

Original Article

Detection of gastritis-associated pathogens by culturing of gastric juice and mucosa

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Abstract: It is well known that *Helicobacter pylori* (*H. pylori*) is not the only indigenous bacterium in the stomach, as numerous studies have revealed that the gastric microbiota contributes to the pathogenesis of gastric disease. However, the correlation between the gastric bacterial flora and gastritis is unclear. By comparing differences in viable gastric bacteria between a gastritis group and a healthy group, we examined the potential species related to chronic gastritis. We collected juice and mucosa samples from 103 consecutive patients and identified 81 species by culturing and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The positive rates of *Streptococcus* and *Neisseria* were markedly higher in the gastritis group than those in the normal group, suggesting that certain bacterial species may play vital roles in the development of gastritis rather than acting as transient microbes. This finding can be applied to the diagnosis and treatment of chronic gastritis as evidence supporting non-*Helicobacter pylori* infection-related gastritis.

Keywords: Gastric microbiota, helicobacter pylori, gastritis, infection disease, matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Introduction

Chronic inflammation is one of the most common gastric conditions worldwide due to bacterial infection, alcohol consumption and biliary reflux. *Helicobacter pylori* (*H. pylori*) infection is the most well-known risk factor [1]. However, infection with other gastric pathogens has been overlooked. The bacteria of gastritis patients must be reviewed, as they were once considered transient bacteria.

Recent studies have indicated that the gastric microbiota are entirely different from the microbiota of the oral cavity, throat, or other parts of the upper gastrointestinal tract [2]. The gastric microbiota may promote *H. pylori*-associated disease [3]. The interaction between *H. pylori* and the microbiota also influences gastric disease progression [4, 5]. *Candida albicans* is an opportunistic pathogen that induces gastritis in patients taking antibiotics [6]. *Streptococcus*-related gastritis has been described in several case reports, but it was never investigated thoroughly [7, 8]. Exploration of gastric pathogens is essential to gain insight into infectious diseases of the stomach.

The 5th Maastricht V/Florence Consensus [9] presented a workshop to discuss the role of the gastric microbiota in *H. pylori*-associated disease and *H. pylori* eradication effects on the microbiota. Although the evidence level was low and the grade of the recommendation was weak, maintaining the balance of the gastric ecosystem was reported to be crucial. Similar to pneumonia, chronic gastritis can also be induced by different pathogens. However, all the microbes cultured from the stomach have been considered transient bacteria, and no solid evidence is available regarding potential pathogens in the gastric microbiota. Marshall's experiment [10] must be repeated to identify other bacteria in the stomachs of patients with chronic gastritis.

MALDI-TOF MS is an innovative tool that is easy to use, rapid, accurate, and cost-effective, and it has revolutionized the identification of highly pathogenic bacteria in clinical microbiology laboratories [11, 12]. By immediately culturing gastric specimens and implementing MALDI-TOF MS in the hospital laboratory, we attempted to examine gastritis pathogens from 50 healthy subjects and 53 gastritis patients. Two

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Table 1. Demographic characteristics of the study group and the control group

	No. (%)		P value
	Gastritis (n=53)	Normal (n=50)	
Males/Females	26/27	1	0.924
Mean age (SD)	44.32 (10.84)	43.16 (11.33)	0.597
<i>H. pylori</i> -positive	22	2	< 0.001
Mean pH value	2.7 (1.32)	2.49 (1.21)	0.403
Bile reflux	13 (24.5)	18 (36)	0.205
Gastric juice growth	33 (62.3)	27 (54)	0.395
Mucosal growth	46 (86.8)	24 (48)	< 0.001
Mucosal growth (no <i>H. pylori</i>)	38 (71.7)	24 (48)	0.014

types of medium were used to culture gastric juice and mucosa samples under three conditions. Four species, including *H. pylori*, were found to be significantly associated with chronic gastritis. Additionally, bacterial overgrowth and an abnormal pH were associated with the severity of gastritis. These species may facilitate the understanding of other infection factors that predispose individuals to gastritis.

Materials and methods

Overview of the study

From May 2015 to May 2016, 103 patients referred for endoscopy and histologic examination at Peking University Third Hospital were recruited for this research. Gastric juice and mucosa samples of the corpus and antrum were collected into sterile containers and were sent to the microbiology laboratory for immediate bacterial culture. All enrolled patients provided informed consent for clinical sample collection. This research was approved by the Medical Scientific Ethics Committees of Peking University Third Hospital (IRB00006761-2015172).

Patients and sample culturing

Of the participants enrolled in this study, 53 did not have gastritis and 50 had chronic gastritis. Individuals were recruited if they were 20 to 60 years old without severe complications or other systemic disease and normal lifestyle. Persons diagnosed with cancer, or those who had taken antibiotics or acid inhibitory drugs in recent months before the endoscopy examination were excluded. Mucosal biopsies and two 3-mm diameter ceramic beads were placed

into a homogenizing pipe (Ependorf Corporation, Germany) containing 400 µl of sterile normal saline. The auto homogenizer (Retsch Corporation, Germany) was set to 30 times per second to produce tissue homogenate. According to the pH of the gastric juice, an appropriate volume of 10-100 µl of tissue homogenate and gastric juice were incubated on agar plates. All samples were cultured on Columbia blood agar (Oxoid LTD, Basingstoke,

Hampshire, England) and *Helicobacter pylori*-selective culture medium (Oxoid LTD, Basingstoke, Hampshire, England) for 1, 5 and 3 days under aerobic, anaerobic and microaerophilic conditions respectively. Colony-forming units (CFU) were detected by morphology and microscopy examination.

Identification of isolates

Various forms of bacterial colonies were identified using the direct transfer approach [13] of the Bruker IVD MALDI (Matrix Assisted Laser Desorption Ionization) Biotyper (Bruker, Germany) according to the standard of operation based on the instruction of the facility [14]. A single colony was transferred to the MALDI target plate using an inoculating loop and smeared gently as a thin film within a spot on the plate. The dyed film was overlain with formic acid and then dyed again. One µl of IVD was added to the HCCA matrix. The film was allowed to dry at ambient temperature. The sample location on the plate was assessed and recorded. The plate was placed in the ionization chamber of the mass spectrometer and the MSP identification IVD method was selected.

A laser was applied to the spots subjected to analysis to desorb and ionize microbial and matrix molecules on the target plate. The cloud of ionized molecules was directed into the TOF mass analyzer, toward a detector. Lighter molecules travel faster, followed by progressively heavier analytes. A mass spectrum was generated, representing the number of ions hitting the detector over time [15]. The details of the parameter settings of the operation are available in the brochure (https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separa-

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Table 2. Gastric mucosal bacteria

Mucosal bacteria	Aerobic	Microaerobic	Selective	Anaerobic	Colonized patients No. (%)	Gastritis No. (%)	Normal No. (%)	P value
<i>Rothia mucilaginosa</i>	22	11	1	3	24 (23.3)	9 (17.0)	15 (30)	0.188
<i>Helicobacter pylori</i>	0	10	24	0	24 (23.3)	22 (41.5)	2 (4.0)	< 0.001
<i>Neisseria flavescens</i>	16	15	15	0	23 (22.3)	16 (30.2)	7 (14)	0.049
<i>Streptococcus mitis</i>	13	8	0	10	18 (17.5)	14 (26.4)	4 (8)	0.014
<i>Streptococcus salivarius</i>	9	5	2	9	16 (15.5)	10 (18.9)	6 (12)	0.336
<i>Streptococcus oralis</i>	9	5	0	11	16 (15.5)	11 (20.8)	5 (10)	0.132
<i>Neisseria perflava</i>	6	3	5	0	11 (10.7)	10 (18.9)	1 (2)	0.006
<i>Streptococcus parasanguis</i>	5	1	1	2	8 (7.8)	5 (9.4)	3 (6)	0.716
<i>Rothia dentocariosa</i>	1	6	0	5	8 (7.8)	4 (7.5)	4 (8)	>0.999
<i>Staphylococcus aureus</i>	3	6	0	0	7 (6.8)	2 (3.8)	5 (10)	0.261

Table 3. Gastric juice culturable bacteria

Juice species	Aerobic	Microaerobic	Selective	Anaerobic	Colonized patients No. (%)	Gastitis No. (n=53)	Normal No. (n=50)	P value
<i>Candida albicans</i>	9	11	12	9	15 (14.6)	9	6	0.337
<i>Streptococcus oralis</i>	8	4	0	4	10 (9.7)	1	9	0.017
<i>Rothia mucilaginosa</i>	5	6	0	1	9 (8.7)	0	9	0.003
<i>Neisseria flavescens</i>	4	5	5	1	7 (6.8)	2	5	0.438
<i>Streptococcus mitis</i>	1	2	1	6	6 (5.8)	2	4	0.679
<i>Neisseria perflava</i>	6	3	3	0	6 (5.8)	0	6	0.027
<i>Staphylococcus aureus</i>	5	4	0	3	6 (5.8)	4	2	0.428
<i>Streptococcus salivarius</i>	1	2	1	4	5 (4.8)	1	4	0.364
<i>Ralstonia pickettii</i>	1	3	0	1	5 (4.8)	2	3	>0.999

tions_MassSpectrometry/Literature/Brochures/1840190_MBT_Clinical_IVD_brochure_10-2015_eBook.pdf).

Assessment scores were applied to evaluate the quality of identification. A score of ≥ 2.000 indicated reliable identification at the species level, a score of 1.700 to 1.999 indicated reliable identification at the genus level, and a score of < 1.700 indicated unreliable identification [14].

Unreliably identified stains were determined by 16SrDNA sequencing via mass spectrometry. All bacterial colonies that were not identified were subcultured and collected for DNA extraction. Full-length 16 s rDNA was amplified with 1492R (5'-GGTTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCCTFFCTCAG-3') primers. The PCR conditions were as follows: one cycle at 95°C for 5 min, 35 cycles at 94°C for 30 s, 50°C for 45 s and 72°C for 2 min, and a final extension step at 72°C for 10 min [2]. Then, 1500-bp PCR products were purified from the agarose gel and sent to BGI Company

for sequencing. A Fastq file was uploaded to the NICB website (<http://www.ncbi.nlm.nih.gov/BLAST/>) for blasting. The identities of the isolates were determined based on the highest scores. Sequences with a percentage similarity of 97% or higher were assigned to a species category.

Statistical analysis

The data analysis was performed using SPSS (version 20.0). Continuous variables are described as the mean plus the stander error and counting variables are shown as numbers and ratios. Correlations between continuous variables were assessed using Pearson correlation coefficients. Wilcoxon signed rank tests were used to compare continuous variables. Patient characteristics and positive rates of certain bacteria were analyzed by Fisher's test or the chi-squared (X^2) method as appropriate. Odds ratios are summarized as asymptotic standard errors and 95% confidence intervals (95% CI). A P value ≤ 0.05 indicated a significant difference.

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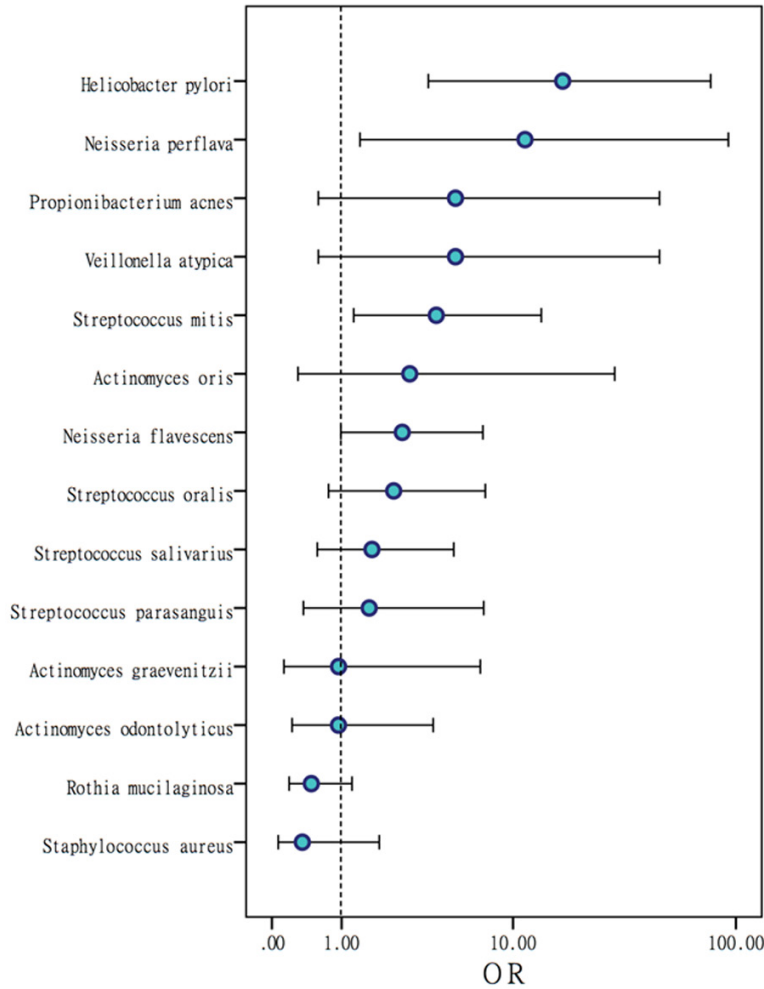


Figure 1. Odds ratios (ORs) of colonized gastric mucosal bacteria. OR values show the risk of gastritis. An OR value greater than 1 reflects a high risk of gastritis.

Results

One hundred three patients were included for culturing of gastric mucosa and gastric juice. According to the histopathology test of gastric biopsy tissues, 53 patients had chronic gastritis and 50 patients were normal. Therefore, the recruited cases were divided into a gastritis group and a control group. The patients in the chronic gastritis group presented with concomitant hemorrhage, atrophy, intestinal metaplasia or hyperplasia. The healthy group included 50 subjects with mild superficial gastritis and served as the control group. The two groups were matched according to mean age, gender, and endoscopy status (**Table 1**). The positive rate of mucosal bacterial growth was higher in the gastritis group (86.7%) than that in the nor-

mal group (48%), with a significant difference between the groups ($P < 0.001$) even after excluding *H. pylori* infection data ($P=0.014$).

Gastric microbiota

Eighty-one species were isolated from all the gastric mucosa and juice samples, of which 56 of which were positive in the gastric juice samples, 53 were positive in the gastric mucosa samples, and 28 were positive in both gastric juice and mucosal biopsies. All the isolated gastric species are listed in **Tables S1** and **S2**. As shown in **Table 2**, *Helicobacter pylori* (23.3%), *Rothia mucilaginosa* (23.3%) and *Neisseria flavescens* (22.3%) were common in the mucosa. In the gastric juice, *Candida albicans* (14.6%), *Streptococcus Oralis* (9.7%) and *Rothia mucilaginosa* (8.7%) were common, as shown in **Table 3**.

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Positive rates of *Helicobacter pylori* ($P < 0.001$), *Streptococcus mitis* ($P=0.014$), *Neisseria flavescens* ($P=0.049$)

and *Neisseria perflava* ($P=0.006$) in the mucosa of the chronic gastritis group were significantly higher than those in the mucosa of the healthy group (**Table 2**). The odds ratios (ORs) of these potential pathogens are shown in **Figures 1** and **2**: *Helicobacter pylori* (OR=17.03, [95% CI, 3.74-77.59]), *Streptococcus mitis* (OR=4.13, [95% CI, 1.26-13.57]), *Neisseria flavescens* (OR=2.66, [95% CI, 1.00-7.16]), and *Neisseria perflava* (OR=11.40, [95% CI, 1.40-92.69]). The positive mucosal rates of *Neisseria* (41.5% vs. 16% $P=0.009$) and *Streptococcus* (50.9% vs. 28% $P=0.017$) were significantly different between the chronic gastritis group and the normal group.

Cultures of gastric juice showed notable differences in the positive rates of *Streptococcus*

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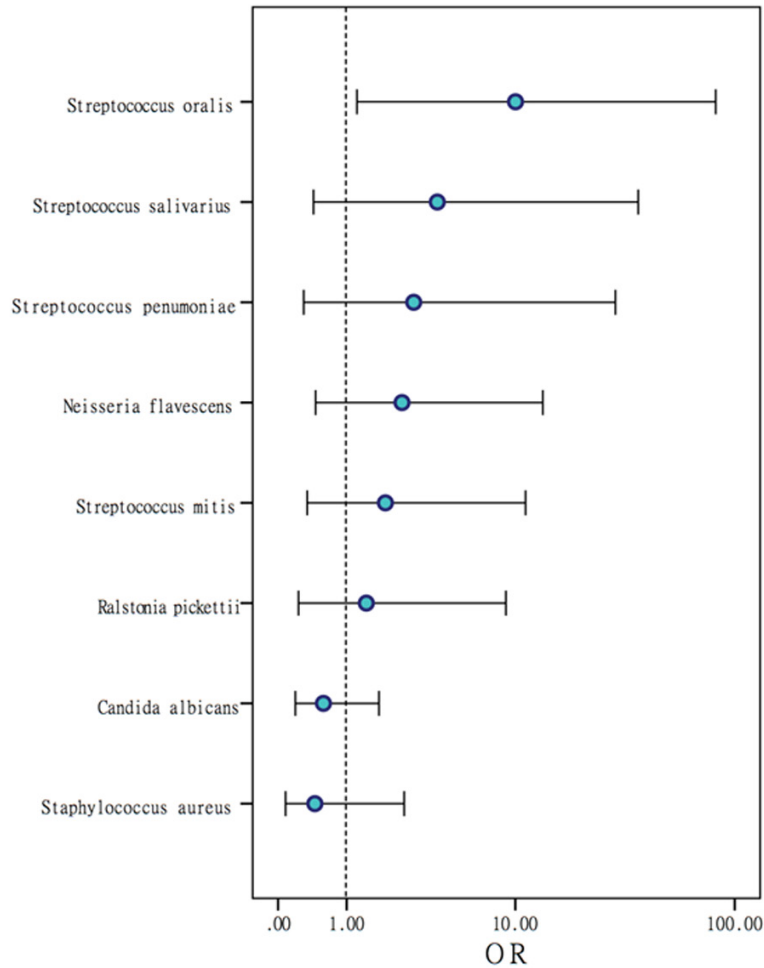


Figure 2. Odds ratios (ORs) of positive gastric juice bacteria. Species with a greater OR value are associated with a higher risk of gastritis.

oralis (P=0.017), *Rothia mucilaginosa* (P=0.003) and *Neisseria perflava* (P=0.027) between the gastritis and normal groups (Table 3). Similar to the mucosal culture results, the positive rates of certain bacterial species were higher in the gastritis group than those in the control group.

Symbiosis

Bacterial overgrowth (> 10⁵ CFU/L) of 16 species was found in 17 gastric juice samples, and overgrowth (> 10⁵ CFU/g) of 8 species was observed in 22 mucosal samples. Bacterial overgrowth in gastric juice (P < 0.001) and mucosa (P=0.009) was significantly associated with an abnormal pH value of gastric juice (pH > 4), which was significantly correlated with chronic gastritis (P=0.036). Sixteen mucosal samples from 24 *Helicobacter pylori*-positive

selective agars were colonized by non-*Helicobacter pylori* bacterial flora, including *Streptococcus*, *Neisseria*, *Ralstonia* and *Staphylococcus*, suggesting that other bacteria live in the gastric mucosa concurrently with *Helicobacter pylori*.

Discussion

This study revealed that *Streptococcus*, *Neisseria* and *H. pylori* were significantly associated with chronic gastritis. As a form of infectious disease, chronic gastritis can be caused by pathogens other than *H. pylori*. Other potential pathogens in the stomach may include a range of bacterial species such as *Mycobacterium* and *Enterococcus* [1]. Evidence-based data regarding other etiologies of chronic gastritis are limited, although several case studies have reported that *Streptococcus* spp. are associated with acute gastritis [16, 17]. Additionally, the clinical diagnosis and treatment of chronic gastritis generally focus on *H. pylori* infection.

H. pylori is a well-known bacterial species in the stomach that can survive among hundreds of species in gastric acid [18]. However, the *H. pylori* sequence can be detected in 60% of conventionally negative samples [19, 20]. Therefore, *H. pylori* is considered a normal microbe that is beneficial to its host for a certain period. Blaser et al [21] investigated a series of possible disorders that may be caused by *H. pylori* loss. Eradicating *H. pylori* is controversial and requires long-term observational and clinical research.

The latest consensus on the management of *H. pylori* discussed the gastric microbiota in terms of its composition as determined by sequencing. Bik et al [22] found 128 species in the stomach and invalidated the concept of *H. pylori* as the only gastric colonizer. Schulz identified 687 phlotypes from 24 individuals by RNA

amplification and sequencing [23]. However, comparisons of the gastric microbiota between gastritis and non-gastritis subjects have been neglected. In our study, specimens from 103 individuals with or without gastritis were cultured for live bacterial detection. We identified 81 species in samples from healthy subjects and gastritis patients by MLDI-TOF. Delgado et al [2] isolated 16 species from mucosal and gastric samples of healthy human stomachs; the dominant genus was entirely different for the various community sources of the samples.

The prevalence of non-*H. pylori* pathogens in the stomach is associated with the pH value of gastric juice. A pH value > 4 reflects a risk of gastric flora overgrowth [24]. Rosen et al [25] reported that acid suppression resulted in gastric bacterial overgrowth of genera including *Staphylococcus* and *Streptococcus*. We observed the same phenomenon. In addition, we found that *Streptococcus* could inhibit the growth of *H. pylori* on culture medium. Khosravi et al [26] reported that *Streptococcus mitis* induced the conversion of *H. pylori* to coccid cells and growth inhibition. Therefore, we can infer that the gastric microbiota is the key to restricting the overgrowth of *H. pylori* or other pathogens.

Gastritis is similar to pneumonia and nephritis in that it can be induced by many kinds of bacteria. In addition to *H. pylori*, *Streptococcus* and *Neisseria* may also contribute to the pathogenesis of gastritis and even gastric cancer [27]. To our best knowledge, our study is the first to show the relationship between the risk of gastritis and infection with non-*H. pylori* bacteria. Our results are consistent with current reports on *H. pylori*-related disease. Unlike Marshall et al [10], we identified all cultivable colonies, including *H. pylori*, on the available medium, which could eventually lead to the identification of other pathogens. Until now, no studies have clearly linked specific gastric microbiomes besides *H. pylori* to an increased risk of gastritis. Future work should focus on the pathogenic effects of *Streptococcus* and *Neisseria*.

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Disclosure of conflict of interest

None.

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Table S1. Gastric juice culturing positive individuals' number

Bacteria from gastric juice	Aerobic	Microaerobic	Antibiotic	Anaerobic	Total Individuals (n)
<i>Streptococcus salivarius</i>	1	2	1	4	5
<i>Streptococcus mitis</i>	1	2	1	6	6
<i>Streptococcus pneumoniae</i>	0	2	0	3	4
<i>Streptococcus parasanguis</i>	0	1	0	1	2
<i>Streptococcus sanguis</i>	1	0	0	0	1
<i>Streptococcus oralis</i>	8	4	0	4	10
<i>Neisseria flavescens</i>	4	5	5	1	7
<i>Neisseria perflava</i>	6	3	3	0	6
<i>Neisseria mucosa</i>	1	1	1	0	2
<i>Rothia mucilaginosa</i>	5	6	0	1	9
<i>Rothia dentocariosa</i>	1	2	1	0	2
<i>Actinomyces odontolyticus</i>	0	0	0	1	1
<i>Candida albicans</i>	9	11	12	9	15
<i>Corynebacterium argenteratense</i>	1	0	0	1	1
<i>Staphylococcus aureus</i>	5	4	0	3	6
<i>Klebsiella pneumoniae</i>	1	1	0	0	1
<i>Paenibacillus huicus</i>	1	1	0	0	2
<i>Streptococcus australis</i>	1	0	0	0	1
<i>Rotia aerea</i>	1	2	0	0	2
<i>Ralstonia pickettii</i>	1	3	0	1	5
<i>Haemophilus parainfluenzae</i>	1	0	1	0	2
<i>Mycobacterium abscessus</i>	0	2	0	0	2
<i>Bacillus cereus</i>	1	0	0	0	1
<i>Veillonella atypica</i>	0	0	0	1	1
<i>Veillonella dispar</i>	0	0	0	1	1
<i>Propionibacterium acnes</i>	0	0	0	2	2
<i>Fusobacterium periodonticum</i>	0	0	0	1	1
<i>Lactobacillus crispatus</i>	0	0	0	1	1
<i>Spingomonas melonis</i>	1	1	1	0	2
<i>Brevibacillus parabrevis</i>	1	0	0	0	1
<i>Arthrobacter psychrolactophilus</i>	1	0	0	0	1
<i>Bacillus subtilis</i>	4	4	2	0	7
<i>Bacillus atropheus</i>	1	0	0	0	1
<i>Bacillus thuringiensis</i>	1	0	0	0	1
<i>Paenibacillus pasadenensis</i>	0	1	0	0	1
<i>Serratia marcescens</i>	1	1	1	0	1
<i>Candida glabrata</i>	1	1	1	0	1
<i>Aspergillus niger</i>	3	0	1	0	3
<i>Micrococcus flavus</i>	1	0	0	0	1
<i>Burkholderia seminalis</i>	1	0	0	0	1
<i>Lactobacillus gasseri</i>	1	6	1	3	6
<i>Ralstonia insidiosa</i>	1	1	2	1	2
<i>Klebsiella oxytoca</i>	0	1	0	0	1
<i>Microbacterium trichothecenolyticum</i>	1	0	0	0	1
<i>Meyerozyma guilliermondii</i>	0	0	1	0	1
<i>Lactobacillus oris</i>	0	0	0	1	1
<i>Lactobacillus mucosae</i>	0	0	0	1	1
<i>Lactobacillus amylovorus</i>	0	0	0	1	1
<i>Veillonella parvula</i>	0	0	0	2	2
<i>Corynebacterium macginleyi</i>	0	0	0	1	1
<i>Fusobacterium nucleatum</i>	0	0	0	1	1
<i>Fusobacterium canifelinum</i>	0	0	0	1	1
<i>Staphylococcus epidermidis</i>	0	0	0	1	1

Detections of pathogens in stomach

Table S2. Mucosa culturing positive individuals' number

Bacteria from mucosa	Aerobic	Microaerobic	Antibiotic	Anaerobic	Total Individuals (n)
<i>Rothia mucilaginosa</i>	22	11	1	3	24
<i>Helicobacter pylori</i>	0	10	24	0	24
<i>Neisseria flavescens</i>	16	15	15	0	23
<i>Streptococcus mitis</i>	13	8	0	10	18
<i>Streptococcus salivarius</i>	9	5	2	9	16
<i>Streptococcus oralis</i>	9	5	0	11	16
<i>Neisseria perflava</i>	6	3	5	0	11
<i>Streptococcus parasanguis</i>	5	1	1	2	8
<i>Actinomyces odontolyticus</i>	1	6	0	5	8
<i>Staphylococcus aureus</i>	3	6	0	0	7
<i>Neisseria mucosa</i>	1	2	4	2	5
<i>Veillonella atypica</i>	0	0	0	5	5
<i>Propionibacterium acnes</i>	0	0	0	5	5
<i>Actinomyces graevenitzii</i>	3	3	0	4	4
<i>Actinomyces oris</i>	3	1	2	4	4
<i>Streptococcus pneumoniae</i>	0	0	3	3	3
<i>Streptococcus sanguis</i>	0	1	2	3	3
<i>Candida albicans</i>	1	0	0	3	3
<i>Corynebacterium argentoratense</i>	1	0	1	3	3
<i>Haemophilus parainfluenzae</i>	1	3	0	3	3
<i>Veillonella dispar</i>	0	0	3	3	3
<i>Streptococcus infantarius</i>	0	0	1	2	2
<i>Rothia dentocariosa</i>	1	0	0	2	2
<i>Gemella sanguinis</i>	1	0	0	2	2
<i>Ralstonia pickettii</i>	1	1	0	2	2
<i>Prevotella salivae</i>	1	0	2	2	2
<i>Bacillus cereus</i>	0	0	2	2	2
<i>Neisseria subflava</i>	0	0	0	1	1
<i>Staphylococcus capitis</i>	1	0	0	1	1
<i>Staphylococcus warneri</i>	0	1	0	1	1
<i>Microbacterium esteraromaticum</i>	0	0	0	1	1
<i>Bacillus infantis</i>	0	0	0	1	1
<i>Bacillus flexus</i>	0	0	0	1	1
<i>Klebsiella pneumoniae</i>	0	0	0	1	1
<i>Paenibacillus huicus</i>	1	0	0	1	1
<i>Streptococcus anginosus</i>	1	0	0	1	1
<i>Streptococcus australis</i>	1	0	0	1	1
<i>Rotia aeria</i>	1	0	0	1	1
<i>Corynebacterium propinquum</i>	1	0	0	1	1
<i>Arthrobacter globiformis</i>	0	1	0	1	1
<i>Cryptococcus neoformans</i>	0	1	0	1	1
<i>Corynebacterium felinum</i>	0	1	0	1	1
<i>Mycobacterium abscessus</i>	0	1	0	1	1
<i>Lactobacillus sakei</i>	0	0	1	1	1
<i>Lactobacillus sp.</i>	0	0	1	1	1
<i>Staphylococcus haemolyticus</i>	0	0	1	1	1
<i>Veillonella denticariosi</i>	0	0	1	1	1
<i>Bacillus mycetoides</i>	0	0	1	1	1
<i>Streptococcus constellatus</i>	0	0	1	1	1
<i>Atopobium parvulum</i>	0	0	1	1	1
<i>Arthrobacter parietis</i>	0	0	1	1	1
<i>Arthrobacter ramosus</i>	0	0	1	1	1
<i>Fusobacterium periodonticum</i>	0	0	1	1	1
<i>Listeria ivanovii</i>	0	0	1	1	1
<i>Lactobacillus crispatus</i>	0	0	1	1	1
<i>Capnocytophaga gingivalis</i>	0	0	1	1	1