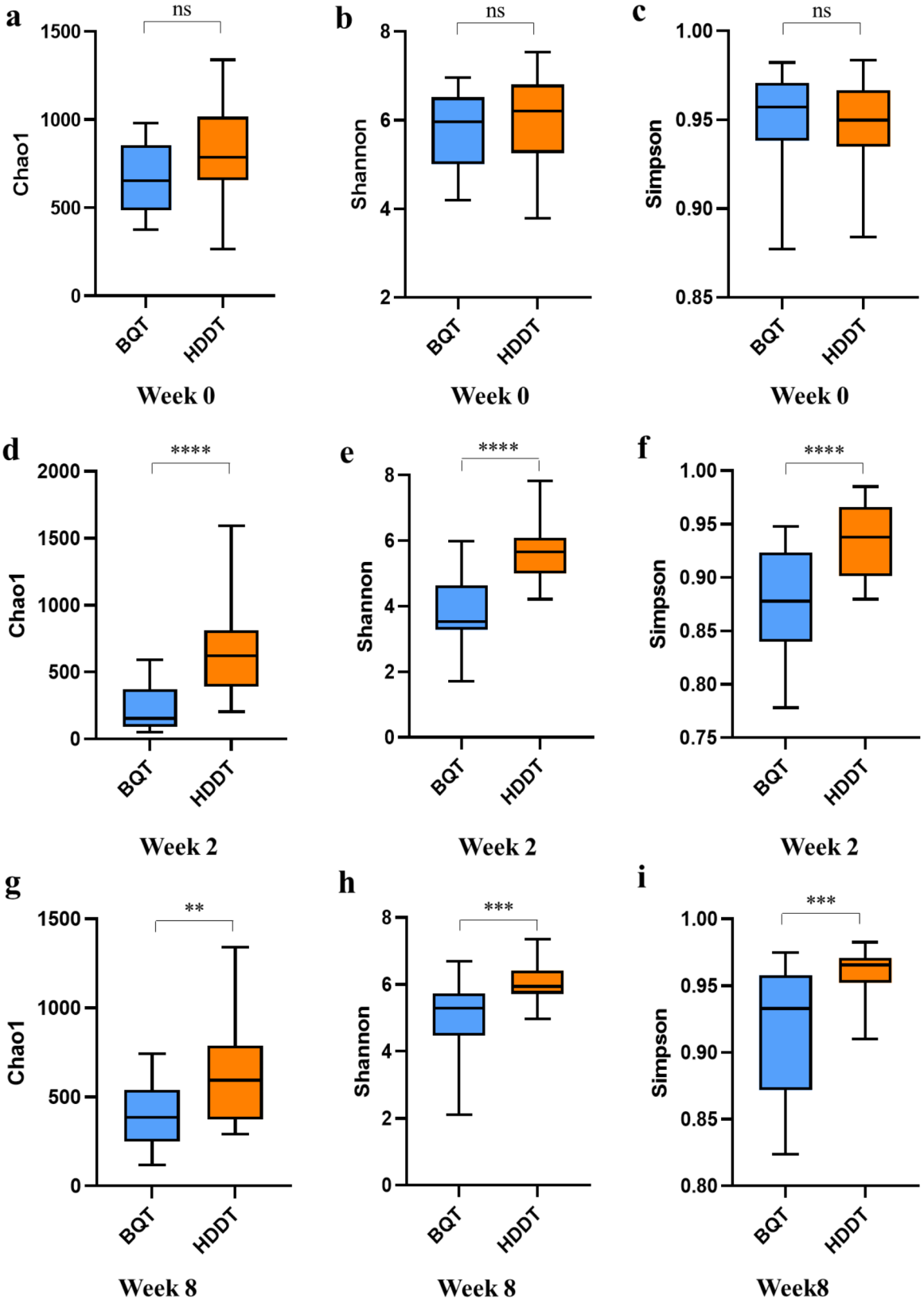

Fig. 3 Comparison of the alpha diversity at the same time point among groups. (**a**-**c**) at week 0, (**d**-**f**) at week 2, (**g**-**i**) at week 8. $^{**}0.001 \le P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns, not significant

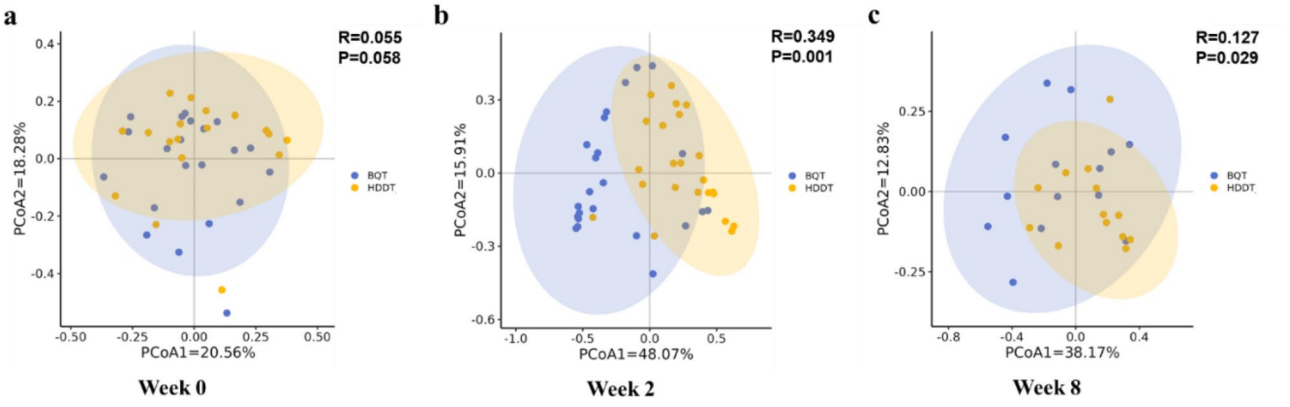

Fig. 4 Comparison of the beta diversity at the same time point among groups. (**a**) at week 0, (**b**) at week 2, (**c**) at week 8

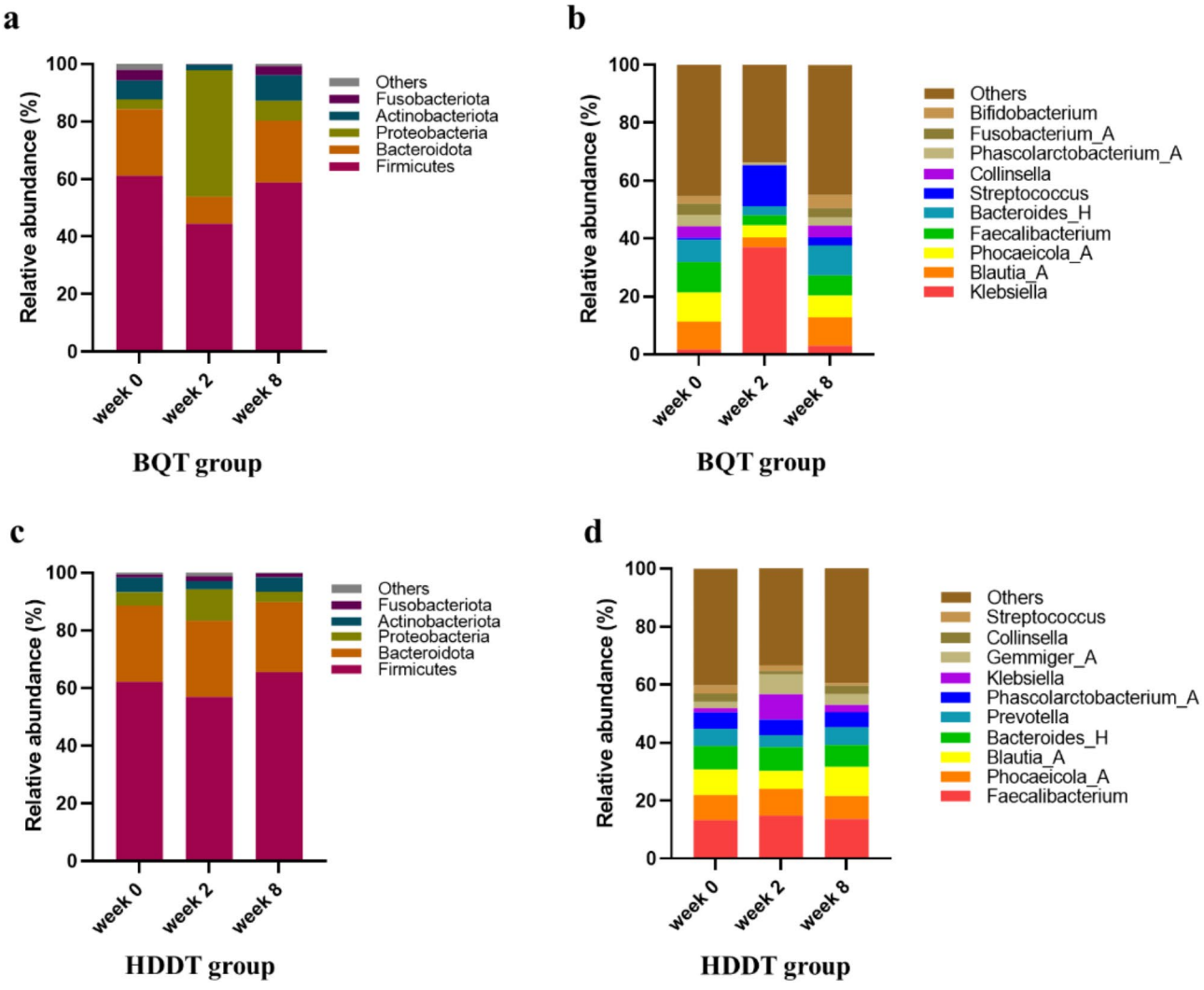

Fig. 5 The relative abundance of the dominant phylum and genus at different time points among groups. (**a**, **c**) at phylum level, (**b**, **d**) at genus level

To gain a more in-depth understanding of the disparity between the impacts of the two treatment regimens on the gut microbiota at the species level, an analysis of the species - level changes across different time points within the two groups was carried out (as presented in Table S2). The species with relative abundances greater than 1% that exhibited significant shifts are depicted in Fig. 6. At week 2, the BQT group demonstrated substantial alterations

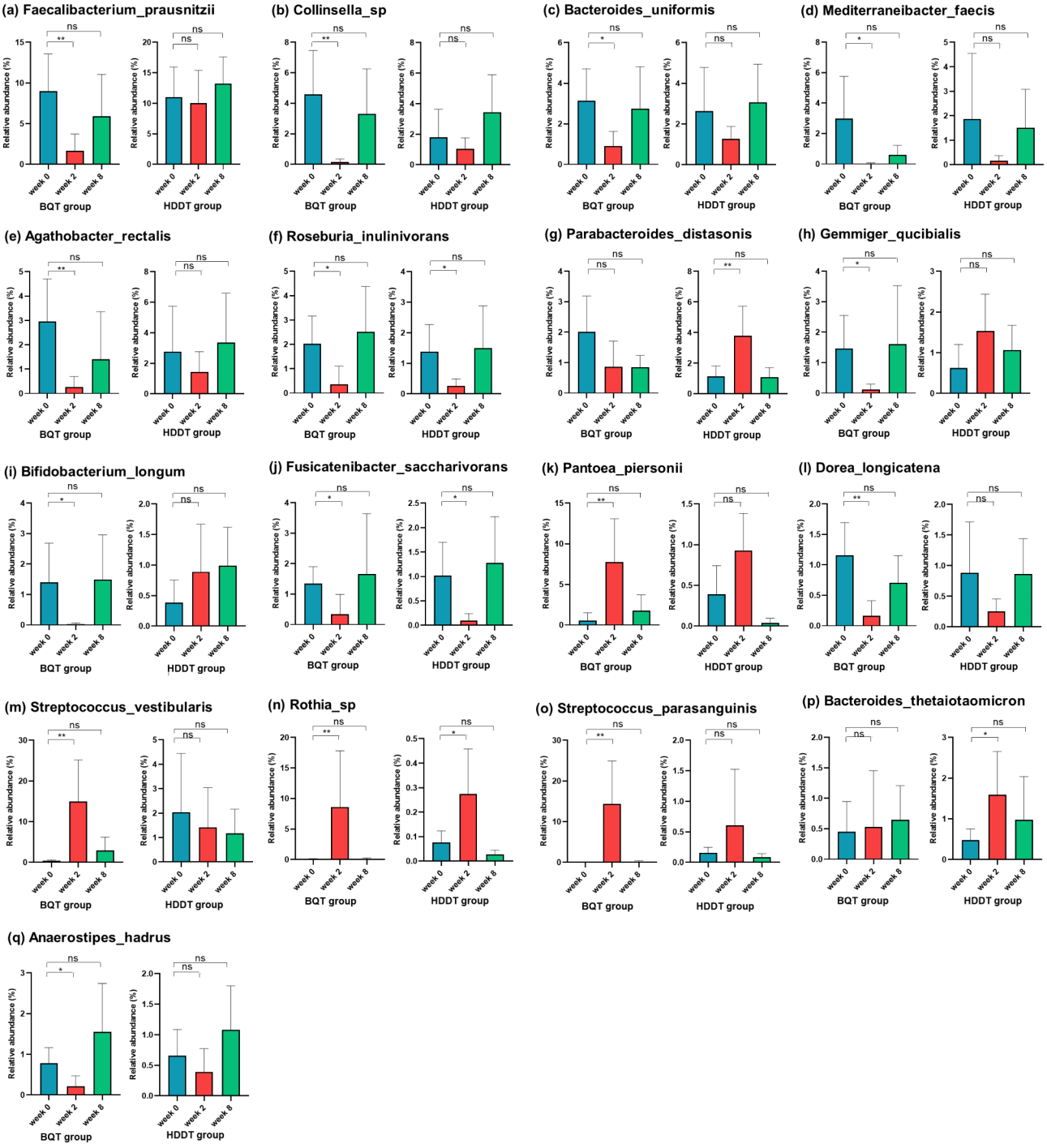

Fig. 6 the change of species with relative abundances > 1% at different time points among groups. *$0.01 \le P < 0.05$, **$0.001 \le P < 0.01$, ns, not significant

in the abundances of fifteen species: *Faecalibacterium_ prausnitzii* (3.78% vs. 9.01%, $P = 0.0058$), *Collinsella_sp* (0.15% vs. 4.59%, $P = 0.0038$), *Bacteroides_H_uniformis* (0.91% vs. 3.13%, $P = 0.0132$), *Mediterraneibacter_ faecis* (0.03% vs. 2.99%, $P = 0.0393$), *Agathobacter_rectalis* (0.26% vs. 2.97%, $P = 0.0043$), *Roseburia_inulinivorans* (0.37% vs. 2.03%, $P = 0.0170$), *Gemmiger_ qucibialis* (0.13% vs. 1.46%, $P = 0.0206$), *Bifidobacterium_longum* (0.02% vs. 1.39%, $P = 0.0412$), *Fusicatenibacter_saccharivorans* (0.35% vs. 1.35%, $P = 0.0187$), *Pantoea_ piersonii* (7.75% vs. 0.56%, $P = 0.0064$), *Dorea_longicatena* (0.16% vs. 1.16%, $P = 0.0015$), *Streptococcus_vestibularis* (14.9% vs. 0.38%, $P = 0.0035$), *Rothia_sp* (5.80% vs. 0.06%, $P = 0.025$), *Streptococcus_parasanguinis* (14.36%

vs. 0.03%, $P = 0.0052$), *Anaerostipes_hadrus* (0.22% vs. 0.79%, $P = 0.0150$). At this time point, the analysis of the HDDT group revealed significant changes in the abundances of the following taxa: *Roseburia_inulinivorans* (0.26% vs. 1.38%, $P = 0.0170$), *Parabacteroides_ distasonis* (3.80% vs. 1.13%, $P = 0.0083$), *Fusicatenibacter_saccharivorans* (0.10% vs. 1.02%, $P = 0.0105$), *Rothia_sp* (0.27% vs. 0.08%, $P = 0.0330$), *Bacteroides_ thetaiotaomicron* (1.60% vs. 0.48%, $P = 0.0369$). It's important to note that all of the above-mentioned changes in the abundances of these species were restored to their previous levels at week 8 in both the HDDT and BQT groups.

Association between specific bacterial taxa and diarrhea occurred during *H. pylori* eradication

Among the 25 patients who received BQT, 5 patients experienced diarrhea. In order to investigate whether the occurrence of diarrhea was associated with specific bacterial taxa, the LEfSe method was utilized to analyze the differential taxa between patients with diarrhea and those without diarrhea within the BQT group. As shown in Supplementary Figure S2a, *Veillonella* and *Klebsiella* were identified as the bacteria that were differentially expressed between these two groups. Specifically, the relative abundance of *Veillonella* was found to be higher in patients with diarrhea compared to those without diarrhea (14.51% vs. 0.06%, $P = 0.0064$). The relative abundance of *Klebsiella* was lower in patients with diarrhea than in those without diarrhea (4.42% vs. 47.94%), however, there was no statistically significant difference between these two groups ($P = 0.0506$). (Supplementary Figure S2b, c)

Functional analysis before and after *H. pylori* eradication

Furthermore, we performed PICRUSt analysis to predict the changes in the functional capacity of the gut microbiota before and after *H. pylori* eradication therapy among the two groups. Our results demonstrated that many important metabolic pathways related to Genetic information processing and Metabolism were significantly changed in both the BQT and HDDT groups (Supplementary Figure S3a, b). Among these, Metabolism of terpenoids and polyketides, and Amino acid metabolism exhibited a decrease, while xenobiotics biodegradation and metabolism, and Carbohydrate metabolism increased.

Discussion

As public health awareness has been steadily growing, an increasing number of people are choosing to proactively undergo testing or eradication treatment for *H. pylori*. Currently, there are numerous regimens available for *H. pylori* eradication. PPI-based triple therapy was formerly the predominant prescribed treatment option, boasting an eradication success rate of around 90% [20, 21]. However, with the increasing antibiotic resistance, the eradication rate of triple therapy has gradually decreased to below 80% in some regions, and is no longer recommended as a first-line eradication regimen in China [22–24]. At present, BQT, which demonstrates a relatively high eradication rate, is the most recommended and commonly utilized first-line eradication regimen in China [25]. In several regions where bismuth is unavailable, non-bismuth quadruple therapies, including sequential therapy, concomitant therapy, and hybrid therapy, can also achieve satisfactory eradication effectiveness and have been recommended as first-line regimens [26]. Nevertheless, these quadruple regimens tend to have a relatively high incidence of side effects and poor patient compliance. Moreover, the long-term use of multiple antibiotics can exacerbate *H. pylori*'s resistance. Consequently, researchers have been constantly searching for new effective regimens with fewer adverse events and less use of antibiotics. In this context, HDDT, which typically consists of only a single antibiotic (usually amoxicillin), was proposed and attracted much attention [27]. It has been reported that higher doses or more potent acid-inhibiting drugs can maintain the gastric pH above 6 [28]. When the gastric pH ranges from 6 to 8, *H. pylori* could develop into the replication state, and became more sensitive to amoxicillin [29, 30]. Additionally, frequent dosing of amoxicillin ensures that its concentration remains higher than the minimum inhibitory concentration for an extended period [31, 32]. Furthermore, the primary resistance rate to amoxicillin is less than 5% in most regions, and even after an unsuccessful eradication attempt, the secondary resistance rate remains relatively low [33]. Hence, this simple dual-drug regimen could achieve high eradication efficacy and good safety [10, 11, 34].

Accumulating evidence has proven the beneficial effects of *H. pylori* eradication on on gastric cancer prevention and peptic ulcer treatment [35–37]. However, the disruption of the gut microbiota caused by PPIs and antibiotics has been a significant concern. Previous studies have demonstrated that *H. pylori* eradication could significantly alter the composition and diversity of the gut microbiota [6, 7, 38]. Liou et al. compared the impacts of BQT, concomitant therapy, and triple therapy on the gut microbiota, and found that the alpha diversity indices and beta diversity of all three groups changed significantly at week 2, the diversity of patients in the triple therapy group returned to baseline levels at week 8, whereas the disruption of the gut microbiota in the concomitant therapy and BQT groups was not completely restored even after a year [6]. Another study also indicated that the eradication of *H. pylori* infection could lead to gut microbiota dysbiosis, and the changes induced by

BQT, concomitant therapy, and sequential therapy nearly returned to the baseline level one year after treatment [7]. In the current study, a comparison was made between the impacts of HDDT and BQT on the gut microbiota. It was found that HDDT caused minimal disruption to the diversity, while BQT significantly altered the diversity at week 2 and these changes persisted at week 8. These results were consistent with previous findings. Horii et al. analyzed the impact of vonoprazan-amoxicillin (VA) dual therapy on the gut microbiota and found that, compared to the baseline, there was no significant difference in the diversity at week 1 and at week 8 [39]. Another study from China compared the changes in the gut microbiota before and after treatment in the VA dual therapy group and the BQT group. It was discovered that the alpha diversity and the relative abundance of phyla in both groups did not change significantly one month after treatment, but the relative abundance of some dominant genera changed markedly in the BQT group [40]. A recent study conducted among servicemen also showed that the disruption caused by HDDT was less than that of BQT [41].

It has been reported that short-term exposure to amoxicillin exerted a relatively minor impact on the diversity of the gut microbiota [42]. In another study, it was demonstrated that after subjects received 500 mg/day of amoxicillin for 7 consecutive days, the composition of the gut microbiota remained similar to that of the placebo group, and the Simpson index did not undergo significant changes before and after the intervention [43]. However, macrolides were found to have a potent effect on the gut microbiota and were capable of inhibiting numerous microorganisms. A previous study indicated that macrolides might have a more persistent effect on the gut microbiota compared to amoxicillin, even the changes of some bacteria persisted for 1–2 years after completing a macrolide course [44]. Results from Nobel et al. also revealed that macrolides had greater effect on the gut microbiota than amoxicillin [45]. Maier et al. found that macrolides could not only inhibit many gut bacteria but also kill several species [46]. They believed that this could partly explain the strong effect of macrolides on gut microbiota. A study further demonstrated that sequential treatments involving two antibiotics led to more severe disruptions in the gut microbiota than exposure to a single antibiotic [45]. In addition, a study showed that simultaneous administration of PPI, amoxicillin and clarithromycin increases the serum concentrations of PPI and the active 14-OH-clarithromycin metabolite significantly [47]. Hence, BQT, which contains both amoxicillin and clarithromycin, might induce a more substantial impact on the gut microbiota than HDDT, which only contained amoxicillin.

When analyzing the gut microbiota composition after receiving HDDT and BQT, we observed that a greater number of species had alterations in their abundances in the BQT group compared to the HDDT group at week 2. Among these species, several play crucial roles in the health and diseases. For instance, *Faecalibacterium_prausnitzii*, *Roseburia_inulinivorans*, *Agathobacter_rectalis*, *and Anaerostipes_hadrus* are involved in gut butyrate production [48–52]. Butyrate is significant as it can impact colonic motility, contribute to immunity maintenance, and possess anti-inflammatory properties [53, 54]. *Bifidobacterium_longum* is one of the most abundant members in the gut, and can protect the intestinal epithelial barrier and tissue structure, balance the gut microbiota to alleviate the symptoms of colitis and maintain the host in a healthy state [55]. *Bacteroides_uniformi* and *Bacteroides_thetaiotaomicron* are capable of degrading and utilizing glycans for capsular polysaccharide synthesis, which is essential for maintaining intestinal health [56]. Moreover, it has been demonstrated that *Parabacteroides_distasonis* could exert protective effects against certain diseases, including colorectal cancer, multiple sclerosis, and inflammatory bowel disease [57]. Consequently, the reduction in the abundances of these beneficial bacterial species can have a negative impact on intestinal health and may promote inflammation and disease development. In addition, after receiving BQT, *Pantoea_piersonii*, *Streptococcus_parasanguinis* and *Streptococcus_vestibularis* tend to overgrow in the gut, which may lead to invasive diseases, such as peritonitis and subacute endocarditis [58–60]. However, in the HDDT group at week 2, the relative abundances of these potential pathogens did not show an increase. These observed changes collectively suggested that the impact of HDDT on the gut microbiota is milder than that of BQT. Chen et al. also demonstrated that BQT had a greater effect on the relative abundance of butyrate-producing bacteria compared to HDDT [41].

Our prior study indicated that patients who received BQT experienced more abdominal discomfort, particularly diarrhea than patients in the HDDT group (4.6% vs. 1.4%, $P=0.014$) [12]. In the current study, we analyzed the association between specific bacteria and diarrhea, and found that patients experiencing diarrhea had a relatively higher abundance of *Veillonella*. *Veillonella* is a Gram-negative anaerobic coccus that has the capacity to produce lipopolysaccharide, stimulate the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-6, and commonly seen in upper respiratory and intestinal infections [61]. A study that aimed to explore the association of fecal bacteria composition and subtypes and symptoms of irritable bowel syndrome showed that an increased abundance of *Veillonella* was associated with loose stools, while a reduced

abundance was linked to constipation [62]. Another study involving 992 children from four low-income countries demonstrated that *Veillonella* exhibited a significant increase in young children with moderate-to-severe diarrhea [63]. Gomez et al. also found that diarrheic calves had a higher abundance of *Veillonella* than control group [64]. Hence, the increased abundance of *Veillonella* might be the underlying cause of the occurrence of diarrhea after receiving BQT, and it's worth of further study.

However, there were some deficiencies in our study. Firstly, the sample size was relatively small in our study, and we could not conduct more stratified analysis. Secondly, this study did not collect gastric samples to analyze the gastric microbiota before and after treatment. Thirdly, analysis of 16 S rRNA can only provide information on the presence of bacteria, information including the changes of antibiotic resistance gene cannot be obtained, and need to be analyzed in our further study. Lastly, HDDT cannot be used for patients who have an allergy to amoxicillin or those with amoxicillin-resistant *H. pylori*. For these infected patients, vonoprazan-tetracycline dual therapy has been suggested as a potentially viable option [65], but still need to be further validated.

Conclusions

This study revealed that HDDT exerted less impact on the diversity and composition of the gut microbiota, whereas BQT led to notable dysbiosis of the gut microbiota with a reduction in the relative abundance of several important beneficial bacteria and the excessive growth of certain pathogenic bacteria. Our findings demonstrated that the impact of HDDT on the gut microbiota was milder than that of BQT, and provided novel information on treatment options for *H. pylori* infection.

Abbreviations

H. pylori	Helicobacter pylori
MALT lymphoma	Mucosa-associated lymphoid tissue lymphoma
BQT	Bismuth quadruple therapy
HDDT	High-dose dual therapy

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions

All authors contributed to the study conception and design. GJL, XTT, LJZ, LPY, LY, MYS, and HBY were involved in the acquisition and interpretation of data. GJL, XSH, and ZYN were involved in the analysis of data, interpretation of data, and drafting of the manuscript. GJL, HYY, LJY, LJZ, LPY were involved in the study design and supervision. All authors read and approved the final version of the manuscript.

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Data availability

The dataset supporting the conclusions of this article is available in the SRA databank [the SRA accession number: PRJNA1125614, http://www.ncbi.nlm.nih.gov/sra].

Declarations

Ethics approval and consent to participate

All patients provided informed consent and the study protocol was approved by the Institutional Ethics Board of Wenchang People's Hospital ([2021]-1).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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