Full Paper

Does *Helicobacter pylori* infection affect the structure of bacteria in the gastric mucosa and fluid in patients with chronic antral gastritis?

(Received March 1, 2019; Accepted August 29, 2020; J-STAGE Advance publication date: May 29, 2021)

Yang Jiang,^{1,†} Fei Meng,^{2,†} Ying Liu,³ Liyun Zheng,² Shufang Ye,^{4,*} and Jianmei Zhang⁴

¹ Department of Science and Education, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang, PR China

² Department of Research service, Zhiyuan Medical Inspection Institute CO., LTD, Hangzhou 310000, PR China

³ Department of Basic Sciences, Zhejiang Tongji Vocational College of Science and Technology, Hangzhou, Zhejiang, PR China

⁴ Department of Gastroenterology, People's Hospital of Lishui City, Lishui, Zhejiang, PR China

This study aimed to evaluate the composition of the gastric microbiota in the gastric mucosa and gastric fluid of patients with chronic antral gastritis. Specifically, we sought to determine whether *Helicobacter pylori* (*Hp*) infection changes the bacterial community in the gastric mucosa or alters the microbiota in the gastric fluid. The bacterial community at another site in the stomach was also investigated. DNA was extracted from 160 samples collected from 40 patients with chronic antral gastritis (20 *Hp*-positive and 20 *Hp*-negative cases). Three tissue samples of the gastric mucosa (gastric angle, body, and antral mucosa) and one tube of gastric fluid were collected from every patient. A 16S rRNA amplification library was created, and high-throughput sequencing was performed. A profile of the community composition was obtained using bioinformatics methods, including cluster, taxonomy, and diversity analyses. Analysis of the gastric bacterial community revealed that the community compositions of the gastric mucosa and gastric fluid of patients without *Hp* are similar to but show differences from those of *Hp*-positive patients. The microbiota in *Hp*-positive patients exhibited reduced microbial diversity, and the gastric fluid of these patients contained a small proportion of *Hp*. The richness of *Leptotrichia* in mucosal samples was greater than that in gastric fluid samples from *Hp*-negative patients with chronic antral gastritis. *Hp* changes the growth of other microbiota in the mucosa and affects the microbiota in the gastric fluid of patients with chronic antral gastritis.

In addition to *Hp*, the presence of other bacteria might be related to the development of chronic antral gastritis.

Key Words: bacterial structure; chronic antral gastritis; gastric fluid; gastric mucosal; *Helicobacter pylori*; 16SrRNA sequencing

Introduction

Chronic antral gastritis, which is a type of chronic gastritis, is a common disease of the digestive system that is often persistent (Terao et al., 2020). Infection with *Helicobacter pylori* (*Hp*) has been discovered to be a major cause of chronic gastritis (Dan-Si et al., 2016). Although *Hp* is considered a noninvasive bacterium, some studies have confirmed that *Hp* is, in fact, invasive (Dubois and Boren, 2010; Liu et al., 2012; Watari et al., 2014). Lipopolysaccharide in the outer membrane component of *Hp* stimulates macrophages and vascular endothelial cells to produce inflammatory factors and increases neutrophil infiltration, which results in the presence of lymphocytes and other cells in the gastric mucosa and the promotion of inflammation (Roman et al., 2016; Zhang et al., 2015). *Hp*-induced mucosal injury that occurs in the stomach as a result of this pathogenesis is an important aspect of this disease. It has been proposed that chronic *Hp* infection leading to reduced acid secretion allows for the successful establishment of new microbiota species and is closely related to the occurrence of gastric cancer and lymphoma (Plottel and Blaser, 2011). However, previous studies have shown that only 50% of patients with chronic gastritis are

*Corresponding author: Shufang Ye, Department of Gastroenterology, People's Hospital of Lishui City, Lishui, Zhejiang, PR China.
E-mail: 13735951698@163.com

† The authors contributed equally.

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

Table 1. Patient information.

Number	Site	Average age	¹³ C breath	Pathological examination	Culture
a	angle	50 ± 4.5	negative	negative	negative
b	body	48 ± 3.7	negative	negative	negative
c	antrum	47 ± 3.4	negative	negative	negative
d	fluid	47 ± 3.6	negative	negative	negative
A	angle	45 ± 3.3	Positive	Positive	Positive
B	body	46 ± 3.9	Positive	Positive	Positive
C	antrum	44 ± 3.5	Positive	Positive	Positive
D	fluid	47 ± 2.5	Positive	Positive	Positive

infected with *Hp*. In addition, other bacteria or mechanisms can lead to the occurrence of chronic gastritis (Mowat et al., 2000; Sanduleanu et al., 2001). For example, a high prevalence of *Streptococcus* and *Staphylococcus* spp. in chronic gastritis in the absence of *Hp* has been reported (Yang et al., 2013; Zilberstein et al., 2007).

In recent years, the rapid development of next-generation sequencing has encouraged studies on the composition of the gastrointestinal microbiota. However, few studies have investigated the microbiota at different sites of the gastric mucosa and gastric fluid of patients with certain diseases, such as chronic gastritis, gastric ulcers and cancers. In general, greater attention should be paid to the composition and structure of the gastric microbiota, including those in different sites of the gastric mucosa and fluid of patients with a certain disease, such as chronic gastritis.

This study explored the effects of *Hp* infection on the microbiota at different sites of the gastric mucosa and the gastric fluid through the high-throughput sequencing of bacterial 16S rRNA using Illumina MiSeq sequencing. Comparisons of the composition and structure of the gastric microbiota between different groups were performed to investigate the changes in the microbiota of the gastric mucosa and gastric fluid induced by *Hp* infection. The composition of the gastric mucosa and gastric fluid of patients might provide a theoretical basis for understanding the development of chronic antral gastritis.

Materials and Methods

Subject selection. This study was approved by the ethics committee of People's Hospital of Lishui City. All patients had a detailed understanding of this study and provided written informed consent. Patients with upper gastrointestinal symptoms were subjected to a ¹³C-urea breath test and *Hp* culture analysis.

Clinical data of the patients. Forty patients with chronic antral gastritis, including 20 *Hp*-positive and 20 *Hp*-negative patients, were enrolled in the study group. The age range of the patients was 35–65 years, with an average of 47.45 ± 7.05 years, and the male-to-female ratio was 1.17:1. No significant difference in age ($p > 0.05$, t -test) or sex ratio ($p > 0.05$, Chi-square test) was found among the *Hp*-positive and negative patients (Table 1).

Collection of gastric mucosal biopsies and fluid samples. All patients underwent endoscopy, and a piece of the gastric mucosa was collected from each angle, body and antrum placed in TE buffer for DNA extraction. In addition,

another two pieces of the antrum of the gastric mucosa were collected. The first biopsy samples of the gastric mucosa antrum were collected for pathological examination, and a second set of samples of the mucosal antrum were collected and cultured in brain heart infusion broth for the isolation and culture of *Hp*. Fluid samples were simultaneously collected from the subjects who underwent standard endoscopy due to dyspepsia. Briefly, gastric fluid was obtained from the patients with clear gastric fluid via a catheter connected to a 5-mL tube during endoscopy. All the samples, with the exception of those used for pathological examination, were stored at –80°C.

Group design. For this study, pathological examinations were performed to determine chronic antral gastritis, and *Hp* positive or negative results from the ¹³C-urea breath test, pathological examination and *Hp* culture. 20 *Hp*-positive chronic antral gastritis patients and 20 *Hp*-negative chronic antral gastritis patients were collected. Samples with *Hp*-negative chronic antral gastritis were designated groups a (angle), b (body), c (antrum), and d (fluid), and those with *Hp*-positive chronic antral gastritis were designated groups A (angle), B (body), C (antrum), and D (fluid), every group has 20 samples.

DNA extraction and amplification of the 16S rRNA gene. Genomic DNA from the microbiota in the gastric mucosa and gastric fluid samples was extracted using an Invitrogen Purelink Genomic DNA kit (Life Technologies, Carlsbad, CA, USA) strictly in accordance with the manufacturer's instructions. The DNA was qualitatively and quantitatively detected by 1% agarose gel electrophoresis and using a Qubit2.0 concentration analyzer, respectively.

A specific primer with a "sequencing linker" was synthesized for the 16S rRNA V3 and V4 regions. The primer sequences used for first-step PCR were as follows: 356F, 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'; and 806R, 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3'. PCR reaction system: 2 × HiFi buffer 10 μL, forward primer 5 μmol/L, reverse primer 5 μmol/L, DNA 10 ng, plus ddH₂O to the total volume of 20 μL. PCR reaction conditions: 95°C 3 min, 25 cycles including 95°C 30 s, 60°C 30 s, 72°C 30 s; 72°C 5 min.

After the first-step PCR amplification, the products were purified with Agencourt AMPure XP (Beckman Coulter Life Science). Briefly, 20 μL of the PCR product was mixed with 16 μL of AMPure XP to absorb the DNA, washed twice with 80% ethanol and eluted with 40 μL of

Fig. 1. Bacterial community α -diversity of the eight groups measured by the ACE, Chao1, Shannon, and Simpson indexes.

(1), (2), (3), (4) represents group A, B, C, D, respectively. a, b, c, d represents group a, b, c, d, respectively. $p < 0.05$ indicates a significant difference among four groups, and $p \geq 0.05$ indicates the absence of a significant difference among four groups.

ddH₂O to obtain the purified PCR product. Subsequently, second-step PCR was performed using the purified products obtained in the first PCR step as the template. PCR reaction system: 2 × HiFi buffer 10 μ L, forward primer 5 μ mol/L, reverse primer 5 μ mol/L, template 2 μ L, plus ddH₂O to the total volume of 20 μ L. PCR reaction conditions: 95°C 3 min, 12 cycles including 95°C 30 s, 60°C 30 s, 72°C 30 s; 72°C 5 min. After completion of the PCR, the product was purified according to the previously mentioned magnetic bead-based purification protocol, which involves elution with 30 μ L of ddH₂O to obtain a DNA library of the mucosal samples.

Quality control of all libraries was conducted using an Agilent 2100 Bioanalyzer and a Qubit 2.0 concentration analyzer. The high-quality libraries were sequenced using an Illumina MiSeq sequencer.

Analysis of 16S rRNA gene sequences. The original data images of the sequencing results were analyzed using CASAVA software (v1.8.2), and sequencing data were obtained after preliminary quality analysis. PANDAseq (v2.7) was used to compare every two sequences and assemble them using the ends of the overlapping sections. The primer and linker sequences, the bases with a mass less than 20 bp at both ends, and the sequences with lengths less than 400 bp were removed using Trimmomatic (v0.30). The remaining sequences were compared with those in the database using USEARCH (v8.0), and the chimeric sequences were removed to obtain the final validated datasets. Operational taxonomic units (OTUs) are

sequences clustered into bins. Clustering was performed using the UCLUST method with a parameter similarity of 97%, and a list of OTUs and OTU representative sequences was obtained. The sequences were randomized using the QIIME platform (v1.7), and dilution curves were constructed using the number of sample sequences and the number of OTUs that might be represented by these sample sequences. ACE, Chao1, Shannon and Simpson analyses were performed to analyze the richness and diversity of the mucosal samples based on the OTU clustering results. Using the QIIME platform (v1.7), a principal component analysis was performed based on the unweighted UniFrac distance matrix.

To guarantee an accurate calculation of the bacterial diversity, we measured Good's coverage, which represents the coverage of each sample library: a higher value indicates a lower probability that the sequence in the sample is not detected. Good's coverage in the patients with chronic antral gastritis ranged from 0.971 to 0.989, which suggests that the 16S rRNA results from each library represent the results for most of the bacteria present in the gastric mucosa and fluid.

Statistical analysis. The statistical analyses were performed using SPSS19.0 software. Continuous variables were compared and analyzed using *t*-tests and the Tukey-Kramer HSD test, and the data were classified and analyzed by the Chi-square test. A *p* value ≤ 0.05 was considered statistically significant.

Table 2. The average of bacterial community α -diversity indexes in four groups.

Group	Reads	OTUs	ACE	Chao1	Shannon	Simpson	Coverage
a	182273.5 $\pm$ 19342	11795	8117.113	7764.049	6.289	0.953	0.987
b	160001 $\pm$ 15045	11173	8525.674	7928.684	6.264	0.955	0.986
p ¹	0.504	0.515	0.395	0.437	0.384	0.999	/
c	149490.75 $\pm$ 16196	9019	6073.992	5315.732	6.118	0.954	0.988
p ²	0.108	0.023	0.006	<0.01	0.844	0.999	/
p ³	0.978	0.026	0.001	<0.01	0.895	0.999	/
d	152253.25 $\pm$ 13883.2	9873	7368.733	6802.867	6.834	0.963	0.973
p ⁴	0.173	0.191	0.126	0.079	0.109	0.560	/
p ⁵	0.996	0.284	0.250	0.051	0.134	0.589	/
p ⁶	1.000	0.221	0.274	0.098	0.558	0.631	/
A	169730 $\pm$ 13390.8	8781	5087.186	4572.488	3.642	0.622	0.984
B	165551 $\pm$ 18428.1	7984	4871.078	4290.48	2.910	0.504	0.993
p ^{1*}	0.945	0.947	0.929	0.382	0.740	0.069	/
C	150109.25 $\pm$ 11918.3	6992	4023.304	3366.175	4.155	0.717	0.986
p ^{2*}	0.651	0.616	0.628	0.677	0.078	0.094	/
p ^{3*}	0.890	0.807	0.772	0.605	0.086	0.089	/
D	155225.75 $\pm$ 17606.6	6649	4013.335	3997.025	5.515	0.922	0.981
p ^{4*}	0.855	0.694	0.949	0.830	0.025	<0.01	/
p ^{5*}	0.980	0.793	0.926	0.877	0.039	<0.01	/
p ^{6*}	0.999	0.766	0.985	0.809	0.048	<0.01	/

Note: a represents *Hp*-negative gastric angle group; b represents *Hp*-negative gastric body group; c represents *Hp*-negative gastric antrum group; d represents *Hp*-negative gastric fluid group; A represents *Hp*-positive gastric angle group; B represents *Hp*-positive gastric body group; C represents *Hp*-positive gastric antrum group; D represents *Hp*-positive gastric fluid group.

¹ represents the *p* value between groups a and b; ² represents the *p* value between groups a and c; ³ represents the *p* value between groups b and c; ⁴ represents the *p* value between groups a and d; ⁵ represents the *p* value between groups b and d; ⁶ represents the *p* value between groups c and d.

^{1*} represents the *p* value between groups A and B; ^{2*} represents the *p* value between groups A and C; ^{3*} represents the *p* value between groups B and C; ^{4*} represents the *p* value between groups A and D; ^{5*} represents the *p* value between groups B and D; ^{6*} represents the *p* value between groups C and D.

Results

Hp infection affects the bacterial community diversity

A total of 19.6 million primitive sequences were obtained by high-throughput sequencing of the 16S rRNA gene of the mucosal samples through two-step PCR amplification. After filtering, 13.6 million of these sequences, with a coverage index of 88.00%, were retained. There was no significant difference in sequence reads. The sequences were sorted based on an OTU similarity of 97%, which resulted in a total of 43,968 OTUs. In addition, 21,850 and 25,322 OTUs were found in the samples from *Hp*-positive and *Hp*-negative antral gastritis patients.

To assess the variations in the microbiota structure between patients infected and not infected with *Hp*, we measured the microbial α diversity and β diversity. By measuring α diversity using the ACE, Chao1, Shannon, and Simpson indexes, we found that patients with *Hp* infection had significantly decreased microbial richness and diversity compared with non-*Hp*-infected patients. As shown in Fig. 1, the richness of gastric microbiota in the angle, body, and antrum of the gastric mucosa and the gastric fluid of the *Hp*-negative gastritis patients were significantly different ($p < 0.05$, Tukey-Kramer HSD test). Additionally, as shown in Table 2, the richness of gastric microbiota in the gastric angle and mucosal body was higher than that in the gastric antrum ($p < 0.05$, Tukey-Kramer HSD test), but no significant difference was found in the *Hp*-positive gastritis patients ($p > 0.05$, Tukey-

Kramer HSD test). However, the diversity of the microbiota in the angle, body, and antrum of the gastric mucosa and the gastric fluid showed no significant differences in the *Hp*-negative gastritis patients ($p > 0.05$, Tukey-Kramer HSD test), but a significant difference in the Shannon and Simpson indexes was found in the *Hp*-positive gastritis patients ($p < 0.05$, Tukey-Kramer HSD test).

Hp infection affects the bacterial similarity

We subsequently performed clustering based on an unweighted distance matrix and a principal component analysis to characterize the β -diversity in both *Hp*-positive and *Hp*-negative patients; specifically, the difference in the bacterial community between every two sets of samples was assessed. As shown in Fig. 2(1), the main axes 1 (PC1) and 2 (PC2) can explain 52.83% and 33.41% of the variation in the *Hp*-positive patients, respectively. The *Hp*-positive gastric mucosa and fluid samples were poorly clustered, and their distribution was relatively dispersed. As shown in Fig. 2(2), the main axes 1 (PC1) and 2 (PC2) can explain 44.89% and 26.47% of the variation in the positive patients, respectively. Notably, the samples from the angle, body, and antrum of the gastric mucosa and gastric fluid of the *Hp*-negative patients mainly clustered together, and their distribution was relatively concentrated. This finding indicated greater community similarity among the *Hp*-negative samples than among the *Hp*-positive samples.

Fig. 2. Unweighted UniFrac distance matrix principal components analysis (PCoA) of the bacterial community β -diversity in eight groups.

(1) PCoA results from the *Hp*-negative patients using PC1 and PC2 as the main axes; (2) PCoA results from the *Hp*-positive patients using PC1 and PC2 as the main axes. Black, red, blue and yellow represent, respectively, gastric fluid, gastric angle mucosa, gastric body mucosa, and gastric antrum samples.

Distribution of predominant bacteria in the gastric mucosa and fluid of patients with or without *Hp* infection

Analysis of the composition of the gastric microbiota revealed that *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were the three most dominant phyla in the gastric antrum mucosa and fluid of the eight groups. The differences in the phylum composition between the gastric mucosa and fluid samples are shown in Fig. 3(1) and Table 3. The composition of *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* showed no significant differences between the gastric mucosa and gastric fluid samples from either the *Hp*-negative or *Hp*-positive patients ($p > 0.05$, Tukey-Kramer HSD test). However, the composition of *Proteobacteria* in the *Hp*-positive patient samples was higher than that in the *Hp*-negative patient samples ($p < 0.01$, Tukey-Kramer HSD test). The richness of *Bacteroidetes* and *Firmicutes* in the *Hp*-positive patient samples were relatively lower than those in the *Hp*-negative patient samples, and these differences were significant ($p < 0.01$, Tukey-Kramer HSD test).

The predominant bacterial genera in the *Hp*-positive gastric fluid were *Prevotella*, *Neisseria*, and *Fusobacterium*, and those in the *Hp*-negative gastric antrum mucosa or gastric fluid were *Prevotella*, *Veillonella*, *Streptococcus*, *Neisseria*, *Fusobacterium*, *Haemophilus* and *Rothia*. The differences in the genus composition between the

gastric mucosa and fluid samples are shown in Fig. 3(2) and Table 3. *Hp* was more abundant in the mucosal samples than the gastric fluid samples from *Hp*-positive patients ($p < 0.01$, Tukey-Kramer HSD test). No difference in the *Hp* content was found among the different sites of the gastric mucosa ($p > 0.05$, Tukey-Kramer HSD test). Due to *Hp* colonization, fewer proportions of *Streptococcus*, *Prevotella*, *Haemophilus* and *Veillonella* were found in the *Hp*-positive samples compared with the *Hp*-negative patients, and these differences were significant ($p < 0.05$, Tukey-Kramer HSD test; Fig. 3(2) and Table 3). The richness of *Leptotrichia* in the mucosal samples from the *Hp*-negative patients was greater than that in the gastric fluid samples from these patients ($p < 0.05$, Tukey-Kramer HSD test).

Discussion

We profiled the gastric microbiota, including that in the gastric mucosa and gastric fluid, associated with chronic antral gastritis, and demonstrated that the gastric microbiota composition in patients with *Hp* infection was significantly different from that in the patients without *Hp* infection. The major finding obtained in this study was confirmation of the existence of *Hp* in gastric fluid. The bacterial compositions at different sites in the gastric mucosa, including the gastric fluid, of the *Hp*-negative patients were similar. After *Hp* infection, the bacterial composition at different sites in the gastric mucosa was similar but differed from that in gastric fluid.

It is generally believed that gastric fluid is rich in digestive enzymes, has a low pH value and thus contains a small number of bacteria. However, this study showed the presence of many microorganisms in gastric juice, which is inconsistent with previous findings.

The gastric microbiota was dominated by five phyla, namely, *Fusobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, and this finding is consistent with that obtained in healthy persons (Peng et al., 2017). Our results revealed that human gastric fluid harbors a diverse microbiota and the existence of differences in the bacterial microbiota of gastric mucosa and gastric fluid of patients with *Hp* infection. In patients without *Hp* infection, the diversity of the gastric fluid microbiota is higher than that of the gastric antrum microbiota. Our findings are supported by those obtained in a previous study, which noted that the bacterial diversity of 30 patients with *Hp* infection was lower than that of 30 healthy persons (Peng et al., 2017).

As expected, *Helicobacter* was the most abundant genus in the samples from *Hp*-infected patients with chronic antral gastritis. The percentage of *Hp* in the gastric fluid was very low, which differs from the findings obtained in previous studies of stomach biopsy samples collected from the mucosa (Yang et al., 2013; Zilberstein et al., 2007). This result was similar to the previous finding that *Hp* is not the dominant species in the gastric fluid microbiota (Rosenvinge et al., 2013; Wu et al., 2014). Antral gastritis caused by *Hp* infection decreased the richness and diversity of the microbiota, as well as the microbiota at other positions of the gastric mucosa. This finding is consistent

Table 3. The percentages of phylum and genus composition in eight groups (%).

Group	Phylum			Genus				
	<i>Proteobacteria</i>	<i>Bacteroidetes</i>	<i>Firmicutes</i>	<i>Hp</i>	<i>Prevotella</i>	<i>Veillonella</i>	<i>Streptococcus</i>	<i>Leptotrichia</i>
a	14.7	38.5	35.3	0	36.3	13.3	11.9	1.6
b	12.5	39.6	36.5	0	36.0	13.9	12.1	1.9
c	15.1	36.6	33.7	0	34.0	12.7	10.0	1.5
d	14.5	44.3	26.6	0	35.3	10.7	5.8	0.4
p ¹	0.936	0.795	0.726	/	0.905	0.683	0.151	0.042
p ²	0.812	0.885	0.650	/	0.926	0.618	0.104	0.047
p ³	0.956	0.716	0.874	/	0.987	0.574	0.124	0.045
A	68.6	13.8	9.8	58.0	10.4	2.8	3.4	1.7
p ¹	<0.01	<0.01	<0.01	/	<0.01	<0.01	<0.01	0.963
B	75.6	9.8	7.9	68.6	8.1	2.6	2.8	1.9
p ²	<0.01	<0.01	<0.01	/	<0.01	<0.01	<0.01	0.989
C	54.5	19.7	13.9	45.5	13.8	3.8	4.6	2.3
p ³	<0.01	<0.01	<0.01	/	<0.01	<0.01	<0.01	0.871
D	46.7	20.8	9.9	0.7	15.5	4.5	2.0	1.2
p ^{4*}	<0.01	<0.01	<0.01	/	<0.01	<0.01	<0.01	<0.01
p ^{5*}	0.358	0.225	0.958	<0.01	0.325	0.609	0.772	0.591
p ^{6*}	0.288	0.106	0.835	<0.01	0.147	0.636	0.803	0.497
p ^{7*}	0.440	0.863	0.726	<0.01	0.449	0.736	0.239	0.436

Note: a represents *Hp*-negative gastric angle group; b represents *Hp*-negative gastric body group; c represents *Hp*-negative gastric antrum group; d represents *Hp*-negative gastric fluid group; A represents *Hp*-positive gastric angle group; B represents *Hp*-positive gastric body group; C represents *Hp*-positive gastric antrum group; D represents *Hp*-positive gastric fluid group;
¹ represents the *p* value between groups a and d; ² represents the *p* value between groups b and d; ³ represents the *p* value between groups c and d.
^{1*} represents the *p* value between groups a and A; ^{2*} represents the *p* value between groups b and B; ^{3*} represents the *p* value between groups c and C; ^{4*} represents the *p* value between groups d and D; ^{5*} represents the *p* value between groups A and D; ^{6*} represents the *p* value between groups B and D; ^{7*} represents the *p* value between groups C and D.

Fig. 3. Bacterial community composition for the four groups identified at the phylum and genus levels.
Note: a represents *Hp*-negative gastric angle group; b represents *Hp*-negative gastric body group; c represents *Hp*-negative gastric antrum group; d represents *Hp*-negative gastric fluid group; A represents *Hp*-positive gastric angle group; B represents *Hp*-positive gastric body group; C represents *Hp*-positive gastric antrum group; D represents *Hp*-positive gastric fluid group.

with the literature (Gross, 1996), which shows that *Hp* colonizes the mucous layer and adheres to gastric epithelial cells. This finding indicates that *Hp* infection will migrate in the stomach and change the microbiota.

In contrast, the bacterial richness of the antrum of patients without *Hp* infection is lower than that at other sites. Many mechanisms lead to chronic antral gastritis (Yakabi et al., 1998), but antral gastritis, which is not caused by

Hp infection, causes a decrease in the survival of bacteria in the gastric antrum but has no effect on the structure of the bacterial microbiota. This finding indicates that the occurrence of chronic antral gastritis inhibits the growth of bacteria but does not affect the structure of the microbiota

Engstrand and Lindberg (2013) studied the gastric microbiota of 13 healthy individuals through pyrosequencing and found 200 germline types and five dominant taxa, *Prevotella*, *Streptococcus*, *Veillonella*, *Rothia*, and *Pasteurellaceae*. These researchers found no significant difference in structure between the microbiota of the gastric antrum and that of the gastric body, which is consistent with our findings. Due to *Hp* colonization, lower proportions of *Streptococcus*, *Prevotella*, *Haemophilus* and *Veillonella* were found in the *Hp*-positive samples compared with the *Hp*-negative patients. *Streptococcus* and *Prevotella* are commensals of the oral cavity and esophagus (Li et al., 2018), and whether they constitute transient or active resident stomach microbes has not yet been clarified. *Haemophilus* and *Veillonella* are bacteria that can reduce the reaction of nitroso to N-nitroso compound by its own nitrozyme, and the synthesis of the nitroso compound increases the risk of gastric cancer (Ma et al., 2012). *Leptotrichia* species typically colonize the oral cavity and genitourinary tract (Eribe and Olsen, 2008). The mucosal barriers are broken, and *Leptotrichia* frequently spread to the bloodstream through these broken barriers (Eribe and Olsen, 2008). *Hp* might interact with *Leptotrichia*, and this interaction leads to the development of gastritis. The richness of *Leptotrichia* in the gastric antrum mucosal samples from the *Hp*-negative patients was greater than that in the gastric fluid samples from these samples. These bacteria may interact to jointly induce the development of antral gastritis.

In summary, *Hp* infection changes the colonization of the gastric mucosa and fluid, and *Hp* exists in the gastric fluid. The occurrence of chronic antral gastritis inhibits the growth of bacteria but does not affect the microbiota structure. The structure of the gastric mucosa and fluid in the absence of *Hp* infection is similar to that in the presence of *Hp* infection. After infection, *Hp* migrates in the stomach and changes the microbiota, and chronic antral gastritis might also be caused by other bacteria.

Author's Contributions

YJ and SFY conceived and designed the study. SFY and JMZ helped with the collection of the samples. FM, YL and LYZ performed the laboratory tests. YJ and FM participated in the data analyses and the writing of the manuscript. All the authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research was supported by the Zhejiang Provincial Natural Science Foundation of China (Grant No. LGF18H090020), the Health Science and Technology Project of Zhejiang Province (Grant No. 2017KY278) and Zhejiang Provincial Public Welfare Technology Research Project (LGF20H030005).

References

- Dan-Si, Q. I., Zhang, X. T., Gao, B. H. et al. (2016) Correlation analysis of *Helicobacter pylori* infection and gastric mucosa pathological changes in patients with chronic gastritis. *Chinese Journal of Nosocomiology*, **26**, 3640–3642.
- Dubois, A. and Borén, T. (2010) *Helicobacter pylori* is invasive and it may be a facultative intracellular organism. *Cell. Microbiol.*, **9**, 1108–1116.
- Engstrand, L. and Lindberg, M. (2013) *Helicobacter pylori* and the gastric microbiota. *Best Prac. Res. Clin. Gastroenterol.*, **27**, 39–45.
- Eribe, E. R. K. and Olsen, I. (2008) *Leptotrichia* species in human infections. *Anaerobe*, **14**, 131–137.
- Gross, M. (1996) When and how to eradicate *Helicobacter pylori*: role of histamine receptor antagonists. *Eur. J. Med. Res.*, **1**, 189.
- Li, D., Jian, Y., Jing, Z., Shan-Rui, M., Hai-Rui, W. et al. (2018) Microbial similarity and preference for specific sites in healthy oral cavity and esophagus. *Front. Microbiol.*, **9**, 1603.
- Liu, H., Semino-Mora, C., and Andre, D. (2012) Mechanism of *H. pylori* intracellular entry: An in vitro study. *Front. Cell. Infect. Microbiol.*, **2**, 13.
- Ma, J. L., Zhang, L., Brown, L. M., Li, J. Y., and Gail, M. H. (2012) Fifteen-year effects of *Helicobacter pylori*, garlic, and vitamin treatments on gastric cancer incidence and mortality. *J. National Cancer Inst.*, **104**, 488–492.
- Mowat, C., Williams, C., Gillen, D., Hossack, M., Gilmour, D. et al. (2000) Omeprazole, *Helicobacter pylori* status, and alterations in the intragastric milieu facilitating bacterial-nitrosation. *Gastroenterology*, **119**, 339–347.
- Peng, X. H., Zhou, L. Y., He, L. H., Song, Z. Q., and Zhang, J. Z. (2017) Analysis of characteristics of gastric flora in patients with *Helicobacter pylori* infection. *J. Gastroenterol. Hepatol.*, **26**, 658–663.
- Plottel, C. and Blaser, M. (2011) Microbiome and malignancy. *Cell Host Microbe*, **10**, 335.
- Roman, L. D., Lukyanchuk, R., Sablin, O. A., Araslanova, E. I., Eklund, C. et al. (2016) Prevalence of *H. pylori* infection and atrophic gastritis in a population-based screening with serum biomarker panel (gastropanel®) in st. petersburg. *Anticancer Res.*, **36**, 4129.
- Rosenvinge, E. C. V., Song, Y., White, J. R., Maddox, C., and Fricke, W. F. (2013) Immune status, antibiotic medication and ph are associated with changes in the stomach fluid microbiota. *ISME J.*, **7**, 1354–1366.
- Sanduleanu, S., Jonkers, D., De, B. A., Hameeteman, W., and Stockbrugger, R. W. (2001) Double gastric infection with *Helicobacter pylori* and non-*Helicobacter pylori* bacteria during acid-suppressive therapy: increase of pro-inflammatory cytokines and development of atrophic gastritis. *Aliment. Pharmacol. Ther.*, **15**, 1163–1175.
- Terao, S., Suzuki, S., Yaita, H. et al. (2020) Multicenter study of autoimmune gastritis in Japan: Clinical and endoscopic characteristics. *Dig. Endosc.*, **32**, 364–372.
- Watari, J., Chen, N., Amenta, P. S., Fukui, H., Oshima, T. et al. (2014) *Helicobacter pylori* associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J. Gastroenterol.*, **20**, 5461–5473.
- Wu, W. M., Yang, Y. S., and Peng, L. H. (2014) Microbiota in the stomach: new insights. *J. Dig. Dis.*, **15**, 54–61.
- Yakabi, K., Mimura, H., Iwabuchi, H., Ro, S., and Nakamura, T. (1998) Interleukin-8 enhances tetragastrin-stimulated acid secretion in vivo. *Dig. Dis. Sci.*, **43**, 2317–2321.
- Yang, I., Nell, S., and Suerbaum, S. (2013) Survival in hostile territory: the microbiota of the stomach. *FEMS Microbiol. Rev.*, **37**, 736–761.
- Zhang, J. Z., Feng, Q. L., Hu, Y. L., Yang, T., and Zhang, Y. (2015) Correlation study on Chinese medical syndrome types of chronic atrophic gastritis children, *Hp*, and IL-1 B Polymorphism[J]. *Zhongguo Zhong Xi Yi Jie He za Zhi*, **35**, 1433–1436.
- Zilberstein, B., Quintanilha, A. G., Santos, M. A. A., Pajecski, D., Moura, E. G. et al. (2007) Digestive tract microbiota in healthy volunteers. *Clinics (São Paulo, Brazil)*, **62**, 47–54.