ORIGINAL PAPER
Polish Journal of Microbiology
2022, Vol. 71, No 2, 227–239
https://doi.org/10.33073/pjm-2022-020

Oral Microbiota, a Potential Determinant for the Treatment Efficacy of Gastric *Helicobacter pylori* Eradication in Humans

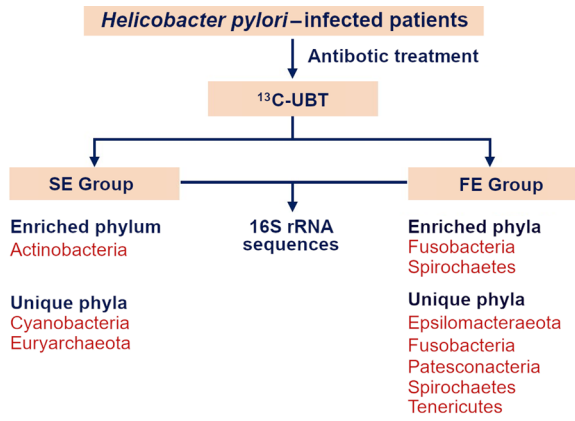
HUIXIA CHEN[1, 2, 3], HUI XIE[3], DONG SHAO[4], LIJU CHEN[3], SIYU CHEN[5]*, LIN WANG[2]* and XIAO HAN[1]*

[1] Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, China
[2] Jiangsu Key Laboratory of Oral Diseases, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing, China
[3] Department of Orthodontics, Changzhou Traditional Chinese Medicine Hospital, Changzhou, China
[4] The Third Affiliated Hospital of Soochow University, The First People's Hospital of Changzhou, Changzhou, China
[5] State Key Laboratory of Natural Medicines and School of Life Science and Technology, China Pharmaceutical University, Nanjing, China

Submitted 5 January 2022, accepted 22 March 2022, published online 31 May 2022

Abstract

The oral cavity serves as another reservoir for gastric *Helicobacter pylori* and may contribute to the failure of gastric *H. pylori* eradication therapy. However, changes to the oral microbial composition after gastric *H. pylori* eradication therapy has not yet been identified. This study aims to dissect whether the oral microbiota is involved and which bacterium mediates the clinic failure in *H. pylori* eradication. In the present study, the oral microorganisms from patients who had received the gastric *H. pylori* eradication treatment were analyzed by a high-throughput 16S rRNA deep sequencing. We found that the β diversity and composition of oral microbiota were remarkably changed in the patients who had experienced successful gastric *H. pylori* eradication treatment (SE group) compared to the failure group (FE group). Significantly enriched families, including *Prevotellaceae*, *Streptococcaceae*, Caulobacteraceae, and Lactobacillaceae, were detected in the SE group. In contrast, the bacterial families, such as Weeksellaceae, Neisseriaceae, Peptostreptococcaceae, Spirochaetaceae, and Veillonellaceae, were abundantly expressed in the FE group. Five operational taxonomic units (OTUs) were positively correlated with DOB values, while two OTUs exhibited negative trends. These different enriched OTUs were extensively involved in the 20 metabolic pathways. These results suggest that a balanced environment in the oral microbiota contributes to *H. pylori* eradication and metabolic homeostasis in humans. Our data demonstrated that the changes in oral microbiota might contribute to the therapeutic effects of antibiotic therapy. Therefore, a different therapy on the detrimental oral microbiota will increase the therapeutic efficacy of antibiotics on *H. pylori* infection.



Keywords: *Helicobacter pylori* eradication, therapeutic effects, oral microbiota, sequencing

Introduction

Helicobacter pylori is a highly adaptive Gram-negative bacterium in the human stomach. *H. pylori* infection affects nearly half of the world's population and is extensively associated with various diseases, including chronic gastritis, peptic ulcer, and even gastric cancers (Wroblewski et al. 2010; Salama et al. 2013). Clinically, due to differences in host defenses and responses, only approximately 3% of *H. pylori*-infected patients

* Corresponding authors: X. Han, Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, China; **e-mail: hanxiao@njmu.edu.cn**
L. Wang, Jiangsu Key Laboratory of Oral Diseases, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing, China; **e-mail: lw603@njmu.edu.cn**
S. Chen, State Key Laboratory of Natural Medicines and School of Life Science and Technology, China Pharmaceutical University, Nanjing, China; **e-mail: siyuchen@cpu.edu.cn**

develop gastric cancers, indicating that other facilitators are involved in the pathogenicity of *H. pylori* strains (Uemura et al. 2001).

Systemic antibiotics have been used in the clinic to achieve therapeutic effects. However, their therapeutic efficacy is not consistent across different patients. It may be caused by either the treatment failure or reinfection after successful eradication. Notably, along with the stomach, *H. pylori* has been detected in dental plaque and saliva, tonsillar tissue, and the esophagus in humans (Dağtekin-Ergür et al. 2008; Anand et al. 2014). Hence, the oral cavity serves as another reservoir for *H. pylori* and provides a conducive microenvironment for its growth and colonization (Goosen et al. 2002; Teoman et al. 2007; Agarwal and Jithendra 2012; Wang et al. 2014). Intriguingly, the presence of *H. pylori* in the oral cavity negatively impacts the bacterium eradication in the stomach, whereas the treatment of oral infection significantly increases *H. pylori* eradication in the stomach (Desai et al. 1991; Jia et al. 2012). Therefore, the oral cavity may contribute to the failure of gastric *H. pylori* eradication therapy.

As is known, the oral cavity is an upstream organ of the digestive tract. Interestingly, oral bacteria are similar to overall gastric mucosa microbiota (Wu et al. 2021). Microorganisms in the oral cavity may disrupt gastric homeostasis, leading to inflammation and carcinogenesis (Bakhti and Latifi-Navid 2021). For example, oral bacteria, such as *Fusobacterium*, *Porphyromonas*, and *Prevotella*, produce inflammatory metabolites and carcinogenic substances, which induce chronic inflammation and, subsequently, gastric cancer (Ahn et al. 2012). Further, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* may inhibit the host immune response and accelerate cancer growth (Colucci 2015; Nosho et al. 2016; Le Bars et al. 2017). Among these bacteria, oral *H. pylori* have been gradually recognized as one of the harmful bacteria in disrupting gastric homeostasis and even aggravating gastric cancer. Epidemically, the global annual recurrence, reinfection, and recrudescence rates of *H. pylori* are 4.3%, 3.1%, and 2.2%, respectively (Hu et al. 2017). In China, this rate reaches 1.5% yearly and is independently associated with minority groups and those with lower education levels, family histories of gastric cancer, and residences located in Western and Central China (Xie et al. 2020). Because the oral cavity and stomach are tightly linked through saliva and food, the coexistence of bacteria in both the oral cavity and gastric mucosal microbiota may lead to the reinfection and recurrence of *H. pylori*. Indeed, it has been demonstrated that oral *H. pylori* affects the outcome of eradication therapy and is associated with the recurrence of gastric infection (Miyabayashi et al. 2000). More importantly, it is more difficult to eradicate *H. pylori* in the mouth than in the stomach. Clinical investigations indicate that recurrence of gastric *H. pylori* infection is observed in approximately 30% of successfully treated patients suffering from oral *H. pylori* (Yee 2016). However, most studies have focused on changes in the oral microbiota in response to gastric *H. pylori* infections, ignoring the potential variations among specific bacteria corresponding to gastric *H. pylori* eradication. Hence, this study was performed to identify whether the oral microbiota is involved and which bacterium could mediate the failure of gastric *H. pylori* eradication therapy in the clinic.

In the present study, we analyzed the oral microorganisms from patients who had received either successful or failed gastric *H. pylori* eradication treatment. Using 13C-UBT analysis, we evaluated the onset of sixteen patients' infection and treatment status. Next, we performed 16S rRNA sequencing to analyze the members and structures of the oral microbiota. We found that the β diversity and composition of oral microbiota were significantly different among patients from the successful and failed gastric *H. pylori* eradication treatment groups, suggesting that changes in the oral microbiota may contribute to the therapeutic effects of antibiotic therapy targeting *H. pylori*.

Experimental

Materials and Methods

General Information. Sixteen gastric – *H. pylori*-infected patients were recruited, and received antibiotic treatment. All procedures conducted in this study conformed to institutional and national ethical standards and were approved by the ethics committee in the Changzhou Traditional Chinese Medicine Hospital (Permit Number: 2017-LL-05(L)). The detailed process involved in the sample collection is presented in Fig. 1A.

For the gastric *H. pylori* eradication treatment, all patients received standard drug treatment according to the quadruple therapeutic strategy, which is based on a proton-pump inhibitor, amoxicillin, furazolidone, and the bismuth agent for ten days. In addition, the proton-pump inhibitor was orally administered 30 min before meals to reduce the secretion of gastric acid. In contrast, other drugs were orally administered after meals to inhibit *H. pylori* growth and protect the stomach by activating the secretion of gastric mucus. All patients received a ^{13}C-urea breath test examination again to evaluate the successfulness of gastric *H. pylori* eradication ten days after treatment. During this period, the patients were asked to have a light diet and maintain oral hygiene by tooth brushing twice daily.

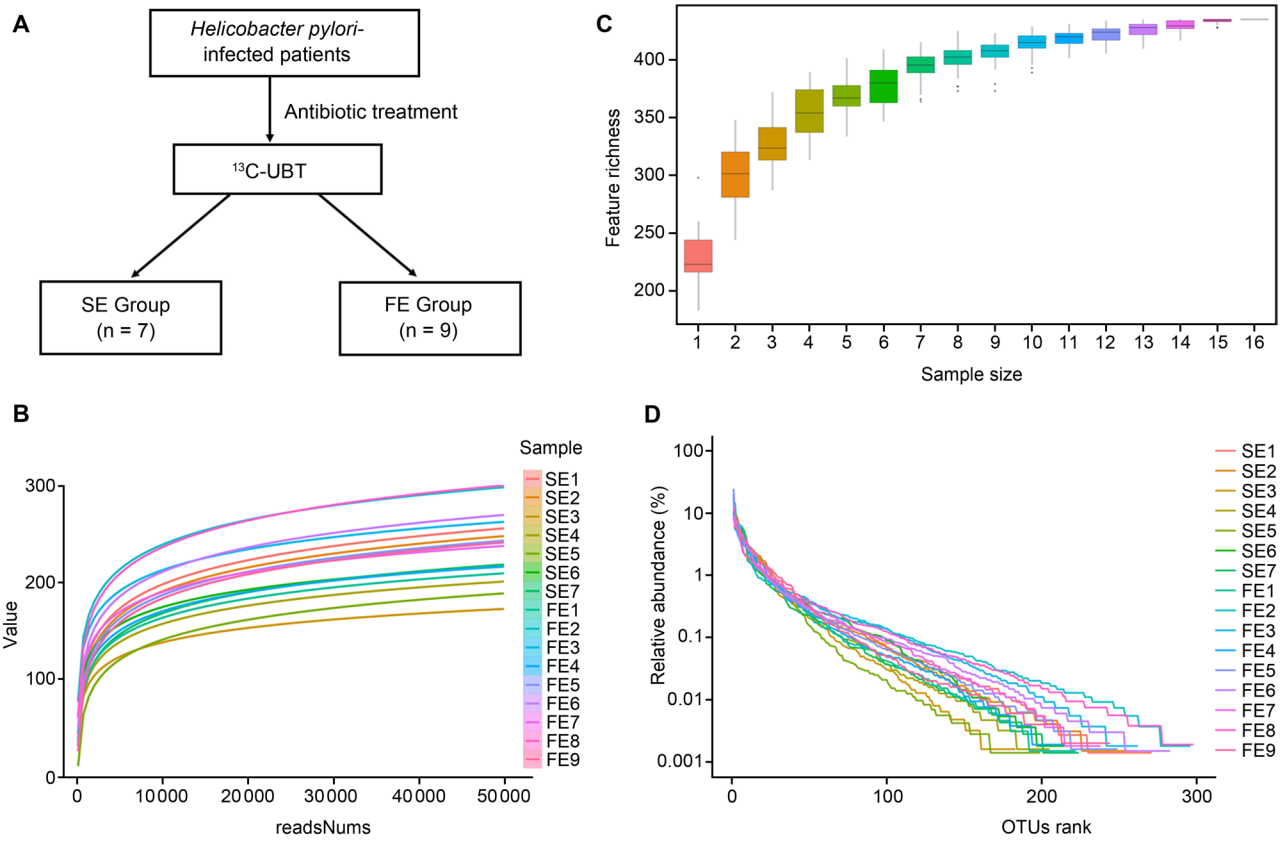


Fig. 1. Sample collection and qualification of 16S rRNA sequencing.

A) A schematic diagram illustrating the sample collection process; B) rarefaction curve analysis; C) specaccum curve analyses; D) rank abundance analysis; n = 7 for the SE group, n = 9 for the FE group; FE – failure eradication, SE – successful eradication, HP – *Helicobacter pylori*.

^{13}C-Urea breath test (UBT). After 4 h fasting, YH08 ^{13}C infrared spectrometer (Anhui Yanghe Medical Equipment Co., Ltd.) was used to measure the $^{13}CO_2$ values. The difference was expressed in DOB values. The positive or negative results were evaluated according to the device algorithm.

Oral sample collection, DNA extraction, and 16S rRNA sequencing. All the subjects were recruited in Changzhou (Jiangsu Province, China). The inclusion criteria for the volunteers were listed as follows: (i) ages ranged from 30 to 70 years old; (ii) the sexes were evenly distributed into two groups; (iii) the remained teeth numbers were over 20; (iv) none of the volunteers suffered from systemic or chronic diseases; (v) none of the volunteers suffered from long-term administration with drugs; (vi) non-pregnant and lactating women were involved; (vii) according to the doctors' instructions, all the *H. pylori*-infected subjects were treated with antibiotics. The exclusion criteria: (i) all the volunteers were native residents who had lived in Changzhou for more than ten years; (ii) patients who suffered from prolonged drug administration; (iii) patients who suffered from gastrointestinal cancers. A total of 2–5 ml of the patients' saliva was collected by the same dentist and was immediately frozen in liquid nitrogen and stored at −80°C. Genomic DNA was extracted by using a Qiagen DNA isolation kit. Extracted DNA was used as a template to amplify the V3-V4 region of the 16S rRNA gene for sequencing using the Illumina HiSeq 2500 platform at CapitalBio (China). 16S rRNA sequence data files have been deposited into the SRA database (https://www.ncbi.nlm.nih.gov/sra) with accession number PRJNA793997.

Bioinformatics analyses. Analysis of sequencing results: the original data were processed by QIIME software and compared with the Silva database (Release128 http://www.arb-silva.de). The clean reads were obtained by following analysis: (i) Trimmomatic 0.32 (http://www.usadellab.org/cms/?page=trimmomatic) was used to remove sequences shorter than 50 bp in length and a quality score lower than 20 in the raw reads (Bolger et al. 2014); (ii) FLASH 1.2.7 (http://ccb.jhu.edu/software/FLASH/index.shtml) analysis was performed to merge the paired-end reads from the filtered DNA fragments (Magoč and Salzberg 2011); (iii) chimera sequences in merged sequences were diminished through the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar et al. 2011). A STAMP software analyzed the distribution differences of species phyla. The Hellinger transformation

normalized the abundance of each OTU and was further used to construct redundancy analysis (RDA) models. The potential functions of specific OTUs were predicted using the phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) software.

Statistical analysis. SPSS21.0 software was used for analysis. The numerical variables were recorded as mean ± SD, which were compared by *t*-test or analysis of variance. The categorical variables were compared by count and percentage, and differences were determined to be statistically significant by a chi-square test. A p-value < 0.05 was considered statistically significant.

Results

Patients' information. Sixteen *H. pylori*-infected volunteers were treated with antibiotics and received ^{13}C-UBT. Among these patients, seven cases involved successful *H. pylori* eradication (Successful Eradication, SE), whereas nine cases involved failed eradication (Failed Eradication, FE). Detailed information on these patients, including their ages, genders, and DOB values, is presented in Table SI. We determined the success or failure of the therapy by evaluating the DOB values. The DOB value less than 4 was an expected value in the clinical evaluation of *H. pylori* infection. As shown in Table SI, the DOB value of the SE group was 0.13 ± 1.23, suggesting that *H. pylori* in these patients were eradicated successfully.

16S rRNA sequencing. To identify the mechanism behind the inconsistent treatment efficacy, we performed 16S rRNA sequencing (a region) to examine the composition and structure of bacteria in the oral cavity. As shown in Table SII, 924,981 clean reads were generated. Each sample in the SE group produced an average of 66,897 ± 6,449 effective reads, while an average of 58,102 ± 6,281 effective reads was obtained in the FE group. The average read length was 423.48 ± 1.11 and 422.10 ± 1.31, respectively, for the SE and FE groups. Of note, the effective reads and read lengths were significantly enriched in the SE group, indicating the abundant composition of the oral bacteria after successful treatment.

Rarefaction and specaccum analyses indicated that the sequencing depth sufficiently covered rare new microbiota (Fig. 1B and 1C). Further, rank abundance analysis revealed that bacterial communities were similar in their composition, as evidenced by the comparable trends for the rank frequency and abundance within each sample (Fig. 1D).

Diversity analysis of oral microbiota. We next evaluated the α diversity of the oral bacterial community. To achieve this result, the observed species, Chao1 richness, ACE index, Shannon index, Simpson index, and J index were calculated from the sequencing results of either the SE or FE group. As depicted in Fig. 2A, no significant difference existed in these parameters, indicating that the diversity and richness of the microbial community were minimally affected by the treatment efficacy. On the other hand, the Weighted UniFrac Principal Coordinates Analysis (PCoA) revealed a significantly different clustering of oral microbiota composition in both the SE and FE groups (Fig. 2B). Meanwhile, the beta diversity between the two groups significantly differed, as evidenced by the adonis analysis accomplished with $R^2 = 0.255$ and $p = 0.003$. These results were confirmed by other two analyses, including MRPP (delta 0.115, $p = 0.003$) and ANOSIM (r = 0.204, $p = 0.028$).

Characterization of the oral microbiota. As shown in Fig. 3A and 3B, the abundance levels of *Bacteroidetes* and *Firmicutes* in the FE group were lower than those

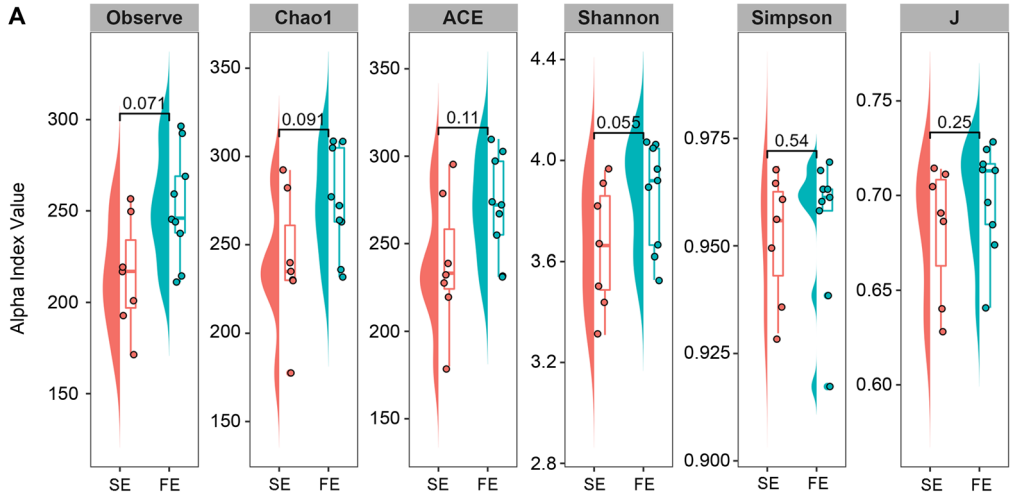


Fig. 2. α and β Diversity analyses.

A) Observed species, Chao1 richness, ACE index, Shannon index, Simpson index, and J index were calculated for α diversity of the oral microbiota.

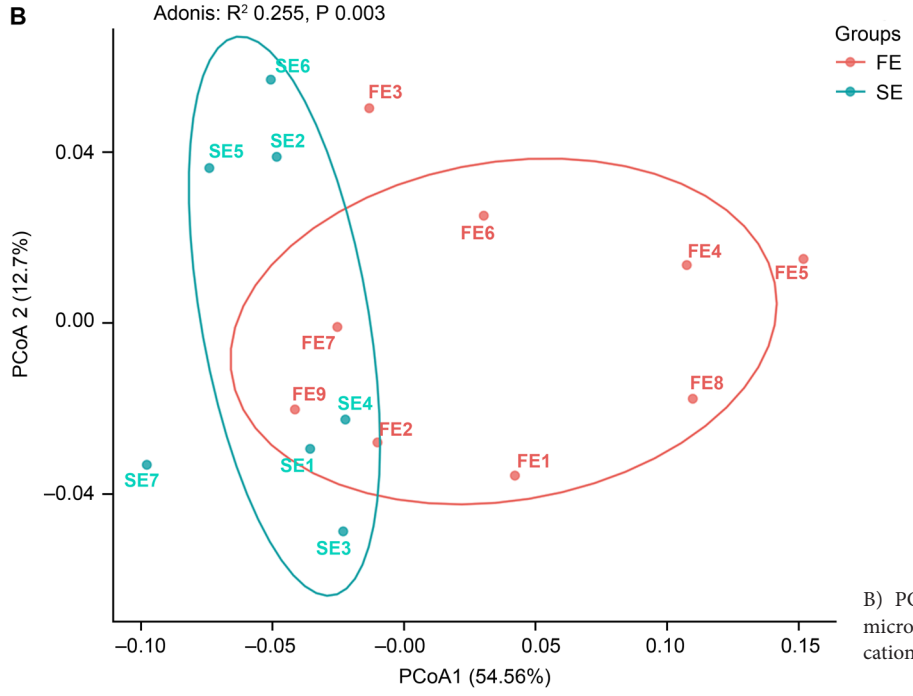


Fig. 2. α and β Diversity analyses.
B) PCoA analysis for β diversity of the oral microbiota; SE – patients with successful eradication therapy, FE – patients with failed eradication therapy.

from the SE group, by 19.23% and 28.25%, respectively. In contrast, the abundance levels of Proteobacteria and Fusobacteria were higher in the FE group than in the SE group. In particular, STAMP analysis demonstrated that Fusobacteria ($p = 0.00062$) and Spirochaetes ($p = 0.04$) were enriched in the oral cavities of the FE group, while *Actinobacteria* was the predominant bacterium in the oral cavities of the SE group (Fig. 3C).

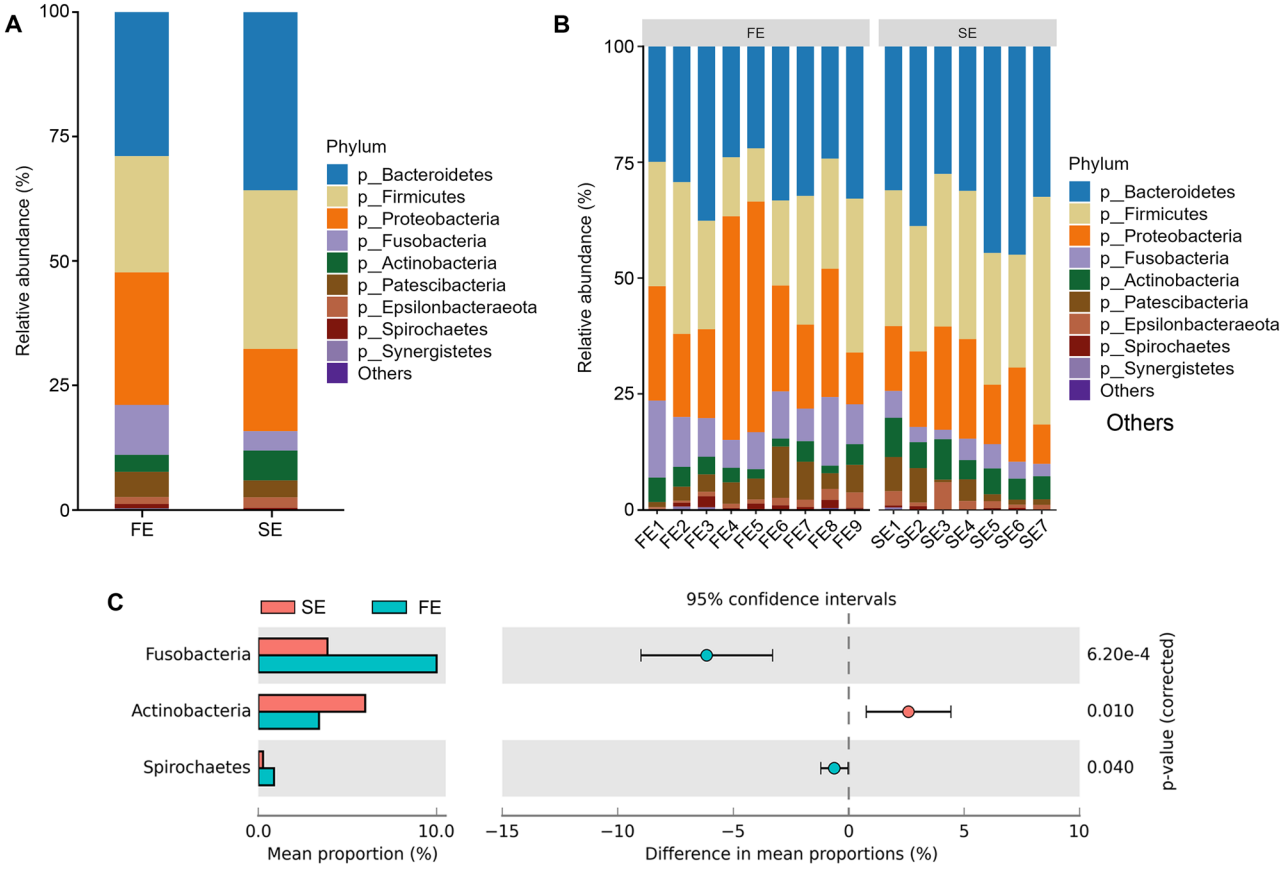


Fig. 3. Component analysis of community structure.
A) Relative abundances of the ten most abundant bacterial phyla in the FE and SE groups; B) relative abundances of the indicated individuals' ten most abundant bacterial phyla; C) *t*-test analysis comparing oral microbiota composition between the FE and SE groups.

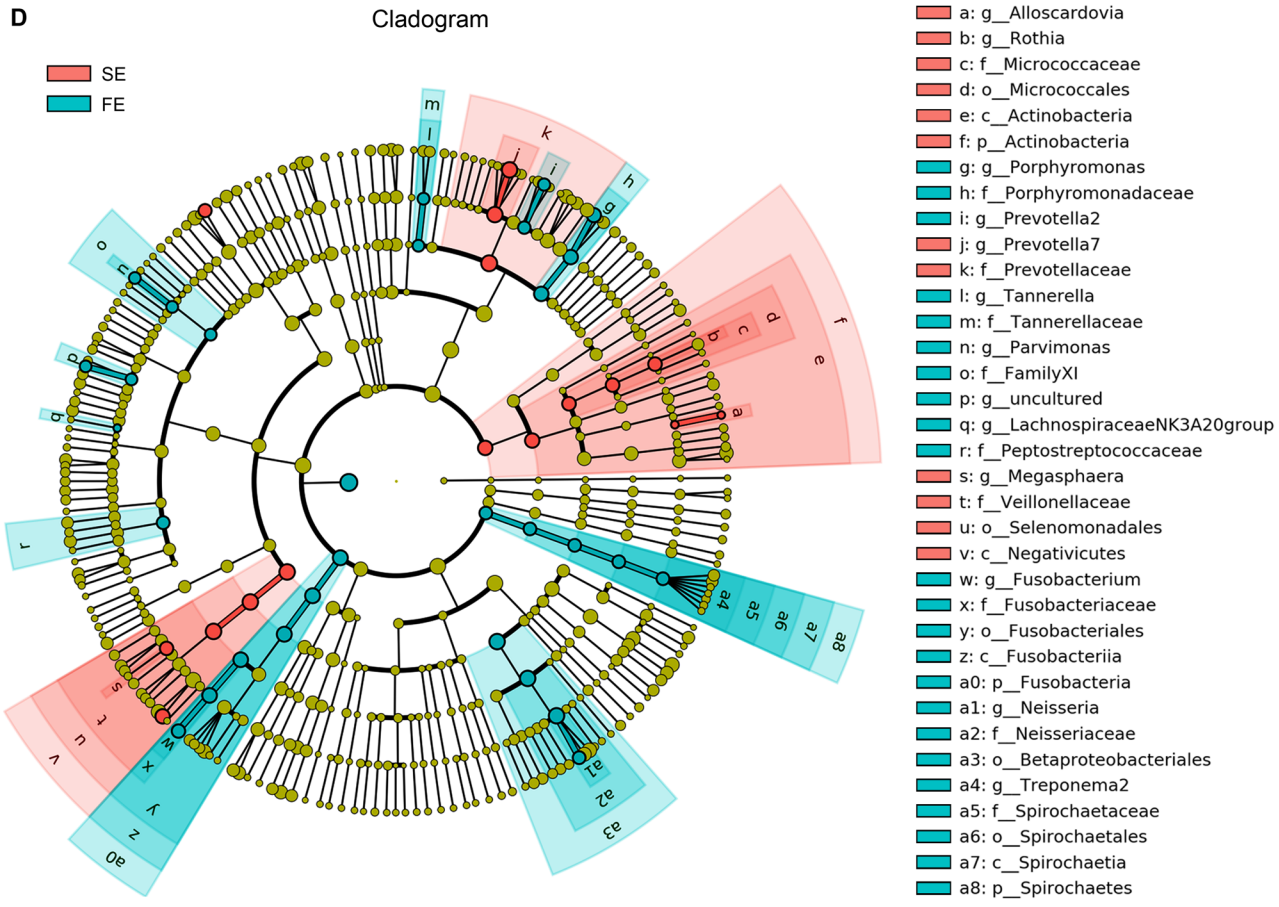


Fig. 3. Component analysis of community structure.
D) cladogram generated from LEfSe analysis.

These results were further confirmed by linear discriminant analysis (LDA) effect size (LEfSe) analysis (Fig. 3D and 3E).

RDA analyses and bacterial function prediction. To dissect the specific bacterial types in the oral cavities of patients with failed or successful treatment, we performed a combined analysis based on DESeq2 and RDA, finding that 14 OTUs were differently altered between the SE and FE groups. As shown in Fig. 4A and 4B, OTUs, including OTU21, OTU13, OTU108, OTU191, OTU226, and OTU231, were enriched in the SE group compared to the FE group with these belonging to the families of *Prevotellaceae*, *Streptococcaceae*, *Caulobacteraceae*, and *Lactobacillaceae*. Conversely, the abundance levels of OTU184, OTU141, OTU212, OTU174, OTU199, OTU154, OTU105, and OTU171 were relatively low in the SE group. These OTUs are classified into the families of Weeksellaceae, Neisseriaceae, Peptostreptococcaceae, Spirochaetaceae, and Veillonellaceae. To further identify the correlations between these OTUs and clinical parameters, we performed the Spearman analysis, finding that the DOB values were positively correlated with the abundance levels of OTUs, including OTU212, OTU171, OTU105, OTU154, and OTU199, whereas they were negatively correlated with OTU13 and OTU21 (Fig. 4C).

We used the PICRUSt software to analyze the potential function of these altered OTUs. Notably, we focused on the L3 channel prediction results of the KEGG database. As shown in Fig. 4D, principal component analysis (PCA) indicated that unique KEGG pathways existed in either the SE or the FE group. After functional annotation and calculation (ANOVA and Duncan's tests), 20 KEGG pathways were altered. Compared to the FE group, the oral microbiota of the SE patients were enriched for pathways involved in folate biosynthesis, the biosynthesis of the siderophore group, nonribosomal peptides, nitrogen metabolism, ubiquinone and other terpenoid quinone biosynthesis pathways, steroid hormone biosynthesis, glycosaminoglycan degradation, the sphingolipid signaling pathway, carbohydrate digestion and absorption, and proteasomes. In contrast, the enrichment pathways in the FE group were aminobenzoate degradation, styrene degradation, lysine degradation, chloroalkane and chloroalkene degradation, taurine and hypotaurine metabolism, tryptophan metabolism, stilbenoid dialylheptanoid and gingerol biosynthesis, flavonoid biosynthesis, biosynthesis of

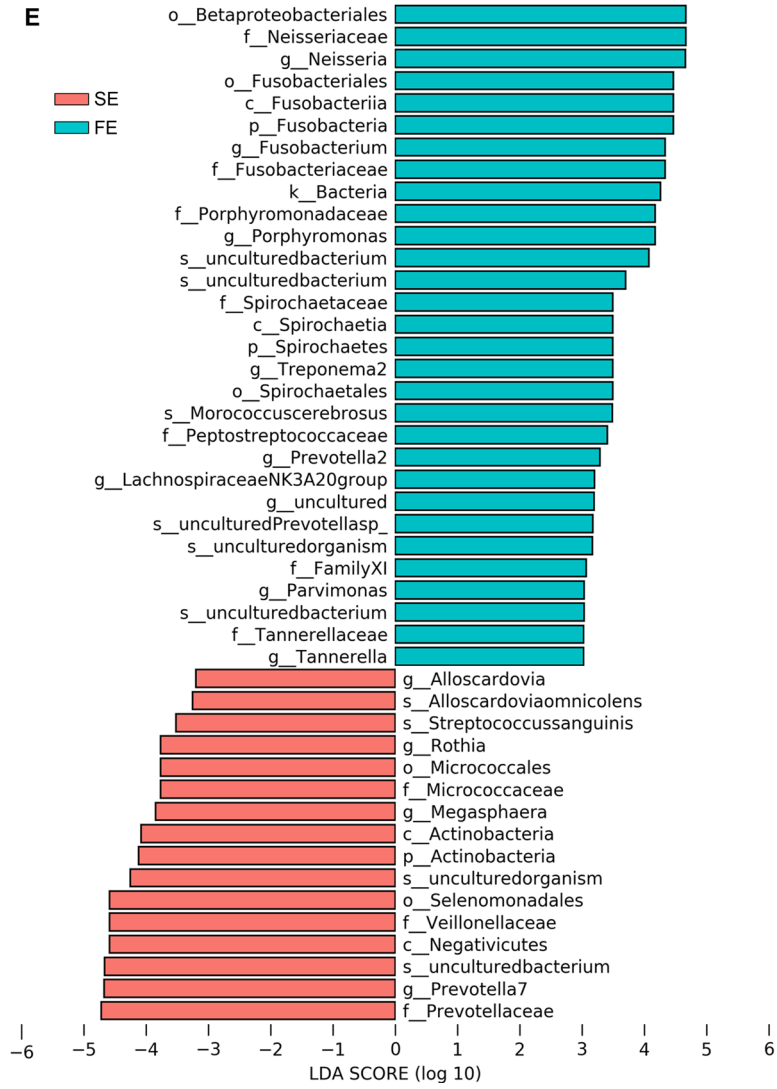


Fig. 3. Component analysis of community structure. E) LDA scores of significantly altered taxa presented in panel D; n = 7 for the SE group, n = 9 for the FE group; SE – patients with successful eradication therapy, FE – patients with failed eradication therapy.

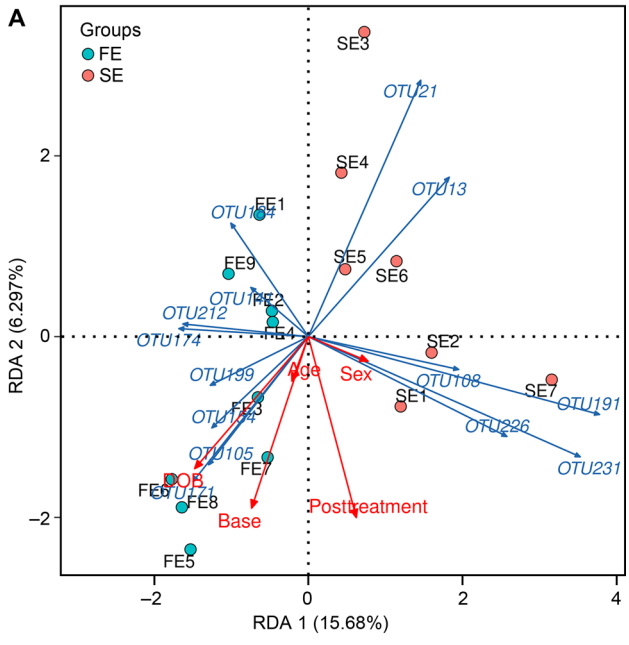


Fig. 4. Correlation analysis of microbial environmental factors. A) RDA analysis comparing OTUs between the FE and SE groups.

vancomycin group antibiotics, and fatty acid degradation (Fig. 4E). Collectively, the microorganisms in the oral cavities of the SE group mainly affected folate biosynthesis and steroid hormone biosynthesis. In contrast, the oral bacteria in the FE group were enriched in the degradation process for amino acids and fatty acids, suggesting that oral microorganisms in the patients of the FE group exerted potential harmful regulatory properties on human metabolic homeostasis.

Venn analysis. Lastly, we performed a Venn analysis to identify the unique oral bacterial species leading to different treatment efficacies among *H. pylori*-infected patients. As shown in Fig. 5, 347 OTUs were found within the two groups. More importantly, 29 unique OTUs were enriched in the SE group, and 59 unique OTUs were abundantly expressed in the FE group. Even more importantly, Epsilonbacteraeota, Fusobacteria, Patescibacteria, Spirochaetes, and Tenericutes were the only phyla found in the oral cavities of the FE group, indicating that the bacteria belonging to these phyla may contribute to failed *H. pylori* eradication in the

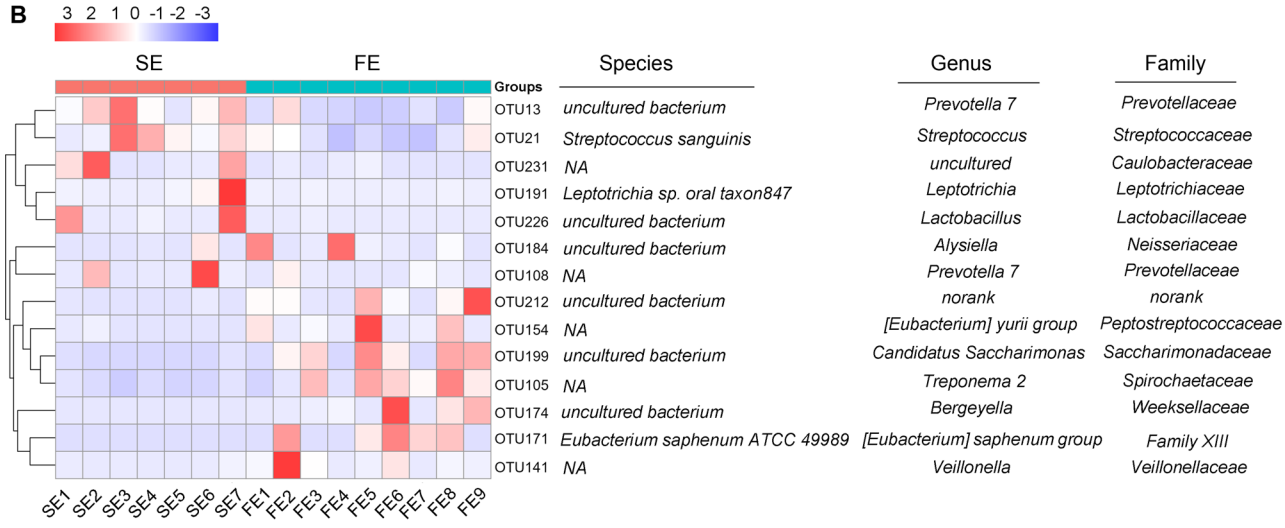


Fig. 4. Correlation analysis of microbial environmental factors.

B) heat map analysis based on RDA analysis showing the abundance of 14 OTUs influenced by *Helicobacter pylori* infection.

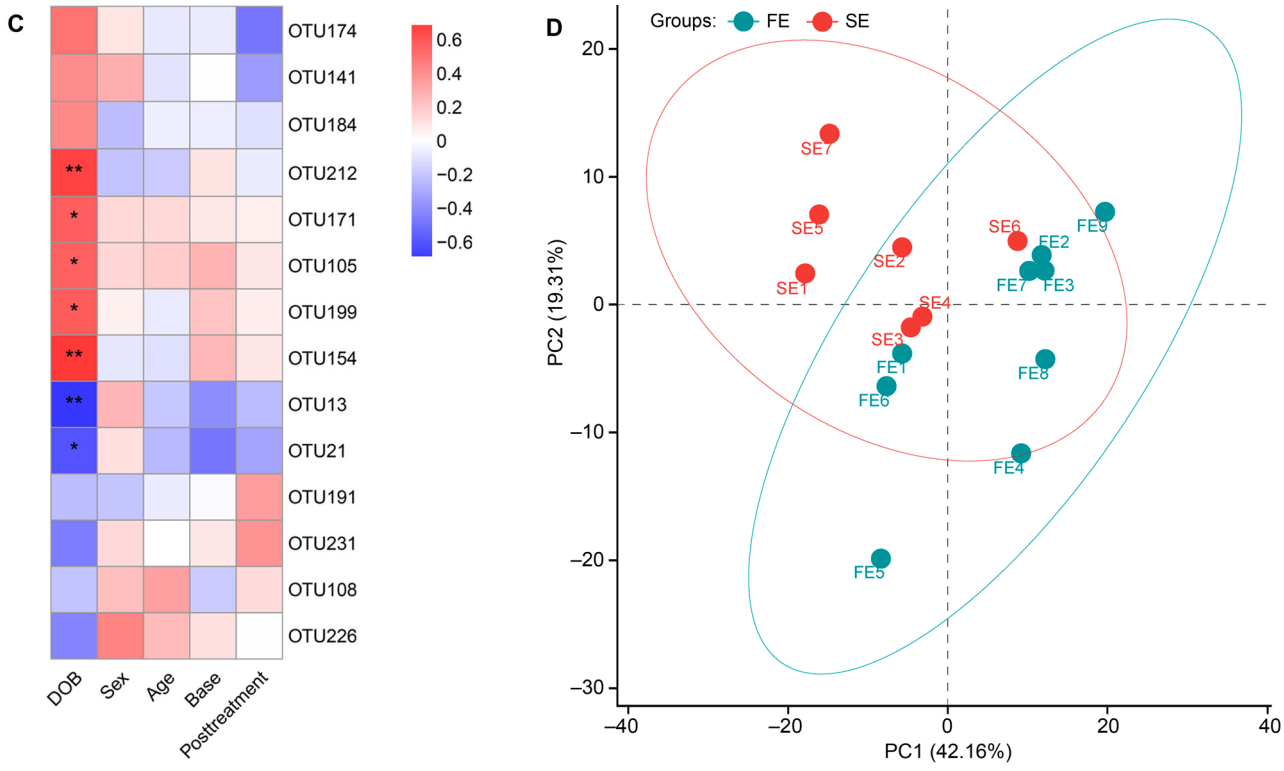


Fig. 4. Correlation analysis of microbial environmental factors.

C) correlations of the abundances of 14 OTUs with DOB values, ages, sex, and treatment status in patients from either FE or SE groups; $^{*}p < 0.05$, $^{**}p < 0.01$; PICRUSt2 function prediction of changed OTUs; D) PCA analysis.

clinic. In contrast, Cyanobacteria and Euryarchaeota were the unique phyla found in the SE group, suggesting these two phyla may function as antagonists against the recurrence of *H. pylori* infection. In addition, when we performed the taxonomic assignation at the species level, we found that 13 unique species were presented in the FE group. These species include *Treponema* genomosp. P4 oral clone MB2_G19, *Treponema parvum*, *Porphyromonas uenonis*, *Phocaeicola abscessus*, *Prevotella heparinolytica*, SR1 bacterium human oral taxon HOT-345, *Actinomyces weissii*, *Simonsiella muelleri* ATCC 29453, *Cobetia marina*, *Eubacterium saphenum* ATCC 49989, *Syntrophomonas palmitatica*, *Desulfomicrobium orale*, and *Mycoplasma lipophilum* (Table SIII). These data suggested that these bacterial species may be involved in gastric *H. pylori* infection recurrence.

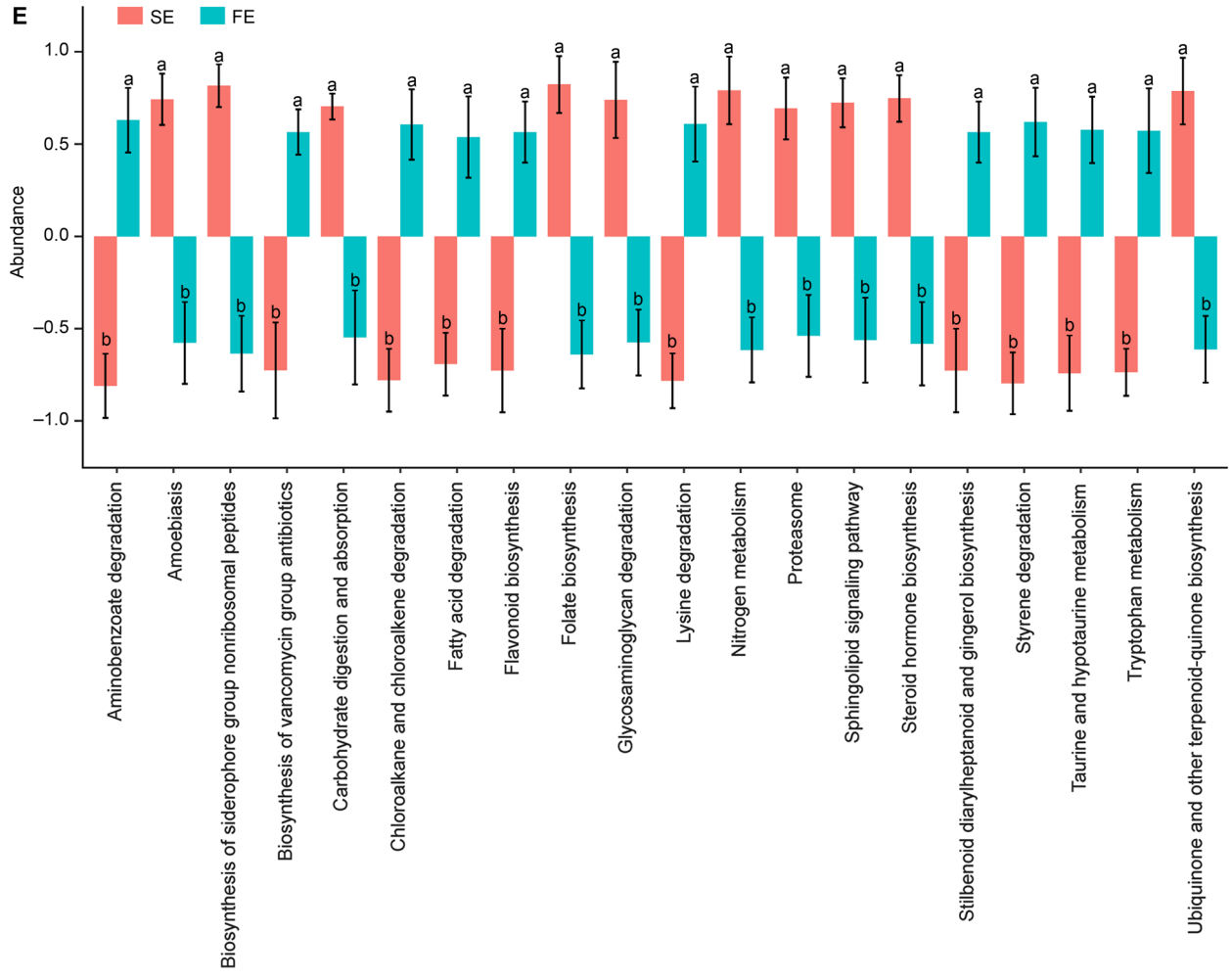


Fig. 4. Correlation analysis of microbial environmental factors.
E) KEGG analysis diagram; n = 7 for the SE group, n = 9 for the FE group; SE – patients with successful eradication therapy, FE – patients with failed eradication therapy.

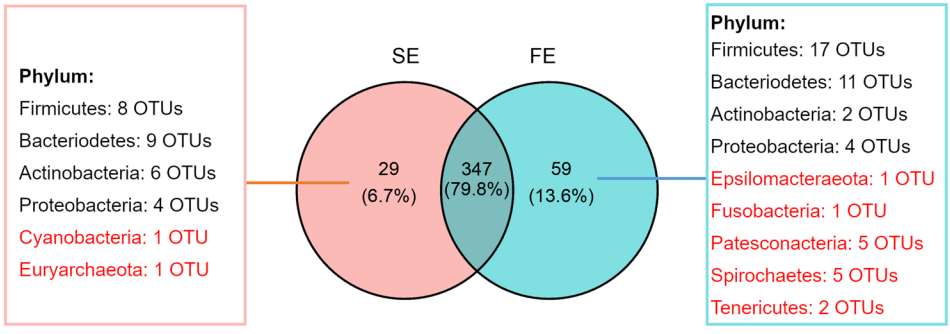


Fig. 5. Venn analysis revealing differently affected and overlapping OTUs between the FE and SE groups; n = 7 for the SE group, n = 9 for the FE group; SE – patients with successful eradication therapy, FE – patients with failed eradication therapy.

Discussion

The oral cavity is connected to the stomach through the esophagus, establishing a relationship between the microorganisms inhabiting these niches. Hence, it is necessary to comprehensively analyze the relationship between oral microbial diversity and gastrointestinal diseases. In addition, *H. pylori* infection is extensively associated with various gastrointestinal diseases, including chronic gastritis, peptic ulcer, and even gastric cancers (Tohidpour 2016). Although antibiotic therapy is known to be a standard treatment for gastric *H. pylori* infection, its therapeutic efficacy varies in the clinic. Considering that *H. pylori* have been

detected in the human mouth the oral cavity may serve as another reservoir for this pathogen contribution to the failure of gastric *H. pylori* eradication therapy (Zou and Li 2011; Bharath et al. 2014; Yee 2016). To prove this hypothesis, we examined the changes in the oral microbial abundance and composition in patients who had received either successful or failed gastric *H. pylori* eradication treatment. We found that the β diversity and composition of oral microbiota were remarkably different in the SE and FE patients. In brief, significantly enriched families, including *Prevotellaceae*, *Streptococcaceae*, Caulobacteraceae, and Lactobacillaceae, were detected in the SE group, while bacterial families such as Weeksellaceae, Neisseriaceae, Peptostreptococcaceae, Spirochaetaceae, and Veillonellaceae were abundantly expressed in the FE group. Five OTUs were positively correlated with DOB values, while two OTUs exhibited negative trends. Functionally, these differently enriched OTUs were extensively involved in multiple metabolic pathways, including folate biosynthesis and steroid hormone biosynthesis (enriched in the SE group), and the degradation process of amino acids and fatty acids (enriched in the FE group). Our results suggest that the maintenance of the healthy oral microbiota conditions contributes to both *H. pylori* eradication and metabolic homeostasis in humans. In addition, maintaining a low abundance of detrimental oral bacteria (e.g., *F. nucleatum*, *P. gingivalis*, and *Bacteroides forsythus*) and prompt therapy on the periodontal and its related diseases could decrease the adhesion of oral *H. pylori* (Bartold and Van Dyke 2019) and improve the oral environment leading to a reduced recurrence of gastric *H. pylori* infections.

The oral microbiota composition is altered by many external factors, including lifestyle, eating habits, and host conditions. Therefore, minimizing the deviation caused by these factors was considered when we selected the samples. In our study, the subjects were selected from the same geographical region to reduce its potential impacts on oral microbiota. Further, we chose the salivary microbiota for analyzing the oral bacteria since it has long-term stability and is a classic routine in reflecting the microbial structure in the human oral cavity. On the other hand, with microecology development, oral microorganisms have been documented to play essential roles in diagnosing and treating gastrointestinal diseases. Hence, a high-throughput sequencing technology will be extensively applied in the clinic, owing to its advantages in analyzing the oral microbiota (Wang et al. 2016).

Fusobacteria and Spirochaetes were highly enriched in the oral cavities of the FE group, implying their potential correlations with the recurrence of gastric *H. pylori* infection. *F. nucleatum*, a representative bacterium of Fusobacteria, is closely associated with the progression of colorectal cancer in the preclinical models (Abed et al. 2016). Interestingly, multi-omics analyses indicated that F. nucleatum localizes to and is enriched in colon adenomas and colorectal adenocarcinoma (Castellarin et al. 2012; Chen et al. 2012; Kostic et al. 2012; McCoy et al. 2013; Flanagan et al. 2014; Ito et al. 2015). Consistently, this bacterium also suppresses antitumor immunity by inhibiting the tumor-killing ability of natural killer cells (Gur et al. 2015), while its abundance exhibits significant correlations with the expression levels of pro-inflammatory genes (Kostic et al. 2013). Functionally and mechanistically, the fusobacterial proteins, including FadA and Fap2, may mediate its potential attachment to colorectal cancer (Rubinstein et al. 2013; Abed et al. 2016). All these findings indicated Fusobacteria might potentially alter the cancer metabolism and systemic inflammation. However, we noticed that Fusobacteria are core members of the human oral microbiome and are less abundant in the healthy gut, raising an interesting question of whether Fusobacteria transfer from the oral cavity to the gut.

Further investigations are needed to track Fusobacteria using a genetically modified GFP-expressing bacterium. Spirochaetes can penetrate tissues and bring the metabolites/endotoxins direct to the target cells. Such a penetration could perturb essential functions of host cells and the immune system (Dahle et al. 1993). Moreover, Spirochetes are suspected to be linked to the iron homeostasis and pathogenesis of neurological diseases, including neurosyphilis or neurodegeneration (Jolivet-Gougeon and Bonnaure-Mallet 2018). Hence, these two bacterial phyla accumulated abundance induced potential dysfunction of immune cells, disruption of iron homeostasis, and systemic inflammation. It might collectively lead to the failure of eradication or gastric *H. pylori* infection recurrence in this group.

On the other hand, gastric *H. pylori* infection is inversely affected by the oral microbiota since the oral cavity is another known reservoir of these bacteria. Strikingly, *H. pylori* may selectively adhere to bacteria. Okuda et al. (2003) reported that *P. gingivalis* and *F. nucleatum* could interact with *H. pylori* strains. Such interactions occur within biofilms and are conducive to *H. pylori* colonization of the human oral cavity. Although *H. pylori* can be detected in the oral cavity by molecular techniques, it is hardly cultured (Chamanrokh et al. 2015). Hence, few studies identify the significance of oral *H. pylori* infection and colonization. Several investigations indicated that the stress-induced transformation of *H. pylori* from its spiral to the coccoid could lead to the different functions of this bacterium (Cellini et al. 1994; Roe et al. 1999; Grzybowski and Rudnicka 2014). The coccoid form is a quiescent state of *H. pylori* in the oral cavity and could not be resurrected in the bacterial culture medium. In contrast, the spiral form of *H. pylori* in the stomach can be

found (Mao et al. 2021). More importantly, the coccoid *H. pylori* trigger the alternations of the gastric tissues when they are gavaged into the stomach for two weeks (Cellini et al. 1994). These findings indicated that even if the oral *H. pylori* were transiently inactive, the stress (e.g., antibiotics and gastric acid) might initiate the oral coccoid *H. pylori* transformation into the spiral form to trigger the gastric diseases in either the oral cavity or the stomach. In addition, microbial diversity may change according to the drug selection. When facing the drug challenge, microorganisms can express antibiotic resistance genes (ARGs), increasing drug resistance risks (Costalonga and Herzberg 2014). Such resistance could increase the overgrowth of pathogenic bacteria, causing a non-dysbiotic state, which may further provide a more suitable environment for *H. pylori* colonization. In addition, the persistent inflammation and the complicated bacterial environment in the periodontal pockets are facilitators of failures to clear *H. pylori* in both the human oral cavity and stomach (Bago et al. 2011). However, neither patients themselves nor gastroenterologists have paid enough attention to oral microbial dysbiosis in the eradication process of gastric *H. pylori* infection.

Function prediction is crucial in understanding the roles oral microbiota plays in host health. In our study, PCA results showed that the metabolic pathways significantly differed, indicating various microbial functions of the two groups. In particular, we found that the predicted function of oral microbiota clustered into fatty acid and protein degradation in the FE group, implying a negative impact on host metabolic homeostasis. Bacterial colonization depends on the adaptation to the host environment. As an important component of bacterial membranes, fatty acids facilitate such adaptation (Aggarwal et al. 2021). In our study, the ability of fatty acid degradation was enriched in the FE group, indicating the enhanced fatty acid utilization in this group. Hence, we speculated that such an enhancement might help detrimental bacteria to colonize host tissues.

Additionally, most bacteria exhibited amino acids' unique consumption and metabolic mode (Liu et al. 2020). ARG-expressing bacteria have been demonstrated to show the priority for the proline metabolism (Halsey et al. 2017). In the present study, we found that the tryptophan metabolism and lysine degradation occurred more often in the FE group, indicating that tryptophan and lysine might be two essential amino acids for the growth and survival of detrimental bacteria belonging to Fusobacteria and Spirochaetes phyla. On the other hand, it should be noted that the mammalian cells could not produce folic acid, and the beneficial bacteria are the source of folic acid *in vivo*. Notably, the folate biosynthesis pathway was inactive in the FE group compared with the SE group. Considering that folic acid is an essential substrate for the host metabolism (Sid et al. 2017), the dysbiosis of the oral microbiota may disrupt the host metabolic homeostasis by altering the circulating levels of folic acid.

On the other hand, by using Venn analysis, we found that Epsilonbacteraeota, Fusobacteria, Patescibacteria, Spirochaetes, and Tenericutes were the unique phyla that existed in the oral cavity of the FE group. At the same time, Cyanobacteria and Euryarchaeota were the unique phyla that were enriched in the SE group, suggesting these phyla may be involved in the recurrence of *H. pylori* infection and potentially interact with it. We also identified the unique species in the FE group and found 13 OTUs that could be annotated. Among them *T. parvum* and *P. uenonis* were closely related to periodontal or its related diseases, as well as the oral inflammation (Cobo et al. 2021; Zeng and Watt 2021,), while the proportions of *D. orale* were significantly higher in periodontitis than in peri-implantitis (Maruyama et al. 2014). All the findings implied that periodontal diseases and the related dysbiosis exhibited strong correlations with the recurrence of gastric *H. pylori* infections. Further studies are of great importance to identify the interactions between *H. pylori* and each bacterium, and the underlying mechanisms for the continuous recurrence of *H. pylori* infections. However, we could not perform this investigation because of the limitations both in the funding and techniques.

Conclusions

Our study found that the relative abundances of Fusobacteria and Spirochaetes were highly enriched in the FE group, which may be the possible reason for the failure of gastric *H. pylori* infection eradication therapy. Functionally, the highly enriched bacteria found in the oral cavities of the FE patients may potentially lead to metabolic disorders in humans. Hence, maintaining balanced microbiota in the oral cavity is important for *H. pylori*-infected patients, as it can effectively decrease the incidence of gastric *H. pylori* infection or recurrence. However, limitations still existed in our study. The samples were collected within a city, which may lead to district-specific results. Meanwhile, the sequencing methods could only reflect the relative abundance of bacteria. Hence, both the increase in sample numbers and multi-omics analyses are needed for further studies, which will have great potential for gastric *H. pylori* infection therapy.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical statement

Sixteen gastric *H. pylori*-infected patients were recruited, and they received antibiotic treatment. All procedures conducted in this study conformed to institutional and national ethical standards and were approved by the ethics committee in the Changzhou Traditional Chinese Medicine Hospital (Permit Number: 2017-LL-05(L)).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abed J, Emgård JEM, Zamir G, Faroja M, Almogy G, Grenov A, Sol A, Naor R, Pikarsky E, Atlan KA, et al. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. Cell Host Microbe. 2016 Aug; 20(2):215–225. https://doi.org/10.1016/j.chom.2016.07.006

Agarwal S, Jithendra KD. Presence of *Helicobacter pylori* in subgingival plaque of periodontitis patients with and without dyspepsia, detected by polymerase chain reaction and culture. J Indian Soc Periodontol. 2012;16(3):398–403. https://doi.org/10.4103/0972-124X.100919

Aggarwal SD, Gullett JM, Fedder T, Safi JPF, Rock CO, Hiller NL. Competence-associated peptide BriC alters fatty acid biosynthesis in *Streptococcus pneumoniae*. MSphere. 2021 Jun 30;6(3):e00145-21. https://doi.org/10.1128/mSphere.00145-21

Ahn J, Chen CY, Hayes RB. Oral microbiome and oral and gastrointestinal cancer risk. Cancer Causes Control. 2012 Mar;23(3):399–404. https://doi.org/10.1007/s10552-011-9892-7

Anand PS, Kamath KP, Anil S. Role of dental plaque, saliva and periodontal disease in *Helicobacter pylori* infection. World J Gastroenterol. 2014;20(19):5639–5653. https://doi.org/10.3748/wjg.v20.i19.5639

Bago I, Bago J, Plečko V, Aurer A, Majstorović K, Budimir A. The effectiveness of systemic eradication therapy against oral *Helicobacter pylori*. J Oral Pathol Med. 2011 May;40(5):428–432. https://doi.org/10.1111/j.1600-0714.2010.00989.x

Bakhti SZ, Latifi-Navid S. Oral microbiota and *Helicobacter pylori* in gastric carcinogenesis: what do we know and where next? BMC Microbiol. 2021 Dec;21(1):71. https://doi.org/10.1186/s12866-021-02130-4

Bartold PM, Van Dyke TE. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. J Clin Periodontol. 2019 Jan;46(1):6–11. https://doi.org/10.1111/jcpe.13046

Bharath TS, Reddy MS, Dhanapal R, Raj Kumar NG, Neeladri Raju PV, Saraswathi TR. Molecular detection and corelation of *Helicobacter pylori* in dental plaque and gastric biopsies of dyspeptic patients. J Oral Maxillofac Pathol. 2014;18(1):19–24. https://doi.org/10.4103/0973-029X.131885

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014 Aug 01;30(15): 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, et al. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. Genome Res. 2012 Feb;22(2):299–306. https://doi.org/10.1101/gr.126516.111

Cellini L, Allocati N, Di Campli E, Dainelli B. *Helicobacter pylori*: A fickle germ. Microbiol Immunol. 1994 Jan;38(1):25–30. https://doi.org/10.1111/j.1348-0421.1994.tb01740.x

Chamanrokh P, Shahhosseiny MH, Mazaheri Assadi M, Nejadsattari T, Esmaili D. Three tests used to identify non-culturable form of *Helicobacter pylori* in water samples. Jundishapur J Microbiol. 2015 Apr 18;8(4):e16811. https://doi.org/10.5812/jjm.8(4)2015.16811

Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. PLoS One. 2012 Jun 28;7(6):e39743. https://doi.org/10.1371/journal.pone.0039743

Cobo F, Pérez-Carrasco V, Sánchez-Martin V, García-Salcedo JA, Martín EB, Navarro-Marí JM. A rare cause of bacteremia due to *Porphyromonas asaccharolytica* in a patient with necrotizing fasciitis. Anaerobe. 2021 Oct;71:102442. https://doi.org/10.1016/j.anaerobe.2021.102442

Colucci F. An oral commensal associates with disease: chicken, egg, or red herring? Immunity. 2015 Feb;42(2):208–210. https://doi.org/10.1016/j.immuni.2015.01.024

Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett. 2014 Dec; 162(2) 2 Pt A:22–38. https://doi.org/10.1016/j.imlet.2014.08.017

Dağtekin-Ergür EN, Eren F, Ustün MB, Eren Y, Taş E, Gürsel AO. [Investigation of *Helicobacter pylori* colonization in pharyngeal and palatine tonsils with rapid urease test and immunohistochemical analysis] (in Turkish). Kulak Burun Bogaz Ihtis Derg. 2008 Mar-Apr; 18(2):85–89.

Dahle UR, Tronstad L, Olsen I. Spirochaetes in oral infections. Dent Traumatol. 1993 Jun;9(3):87–94. https://doi.org/10.1111/j.1600-9657.1993.tb00257.x

Desai HG, Gill HH, Shankaran K, Mehta PR, Prabhu SR. Dental plaque: a permanent reservoir of *Helicobacter pylori*? Scand J Gastroenterol. 1991 Jan;26(11):1205–1208. https://doi.org/10.3109/00365529108998615

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011 Aug 15;27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Flanagan L, Schmid J, Ebert M, Soucek P, Kunicka T, Liska V, Bruha J, Neary P, Dezeeuw N, Tommasino M, et al. *Fusobacterium nucleatum* associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. Eur J Clin Microbiol Infect Dis. 2014 Aug;33(8):1381–1390. https://doi.org/10.1007/s10096-014-2081-3

Goosen C, Theron J, Ntsala M, Maree FF, Olckers A, Botha SJ, Lastovica AJ, van der Merwe SW. Evaluation of a novel heminested PCR assay based on the phosphoglucosamine mutase gene for detection of *Helicobacter pylori* in saliva and dental plaque. J Clin Microbiol. 2002 Jan;40(1):205–209. https://doi.org/10.1128/JCM.40.1.205-209.2002

Grzybowski A, Rudnicka AR. Re: Rudnisky et al.: Antibiotic choice for the prophylaxis of post-cataract extraction endophthalmitis (Ophthalmology 2014;121:835-41). Ophthalmology. 2014 Nov; 121(11): e64. https://doi.org/10.1016/j.ophtha.2014.06.021

Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, Coppenhagen-Glazer S, et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity. 2015 Feb;42(2):344–355. https://doi.org/10.1016/j.immuni.2015.01.010

Halsey CR, Lei S, Wax JK, Lehman MK, Nuxoll AS, Steinke L, Sadykov M, Powers R, Fey PD. Amino acid catabolism in *Staphylococcus aureus* and the function of carbon catabolite repression. MBio. 2017 Mar 08;8(1):e01434-16. https://doi.org/10.1128/mBio.01434-16

Hu Y, Wan JH, Li XY, Zhu Y, Graham DY, Lu NH. Systematic review with meta-analysis: the global recurrence rate of *Helicobacter pylori*. Aliment Pharmacol Ther. 2017 Nov;46(9):773–779. https://doi.org/10.1111/apt.14319

Ito M, Kanno S, Nosho K, Sukawa Y, Mitsuhashi K, Kurihara H, Igarashi H, Takahashi T, Tachibana M, Takahashi H, et al. Association of *Fusobacterium nucleatum* with clinical and molecular features in colorectal serrated pathway. Int J Cancer. 2015 Sep; 137(6): 1258–1268. https://doi.org/10.1002/ijc.29488

Jia CL, Jiang GS, Li CH, Li CR. Effect of dental plaque control on infection of *Helicobacter pylori* in gastric mucosa. Tex Dent J. 2012 Oct;129(10):1069–1073.

Jolivet-Gougeon A, Bonnaure-Mallet M. Treponema, iron and neurodegeneration. Curr Alzheimer Res. 2018 Jun 11;15(8):716–722. https://doi.org/10.2174/1567205013666161122093404

Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013 Aug;14(2):207–215. https://doi.org/10.1016/j.chom.2013.07.007

Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, et al. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. Genome Res. 2012 Feb;22(2):292–298. https://doi.org/10.1101/gr.126573.111

Le Bars P, Matamoros S, Montassier E, Le Vacon F, Potel G, Soueidan A, Jordana F, de La Cochetière MF. The oral cavity microbiota: between health, oral disease, and cancers of the aerodigestive tract. Can J Microbiol. 2017 Jun;63(6):475–492. https://doi.org/10.1139/cjm-2016-0603

Liu YK, Kuo HC, Lai CH, Chou CC. Single amino acid utilization for bacterial categorization. Sci Rep. 2020 Dec;10(1):12686. https://doi.org/10.1038/s41598-020-69686-5

Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011 Nov 01;27(21): 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Mao X, Jakubovics NS, Bächle M, Buchalla W, Hiller KA, Maisch T, Hellwig E, Kirschneck C, Gessner A, Al-Ahmad A, et al. Colonization of *Helicobacter pylori* in the oral cavity – an endless controversy? Crit Rev Microbiol. 2021 Sep 03;47(5):612–629. https://doi.org/10.1080/1040841X.2021.1907740

Maruyama N, Maruyama F, Takeuchi Y, Aikawa C, Izumi Y, Nakagawa I. Intraindividual variation in core microbiota in peri-implantitis and periodontitis. Sci Rep. 2014 May;4(1):6602. https://doi.org/10.1038/srep06602

McCoy AN, Araújo-Pérez F, Azcárate-Peril A, Yeh JJ, Sandler RS, Keku TO. *Fusobacterium* is associated with colorectal adenomas. PLoS One. 2013 Jan 15;8(1):e53653. https://doi.org/10.1371/journal.pone.0053653

Miyabayashi H, Furihata K, Shimizu T, Ueno I, Akamatsu T. Influence of oral *Helicobacter pylori* on the success of eradication therapy against gastric *Helicobacter pylori*. Helicobacter. 2000 Mar;5(1):30–37. https://doi.org/10.1046/j.1523-5378.2000.00004.x

Nosho K, Sukawa Y, Adachi Y, Ito M, Mitsuhashi K, Kurihara H, Kanno S, Yamamoto I, Ishigami K, Igarashi H, et al. Association of *Fusobacterium nucleatum* with immunity and molecular alterations in colorectal cancer. World J Gastroenterol. 2016;22(2):557–566. https://doi.org/10.3748/wjg.v22.i2.557

Okuda K, Kimizuka R, Katakura A, Nakagawa T, Ishihara K. Ecological and immunopathological implications of oral bacteria in *Helicobacter pylori*-infected disease. J Periodontol. 2003 Jan; 74(1): 123–128. https://doi.org/10.1902/jop.2003.74.1.123

Roe IH, Son SH, Oh HT, Choi J, Shin JH, Lee JH, Hah YC. Changes in the evolution of the antigenic profiles and morphology during coccoid conversion of *Helicobacter pylori*. Korean J Intern Med. 1999 Jan 30;14(1):9–14. https://doi.org/10.3904/kjim.1999.14.1.9

Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe. 2013 Aug;14(2):195–206. https://doi.org/10.1016/j.chom.2013.07.012

Salama NR, Hartung ML, Müller A. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Nat Rev Microbiol. 2013 Jun;11(6):385–399. https://doi.org/10.1038/nrmicro3016

Sid V, Siow YL, O K. Role of folate in nonalcoholic fatty liver disease. Can J Physiol Pharmacol. 2017 Oct;95(10):1141–1148. https://doi.org/10.1139/cjpp-2016-0681

Teoman I, Özmeriç N, Özcan G, Alaaddinoğlu E, Dumlu Ş, Akyön Y, Baloş K. Comparison of different methods to detect *Helicobacter pylori* in the dental plaque of dyspeptic patients. Clin Oral Investig. 2007 Jul 31;11(3):201–205. https://doi.org/10.1007/s00784-007-0104-5

Tohidpour A. CagA-mediated pathogenesis of *Helicobacter pylori*. Microb Pathog. 2016 Apr;93:44–55. https://doi.org/10.1016/j.micpath.2016.01.005

Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001 Sep 13;345(11):784–789. https://doi.org/10.1056/NEJMoa001999

Wang K, Lu W, Tu Q, Ge Y, He J, Zhou Y, Gou Y, Van Nostrand JD, Qin Y, Li J, et al. Preliminary analysis of salivary microbiome and their potential roles in oral lichen planus. Sci Rep. 2016 Sep;6(1): 22943. https://doi.org/10.1038/srep22943

Wang XM, Yee KC, Hazeki-Taylor N, Li J, Fu HY, Huang ML, Zhang GY. Oral *Helicobacter pylori*, its relationship to successful eradication of gastric *H. pylori* and saliva culture confirmation. J Physiol Pharmacol. 2014 Aug;65(4):559–566.

Wroblewski LE, Peek RM Jr, Wilson KT. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010 Oct;23(4):713–739. https://doi.org/10.1128/CMR.00011-10

Wu ZF, Zou K, Xiang CJ, Jin ZJ, Ding HH, Xu S, Wu GN, Wang YH, Wu XY, Chen C, et al. *Helicobacter pylori* infection is associated with the co-occurrence of bacteria in the oral cavity and the gastric mucosa. Helicobacter. 2021 Apr;26(2):e12786. https://doi.org/10.1111/hel.12786

Xie Y, Song C, Cheng H, Xu C, Zhang Z, Wang J, Huo L, Du Q, Xu J, Chen Y, et al.; Chinese Society of Gastroenterology, Chinese Study Group on Helicobacter pylori and Peptic Ulcer. Long-term follow-up of *Helicobacter pylori* reinfection and its risk factors after initial eradication: a large-scale multicentre, prospective open cohort, observational study. Emerg Microbes Infect. 2020 Jan 01; 9(1):548–557. https://doi.org/10.1080/22221751.2020.1737579

Yee JKC. *Helicobacter pylori* colonization of the oral cavity: A milestone discovery. World J Gastroenterol. 2016;22(2):641–648. https://doi.org/10.3748/wjg.v22.i2.641

Zeng H, Watt RM. Complete genome sequences of three human oral *Treponema parvum* isolates. Microbiol Resour Announce. 2021 Jul 08;10(27):e00394-21. https://doi.org/10.1128/MRA.00394-21

Zou QH, Li RQ. *Helicobacter pylori* in the oral cavity and gastric mucosa: a meta-analysis. J Oral Pathol Med. 2011 Apr;40(4):317–324. https://doi.org/10.1111/j.1600-0714.2011.01006.x

Supplementary materials are available on the journal's website.