

# Effects of eradication of *Helicobacter pylori* on oral malodor and the oral environment: a single-center observational study

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## Research note

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# Abstract

*Objective:* Although a relationship between *Helicobacter pylori* and oral malodor has been suggested, it remains to be confirmed. One reason for this is that many studies assess oral malodor subjectively. Another reason for the uncertainty is that the reduction in oral malodor may be due to the effect of antibiotics on the oral microbiota. In this study, changes in oral malodor and the eradication treatment of *H. pylori* were investigated by organoleptic test and gas chromatography. In addition, the salivary bacterial composition and clinical parameters were analyzed.

*Results:* The organoleptic test score, hydrogen sulfide and dimethyl sulfide concentrations, and all clinical parameters except for tongue-coating score were significantly decreased at 1 week compared with baseline. Although antibiotic treatment also altered the overall composition of the salivary bacterial population, it had recovered at 7 weeks. On the date that *H. pylori* was determined to have been eradicated from all of the subjects (7 weeks after treatment), only the organoleptic test score was significantly lower compared with baseline. The hydrogen sulfide and dimethyl sulfide concentrations were non-significantly lower than those at baseline.

## Introduction

*Helicobacter pylori* is the most frequent cause of gastritis; it is also responsible for peptic ulcers and gastric cancer. The relationship between *H. pylori* and oral malodor has been known since the identification of *H. pylori* [1].

Upper gastrointestinal disorders can cause oral malodor, but oral malodor is linked to the oral cavity in 90% of cases [2]. Several cross-sectional studies have reported that *H. pylori*-infected individuals had oral malodor [3-5], but no difference in exhaled breath or stomach air [5] compared to uninfected individuals. Therefore, it has often been hypothesized that gastric or oral *H. pylori* infection alters oral conditions, possibly leading to oral malodor [6-8].

Observational studies of the effect of *H. pylori* eradication on oral malodor would help better to clarify the relationship between oral malodor and *H. pylori* infection [9-12]. Such studies should consider two points. First, it is necessary to evaluate oral malodor objectively. Prior studies assessed subjective symptoms of oral malodor using questionnaires and interviews; however, it is difficult for an individual to evaluate their own oral malodor accurately [13, 14]. Second, it is necessary to clarify the effects of the antibiotics used for *H. pylori* eradication on the oral microbiota, since those antibiotics are reportedly effective against periodontal disease [15, 16].

In this study, we objectively evaluated oral malodor changes along with the eradication treatment of *H. pylori* by measuring the concentrations of volatile sulfur compounds (VSCs) by gas chromatography (GC) and organoleptic test (OLT) as the primary outcome. The secondary outcomes included oral symptoms and bacterial parameters. In addition, 16S rRNA gene sequencing was performed to evaluate the direct effect of the antibiotics used to eradicate *H. pylori* on the oral microbiota.

# Materials And Methods

## *Study design*

An observational study was conducted between September 2014 and May 2016. The study population comprised patients who presented with gastric symptoms and were diagnosed with *H. pylori* infection based on positive rapid urease tests conducted on gastric biopsy specimens. All were outpatients who presented at the General Surgery Clinic of Fukuoka Dental College Medical and Dental Hospital. The inclusion criteria were as follows: 20 years or older, having own teeth, and no oral symptoms that require immediate treatment. Those with drug allergies and pregnant women were excluded. Twelve patients (5 men, 7 women; mean age  $59.5 \pm 15.3$  years) were analyzed (Additional file 1; Table S<sub>1</sub>). They were prescribed a 1-week course of a triple-drug regimen (30 mg lansoprazole, 750 mg amoxicillin, and 200 mg clarithromycin, twice daily) as the primary eradication regimen. In cases in which the primary treatment failed, 250 mg metronidazole was used instead of clarithromycin as the secondary regimen.

## *Clinical examinations*

Oral malodor and oral clinical symptoms were evaluated on the date of initiation of *H. pylori* eradication treatment and 1 and 7 weeks thereafter. The severity of oral malodor was determined by OLT [13] and GC. A gas chromatograph (model GC2014; Shimazu Works, Kyoto, Japan) was used to assay the concentrations of H<sub>2</sub>S, CH<sub>3</sub>SH, and CH<sub>3</sub>SCH<sub>3</sub> in mouth air. Periodontal health, plaque control assessed with the plaque index (PII) [17], and the degree of tongue coating measured using the tongue coating score (TCS) [14] were evaluated. Periodontal health was assessed using the average probing pocket depth (PPD) and the percentage of bleeding on probing (BOP) values. The ammonia concentration was measured using a portable ammonia-monitoring device (ATTAIN; Taiyo, Osaka, Japan) according to the manufacturer's instructions. Saliva samples were obtained by stimulation using chewing gum (CheckBuf; Morita, Osaka, Japan) and then subjected to sequencing of the 16S rRNA gene amplicons and quantitative analysis of bacterial species. These examinations were conducted at 9 am; the subjects were prohibited from eating, drinking, chewing, smoking, brushing teeth, or rinsing their mouth after waking up. The subjects were also instructed not to undergo dental treatment during the observation period.

## *Quantitative analysis of bacterial species in saliva*

Quantitative analysis of bacterial species in saliva was performed using the fibrous DNA chip Genopal® (Mitsubishi Chemical, Tokyo, Japan). The copy numbers of all oral bacteria and 12 bacterial species were determined. According to the Socransky pyramid classification [18], the bacterial species were classified into the red (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*), orange (*Campylobacter rectus*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Prevotella nigrescens*), green (*Aggregatibacter actinomycetemcomitans* and *Capnocytophaga gingivalis*), and yellow (*Streptococcus gordonii*, *Streptococcus intermedius*, and *Streptococcus mutans*) groups, and the proportions of these groups among the total oral bacteria were evaluated.

### *Sequencing of 16S rRNA gene amplicons*

DNA was extracted from each saliva sample as described previously [19]. The V1-V2 regions of the 16S rRNA gene were amplified by PCR using the universal bacterial primers 8F and 338R with adaptor and sample-specific 8-base tag sequences as described previously [20]. Equal amounts of the purified PCR amplicons were pooled. Following emulsion PCR, sequencing was performed on the Ion PGM using an Ion PGM Hi-Q View sequencing kit (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's instructions. The raw sequencing reads were quality-filtered as described previously [21]. The quality-checked reads were assigned to the appropriate sample by examining the tag sequence. Similar sequences were assigned to operational taxonomic units (OTUs) using UPARSE [22], with a minimum pairwise identity of 97%. The taxonomy of each representative sequence was determined using blast against oral bacterial 16S rRNA gene sequences in the Human Oral Microbiome Database [23]. Nearest-neighbor species with  $\geq 98.5\%$  identity were selected as candidates for each OTU. The taxonomy of an OTU without any hits was determined to the genus level using the Ribosomal Database Project classifier with a minimum support threshold of 80%. The alpha diversity index and UniFrac distances [24] were calculated following rarefaction to 5,000 reads per sample.

### *Statistical analysis*

The Wilcoxon signed-rank test was used to compare malodor, clinical, and bacterial parameters among baseline, 1 week, and 7 weeks. The relative abundance of bacterial genera after 1 and 7 weeks were compared with those at baseline using Dunnett's test. Statistical analyses were conducted using R software, version 3.6.1 [25]. A *P*-value of  $<0.05$  was considered to reflect statistical significance.

## **Results**

### *Oral malodor and clinical parameters*

Table 1 shows the oral malodor and clinical parameters at baseline, the date of treatment completion (1 week), and the date of determining when *H. pylori* had been eradicated from all subjects. The values of all parameters decreased at 1 week compared with baseline, and the decreases in the OLT score, H<sub>2</sub>S and CH<sub>3</sub>SCH<sub>3</sub> concentrations, PPD, BOP, PII, and ammonia concentration were significant. On the date when *H. pylori* had been eradicated from all subjects, the values of all parameters other than the CH<sub>3</sub>SH concentration were lower than those at baseline but tended to be higher than those at 1 week. Only the OLT score was significantly lower than that at baseline (*P* = 0.037). The comparisons between the success and failure groups after primary eradication treatment showed that the OLT score, total VSC concentration, BOP, and PPD in the failure group were increased slightly at 7 weeks, as compared with 1 week, but were unchanged in the success group (Additional file 2: Figure S<sub>1</sub>).

Table 1. Clinical parameters at baseline, the date of treatment completion, and the date of eradication of *H. pylori*. Median [IQR].

Parameter	Baseline	Date of treatment completion	Determination of date of successful eradication
OLT score	2.25 [1.50, 3.00]	1.50 [0.89, 1.56]*	1.75 [1.19, 2.00]*
VSCs (ng/10 mL)	2.29 [1.26, 6.72]	1.69 [0.25, 2.68]	1.57 [0.78, 5.23]
H <sub>2</sub> S (ng/10 mL)	1.19 [0.50, 4.88]	0.51 [0.13, 1.75]*	0.56 [0.35, 3.14]
CH <sub>3</sub> SH (ng/10 mL)	0.55 [0.36, 1.24]	0.29 [0.09, 0.95]	0.58 [0.21, 1.35]
CH <sub>3</sub> SCH <sub>3</sub> (ng/10 mL)	0.60 [0.38, 0.70]	0 [0.00, 0.43]*	0.32 [0.24, 0.55]
PPD (mm)	3.02 [2.98, 3.24]	3.0 [2.92, 3.02]*	3.0 [2.93, 3.02]
BOP (%)	7.64 [2.98, 21.0]	4.27 [1.74, 6.33]*	4.74 [2.22, 14.0]
PII	0.67 [0.21, 0.83]	0.29 [0.21, 0.39]*	0.65 [0.35, 0.84]
TCS	2.00 [1.75, 2.00]	1.00 [1.00, 2.00]	1.50 [1.00, 2.00]
Ammonia (ppm)	39.0 [20.8, 45.8]	14.0 [0.00, 18.8]*	30.0 [20.0, 50.0]

PII, plaque index; TCS, tongue-coating score; \*  $P < 0.05$  vs. baseline by Wilcoxon signed-rank test

#### Quantitative analysis of 12 bacterial species in saliva

The total bacterial amounts (median [IQR], log copies/mL) in saliva at baseline, 1 week, and 7 weeks with the primary eradication regimen were 7.7 [7.4–8.0], 7.4 [7.2–8.0], and 7.9 [7.5–8.1], respectively. The proportions of bacterial groups were compared in the success and failure groups with the primary eradication regimen (Table 2). The proportion of the red group was lower at 7 weeks than at baseline in both groups, although the difference was significant only in the success group ( $P = 0.039$ ). In addition, the proportions of *T. forsythia* ( $P = 0.098$ ) and *T. denticola* ( $P = 0.098$ ) were decreased at 7 weeks compared with baseline and at 1 week, respectively, albeit not significantly so (Additional file 3: Table S<sub>2</sub>). The proportions of *C. rectus*, *F. nucleatum* and *P. nigrescens* at baseline were non-significantly higher in the failure group than in the success group.

Table 2. Bacterial groups in saliva by the primary eradication regimen outcome. Median [IQR].

Bacterial group	Eradication	Baseline	1 week	7 weeks
Red group	Success	0.04 [0.03, 0.07]	0.02 [0.01, 0.04]	0.03 [0.02, 0.03]*
	Failure	0.05 [0.04, 0.05]	0.03 [0.02, 0.06]	0.02 [0.02, 0.02]
Orange group	Success	2.14 [1.40, 5.56]	1.01 [0.12, 4.38]	3.29 [2.62, 7.17]
	Failure	2.63 [2.11, 4.47]	0.78 [0.43, 3.87]	6.17 [5.0, 6.65]
Green group	Success	0.05 [0.01, 0.28]	0.22 [0.01, 0.77]	0.03 [0.0, 0.09]
	Failure	0.05 [0.04, 0.17]	0.35 [0.18, 0.45]	0.02 [0.01, 0.32]
Yellow group	Success	0.55 [0.42, 1.17]	0.28 [0.15, 0.61]	0.75 [0.38, 0.89]
	Failure	0.80 [0.62, 1.08]	1.05 [0.78, 2.24]	0.38 [0.36, 0.67]

\*  $P < 0.05$  vs. baseline by Wilcoxon signed-rank test

1 week, date of treatment completion. 7 weeks, determination of date of eradication.

### *Bacterial diversity and composition in saliva*

The alpha diversity index was decreased at 1 week compared with baseline in every individual and increased to the baseline level again on the determination of the date of *H. pylori* eradication by the primary regimen (Additional file 4: Figure S<sub>2</sub>). A UniFrac analysis demonstrated that antibiotic treatment for *H. pylori* infection altered the overall composition of the salivary bacterial populations, although they had recovered on the determination day of eradication (Figure 1). The relative abundance of *Rothia* was significantly lower in the saliva at 1 week compared with baseline ( $P = 0.005$ ), whereas no significant difference in the relative abundance of the genus was observed between baseline and the date of determining *H. pylori* eradication by the primary regimen (Additional file 5: Figure S<sub>3</sub>).

## **Discussion**

A recent systematic review concluded that oral malodor is reduced by eradication of *H. pylori* [26]. However, most of the studies included in that review assessed subjective symptoms of oral malodor using a questionnaire [11, 12, 27, 28]. Few studies have assessed oral malodor using a portable sulfide monitor or OLT [10, 29]. Oral malodor is influenced by psychological factors, and it is difficult for individuals to evaluate their own oral malodor accurately [13, 14]. To elucidate the relationship between oral malodor and *H. pylori* infection, objective evaluations of oral malodor are needed, such as measuring odor components and OLT performed by experts. This is the first study to measure VSC concentrations by GC and to perform the OLT to evaluate the effect of *H. pylori* eradication on oral malodor objectively. As a result, the OLT score was significantly decreased after *H. pylori* eradication compared with baseline. In contrast, the concentrations of H<sub>2</sub>S and CH<sub>3</sub>SCH<sub>3</sub> were decreased on the date of eradication compared with baseline, albeit not significantly so. The amelioration of oral malodor was not due to a direct effect of antibiotics on oral bacteria, because the bacterial composition was not different from that before eradication and the total oral bacterial count was not significantly different between after *H. pylori* eradication and baseline. Use of antibiotics in combination with mechanical therapy for periodontal disease resulted in significant differences in the richness and dissimilarity of the oral microbiota after 2 months, whereas the evenness and diversity were not affected [30]. It is possible that the effect on the oral microbiota differs between antibiotics alone and antibiotics plus mechanical therapy. Concerning the significant reduction of the OLT score, ammonia, methylamine, dimethylamine, propionic acid, butyric acid, indole, skatole, and cadaverine may cause oral malodor in addition to VSCs [31]. More studies on the involvement of substances other than VSCs in oral malodor associated with *H. pylori* are needed.

After initiating the primary eradication regimen, there were differences in the clinical findings and the proportions of some bacterial species between the success and failure groups. We postulate that these differences are key to successful eradication. With the primary eradication regimen, the OLT score, total VSC concentration, BOP, and PPD tended to increase slightly, after decreasing at 1 week, in the failure

group, but these parameters remained low in the success group. The success group showed a significant decrease in the proportion of red complex species ( $P = 0.039$ ), of which *P. gingivalis* was detected in only 5 of 12 subjects at baseline. In the success group, the proportion of *T. forsythia* was decreased compared with baseline ( $P = 0.098$ ), and the proportion of *T. denticola* was decreased compared with 1 week ( $P = 0.098$ ), albeit not significantly. At 1 week, the proportions of *S. gordonii* and *C. gingivalis* were increased and decreased in the failure and success groups, respectively. The proportions of *C. rectus*, *F. nucleatum*, and *P. nigrescens* at baseline were higher in the failure group than in the success group. Periodontopathic bacteria such as *F. nucleatum* and *P. nigrescens* produce indole, skatole, and acetic, propionic, and butyric acids [32, 33]. Compared with the success group, the higher proportions of these bacteria in the failure group may be related to the higher OLT score at baseline, despite the lower VSC and PPD levels. *C. rectus* and *H. pylori* share several antigens that may play a role in the initiation and progression of periodontal disease [34, 35]. A high proportion of *C. rectus* may indicate active periodontal or gastric infection.

The ability of *H. pylori* to produce  $\text{H}_2\text{S}$  and  $\text{CH}_3\text{SH}$  is strain-specific [36]. *H. pylori* has been detected in oral specimens [6, 7, 37], and its prevalence was associated with the progression of periodontitis. There is disagreement as to whether *H. pylori* is present in the oral cavity. The bacterial communities in the oral cavity are very complex, with over 500 species, and include many VSC-producing bacteria [2]. The oral malodor associated with *H. pylori* is unlikely to be caused by its production of VSCs, but rather by altering the oral environment and the populations of some oral bacteria. Chronic systemic inflammation and periodontitis interact via the systemic inflammatory response [38]. Thus, irrespective of the presence or absence of *H. pylori* infection in the oral cavity, gastric inflammation by *H. pylori* may exacerbate oral malodor via its effect on the oral environment.

## Limitations

The limitations of the current study include the small number of subjects. The primary *H. pylori* eradication regimen failed in only three subjects, which was insufficient to evaluate statistically. In addition, although 16S rRNA gene sequencing analysis confirmed that the antimicrobial agents had no effect on the oral microbiota at 2 months after the start of treatment, a longer observation period might have clarified the relationship between *H. pylori* and oral malodor.

## Abbreviations

BOP: bleeding on probing

GC: gas chromatography

IQR: interquartile range

PII: plaque index

OLT: organoleptic test

OTU: operational taxonomic unit

PPD: probing pocket depth

TCS: tongue coating score

VSC: volatile sulfur compound

## Declarations

### Ethics approval and consent to participate

All participants understood the nature of the research project and provided written informed consent to participate in this study. Permission for this study was obtained from the Ethics Review Committee for Clinical Research of Fukuoka Gakuen (No. 308).

### Consent for publication

Not applicable

### Availability of data and materials

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

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

Suzuki N, Hirofuji T, and Shinohara T designed the study, analyzed the data, and wrote the manuscript. Suzuki N and Yoneda M measured oral malodor and carried out the clinical assessments of the oral cavity. Hanioka T prepared the manuscript, specifically the visualization/data presentation. Beppu R and Shinohara T invited patients to participate in the study and performed the medical assessments. Takeshita T, Asakawa M, and Yamashita Y analyzed the microbiota by 16S rRNA sequencing.

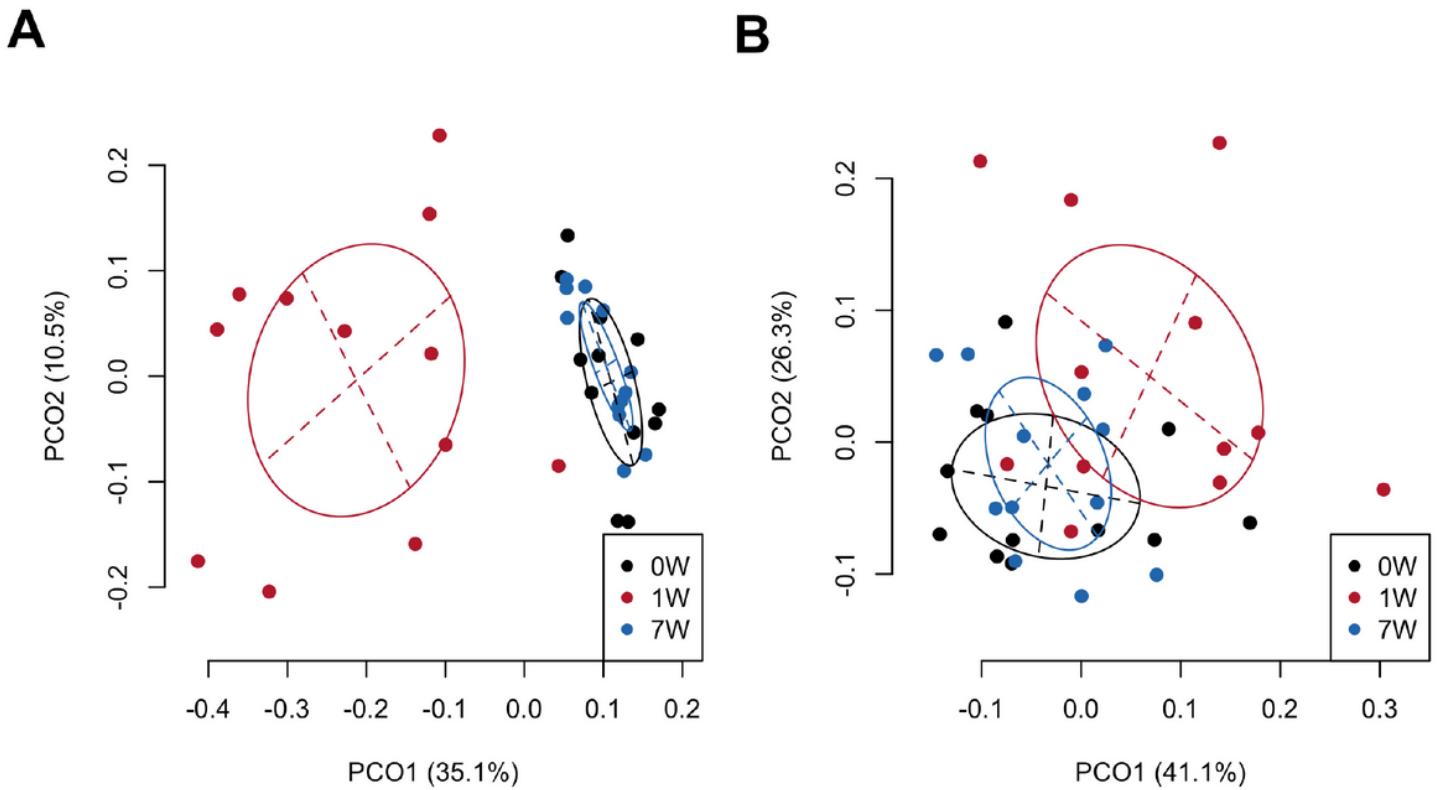
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## Figures



**Figure 1**

A principal coordinate analysis (PCoA) plot showing the similarity relationship between saliva samples from 12 patients at three phases of treatment using unweighted (A) and weighted (B) UniFrac distance matrices with the primary eradication regimen. 0W, baseline; 1W, date of treatment completion (1 week); 7W, determination of date of *H. pylori* eradication (7 weeks). The ellipse covers 67% of the samples belonging to each phase group.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalTablesFigurelegends2.pdf
- FigureS1.pdf
- FigureS2.pdf
- FigureS3.pdf