


# BMJ Open *Helicobacter pylori* infection-induced changes in the intestinal microbiota of 14-year-old or 15-year-old Japanese adolescents: a cross-sectional study

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## ABSTRACT

**Objective** The relationship between *Helicobacter pylori* and the intestinal microbiota has not yet been clearly demonstrated in children and adolescents. The present study aimed at evaluating how *H. pylori* infection could affect the intestinal microbiota in adolescents using genetic analysis.

**Design** Cross-sectional study.

**Setting and participants** We included subjects from a longitudinal project involving *H. pylori* screening and treatment of junior high school third-grade students (aged 14 or 15 years) in Saga Prefecture. The study included a control group (n=79) and an *H. pylori* group (n=80) tested negative and positive for the anti-*H. pylori* antibody in the urine and *H. pylori* antigen in stool specimens, respectively.

**Interventions** The intestinal microbiota was evaluated in stool specimens using 16S rRNA gene/DNA/amplicon sequencing with next-generation sequencing.

**Primary and secondary outcome measures** We assessed alpha and beta diversity, just as well as relative abundances within the bacterial composition at the genus level in both groups.

**Results** As shown by the alpha diversity of the 16S rRNA gene/DNA/amplicon sequence data, the control group exhibited lower microbial species richness with lower alpha diversity compared with the *H. pylori* group (p<0.001). The beta diversity of the intestinal microbiota profile also differed between the two groups (p<0.01). The relative abundance of the *Prevotella* genus was higher in the *H. pylori* group (p<0.01) concomitant with a gain in body mass index (BMI) in the *H. pylori* group (p<0.01) compared with the control group.

**Conclusion** *H. pylori* infection significantly affected the intestinal microbiota in Japanese adolescents. In addition, the prevalence of the *Prevotella* genus is concomitantly increased along with the BMI in *H. pylori*-infected students.

**Trial registration number** UMIN000028721.

## INTRODUCTION

Newborns are exposed to various bacteria that are present in the mother's resident microbiota and the external environment. Bacterial species that comprise the intestinal microbiota change in an age-dependent manner.<sup>1,2</sup> The development of the intestinal

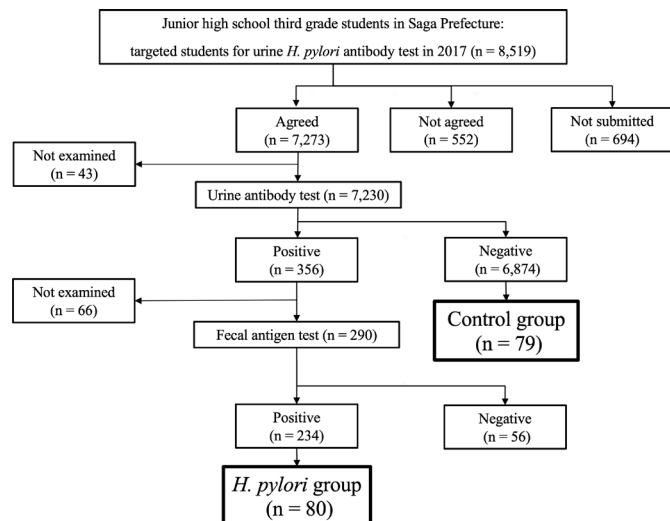
## Strengths and limitations of this study

- The most significant strength of this study is that it clearly demonstrated the effect of *Helicobacter pylori* infection on the intestinal microbiota of children.
- As the participants were Japanese adolescents of almost the same age living in a single prefecture, no major difference would presumably exist between the two groups.
- This study evaluated the intestinal microbiota using faeces specimens, which might differ from the mucosal-associated microbiota.
- The effect of *H. pylori* eradication on the intestinal microbiota could not be analysed, as the eradication therapy is important for intestinal microbiota changes.

microbiota during infancy is affected by several factors, including the maternal resident microbiota,<sup>3,4</sup> the method of nutrition for infants,<sup>5-7</sup> delivery style<sup>5,8,9</sup> and the administration of antibiotics.<sup>3,10,11</sup>

The global *Helicobacter pylori* prevalence in children varies significantly, from 2.5% in Japan to 34.6% in Ethiopia.<sup>12</sup> Sustained infection of *H. pylori* decreases or increases gastric acid secretion, which might affect the gastric microbiota in adults<sup>13-15</sup> and children.<sup>13,14</sup> Several previous reports have suggested that the intestinal microbiota is significantly affected by *H. pylori* infection.<sup>15,16</sup> The effect of *H. pylori* infection on the intestinal microbiota has been demonstrated in adults,<sup>16,17</sup> but has not been fully investigated in children.

Therefore, the present study aimed at examining junior high school students in Japan aged 14–15 years to determine whether *H. pylori* infection changes the intestinal microbiota. Moreover, we also examined how body mass index (BMI) affects the intestinal microbiota, in addition to *H. pylori* infection.



**Figure 1** Flowchart for *Helicobacter pylori* screening and treatment of junior high school students in Saga Prefecture and the selection method used to establish the two groups. The *H. pylori* group comprised 80 students, consented to the study, tested positive for both urinary anti-*H. pylori* IgG antibody and stool antigen test. The control group (n=79) comprised those tested negative for both tests.

## METHODS

### Study design and subjects

The longitudinal project for *H. pylori* screening and treatment among junior high school third-grade students in Saga Prefecture started in 2016 with the aim of primary prevention of stomach cancer.<sup>17</sup> Figure 1 shows a flowchart of the junior high school third grade students in Saga Prefecture in 2017. Among 8519 junior high school students aged 14 or 15 years, 7230 received a screening urinary test (RAPIRAN; Otsuka Pharmaceutical Co., Tokyo, Japan) to detect anti-*H. pylori* IgG antibody by immunochromatography. There is an established screening programme for kidney diseases in Saga Prefecture, targeting third-grade students in junior high schools. Given the full inclusivity of student during this test through simple urine examination, we used the established system to obtain urine samples to screen for *H. pylori* infection.<sup>17</sup> The diagnostic sensitivity, specificity, negative predictive value and positive predictive value of the urinary test was reportedly 78.4, 100, 90.1 and 100%, respectively.<sup>18</sup> A total of 6874 students tested negative for *H. pylori* with the urinary test and 79 of these students were randomly selected as the *H. pylori*-negative group (control group). Students who tested positive in the screening urinary test received an *H. pylori* stool antigen detection test (Testmate rapid pylori antigen; Wakamoto Pharmaceutical Co., Tokyo, Japan). Among 290 students who received the stool antigen test, 234 students tested positive for *H. pylori* infection. Finally, 80 of these students were randomly selected as the *H. pylori*-positive group (*H. pylori* group). The exclusion criteria for the present study were as follows: (1) students who had taken medications, including proton-pump inhibitors (PPIs), H<sub>2</sub> receptor

antagonists, antacids, probiotics, mucosal protective agents and antibiotics within the 6 months prior to enrolment, (2) students who were in the outpatient hospital because of sickness, and (3) students who had undergone eradication therapy for *H. pylori*.

The microbiota distribution was compared between the control and *H. pylori* groups regarding alpha diversity, beta diversity and the relative abundance of the intestinal microbiota. The effect of BMI (low: <15, middle: 15–25 and high: >25) on the microbiota distribution in the two groups was examined.

### Stool sample collection and bacterial DNA extraction from faeces

Each participant collected a stool sample at home for the present study using a paper stool collector and tube that was prefilled with 5 mL of a stool DNA stabiliser. The stool collection method was performed according to the attached document of the stool collection kit. Samples were immediately stored at –20°C and delivered to the project centre within a day. Extraction of bacterial DNA was performed as described previously.<sup>19</sup> A total of 20 mg of faeces was washed three times in 1.0 mL of phosphate-buffered saline and centrifuged (14 000× *g*). The pellets were resuspended in a solution containing 450 µL of extraction buffer (100 mM Tris-HCl, 40 mM EDTA; pH 9.0) and 50 µL of 10% sodium dodecyl sulfate. A total of 300 mg of glass beads (diameter, 0.1 mm) and 500 µL of buffer-saturated phenol were added to the suspension and vortexed vigorously. After centrifugation at 14 000× *g* for 5 min, 400 µL of the supernatant was extracted by phenol–chloroform, and 250 µL of the supernatant was subjected to isopropanol precipitation. Finally, the DNA was suspended in 1.0 mL of Tris-EDTA buffer.

### DNA sequence analysis

We performed the meta-analysis of the bacterial 16S rDNA sequences in the faeces in accordance with a previously described method<sup>20</sup> with minor modifications. Briefly, the V3–V4 region of 16S rDNA were amplified on a Veriti thermal cycler (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The amplicon was purified using AMPure XP magnetic beads (Beckman Coulter, Brea, California, USA). For multiplex sequencing, a PCR was performed with dual eight-base indices (Nextera XT Index kit, Illumina, California, USA). After purification by AMPure XP beads, the purified barcoded library was quantified fluorometrically using a QuantiT PicoGreen ds DNA Assay Kit (Invitrogen, Paisley, UK) and pooled at the same volume. The library pool (10 pM) was spiked with 40% PhiX control DNA (10 pM). Sequencing was conducted on a MiSeq platform with MiSeq Reagent Kit v2 chemistry (Illumina).

### Microbiota analysis

We conducted the removal of low-quality and chimera sequences, construction of operational taxonomic units (OTUs) and taxonomy assignment using the Quantitative

Insights Into Microbial Ecology pipeline (<http://qiime.org/>).<sup>21</sup> Briefly, 50 000 raw reads were randomly obtained from the sequence files for each sample and merged by fastq-join with the default setting. Consequently, sequence reads with an average quality value of <25 were removed and then chimera-checked. Five thousand reliable sequence reads were randomly obtained for each sample and OTUs were constructed by clustering with a 97% identity threshold. The representative reads of each OTU were then assigned to the 16S rRNA gene database using UCLUST with  $\geq 97\%$  identity.<sup>22</sup> A comparison of each taxon in the gut microbiota was conducted at the genus level. Beta diversity was estimated by computing the weighted and unweighted UniFrac distances between the samples.<sup>23</sup> In order to compare the differences in the overall bacterial gut microbiota structure, principal coordinates analysis was applied to reduce the dimensionality of the resulting distance matrix. We calculated the Shannon index, observed OTUs, chao 1, and the abundance-based coverage estimator (ACE) index to investigate the alpha diversity of the microbiota in the samples.

### Statistical analysis

All statistical analyses were conducted with the R statistical software (R Core Team (2018); R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria; <https://www.R-project.org/>). Data are shown as the mean $\pm$ SE. Statistical significance was set at  $p < 0.05$ . During the analyses of the gut microbiotas, the statistical significance was determined by Welch's t-test with Benjamini-Hochberg correlation. The relative abundance data were non-normally distributed. However, we applied Welch's t-test as the Mann-Whitney U-test is reportedly less robust.<sup>24</sup> Beta diversity was analysed using permutational analysis of multivariate dispersions (PERMDISP) for comparisons of gene similarity.

### Patient and public involvement

This study was performed without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient-relevant outcomes or interpret the results. Patients were

not invited to contribute to the writing or editing of the manuscript for readability or accuracy.

## RESULTS

### Student characteristics

A total of 159 students participated in this study. The student characteristics are shown in [table 1](#). No significant differences could be observed in sex, age, BMI, birth delivery style, method of infant nutrition or the prevalence of allergic disease between the groups. The ratio of nursery school graduates to kindergarten graduates was significantly higher in the *H. pylori* group than in the control group ( $p < 0.001$ ). The subjects of this study did not include low-birth-weight infants (birth weight 2500 g or less). In addition, we did not investigate whether symptoms associated with *H. pylori* infection, such as abdominal symptoms, were present in the *H. pylori* group.

### Alpha and beta diversity in the control and the *H. pylori* groups

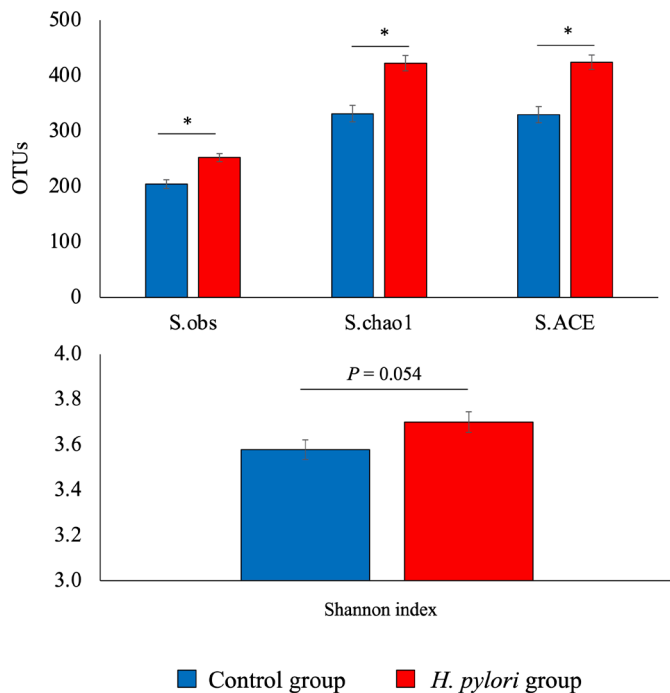
[Figure 2](#) shows the alpha diversity of the 16S rRNA gene/DNA/amplicon sequence data. The control group showed lower microbial species richness with lower alpha diversity compared with the *H. pylori* group. The observed species index, chao 1 index, and ACE index all showed significantly higher diversity in the *H. pylori* group compared with the control group ( $p < 0.001$ ). The Shannon index was not significantly different between the two groups ( $p = 0.054$ ).

[Figure 3](#) shows the beta diversity of the 16S rRNA gene/DNA/amplicon sequence data. The two-dimensional principal coordinate analysis of the weighted and unweighted UniFrac distances of the 16S rRNA gene/DNA/amplicon sequence data showed that the majority of samples were clustered dependent on the *H. pylori* infection status. The similarity analysis showed that the differences were significant for the weighted UniFrac distance ( $p < 0.001$ ), but not for the unweighted UniFrac distance ( $p = 0.643$ ) using PERMDISP.

**Table 1** Background characteristics of junior high school students in the two groups

	Control group (n=79)	<i>Helicobacter pylori</i> group (n=80)	P value
Sex (male/female)	42/37	46/34	0.80
Age (years)	14.73 $\pm$ 0.33	14.76 $\pm$ 0.32	0.71
Body mass index (kg/m <sup>2</sup> )	19.69 $\pm$ 3.48	19.67 $\pm$ 2.41	0.97
Delivery (vaginal/caesarean section)	68/11	60/11	0.79
Nutrition (breast/formula/mix)	37/6/36	27/15/36	0.07
School (nursery/kindergarten/none)	25/54/0	53/25/2	<0.001
Allergies (+/-)	5/75	7/73	0.55

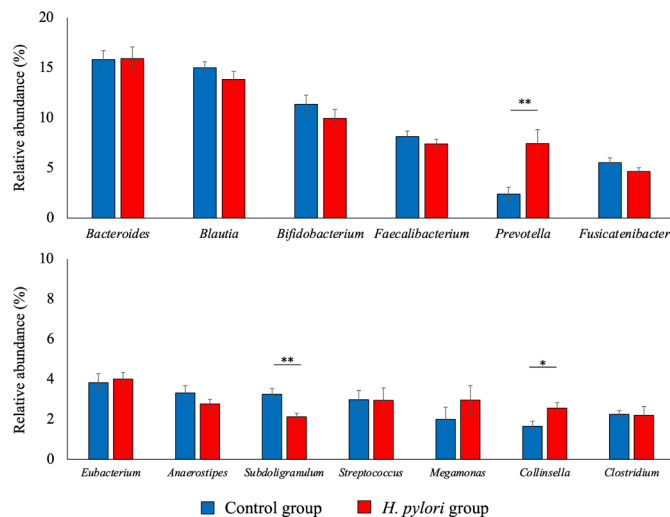
Delivery, birth delivery style; nutrition, method of infant nutrition; school, pre-school situation.



**Figure 2** Alpha diversity of the 16S rRNA sequences in the control and *Helicobacter pylori* groups. The control group exhibited lower microbial species richness compared with the *H. pylori* group. The S.obs index, chao 1 index and ACE index, all showed significantly higher diversity in the *H. pylori* group than in the control group (\* $p < 0.001$ ). The Shannon index was not significantly different between the two groups ( $p = 0.054$ ). ACE, abundance-based coverage estimator; obs, observed; OTU, operational taxonomic unit; S, species.

### Relative abundances within the bacterial composition at the genus level for the two groups

**Figure 4** shows the 13 main bacterial types present in the intestinal microbiota at the genus level as follows: *Bacteroides*, *Blautia*, *Bifidobacterium*, *Faecalibacterium*, *Prevotella*, *Fusicatenibacter*, *Eubacterium*, *Anaerostipes*, *Subdoligranulum*, *Streptococcus*, *Megamonas*, *Collinsella* and *Clostridium*. The relative abundances of the *Prevotella* ( $p < 0.01$ ) and

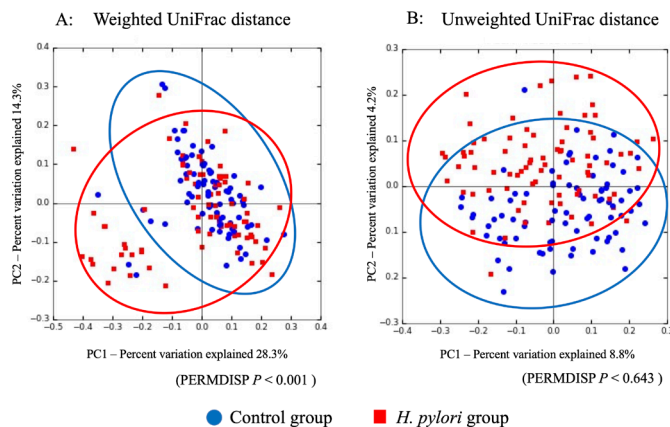


**Figure 4** The main 13 bacterial types present in the intestinal microbiota at the genus level, comparing the *Helicobacter pylori* and control groups (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

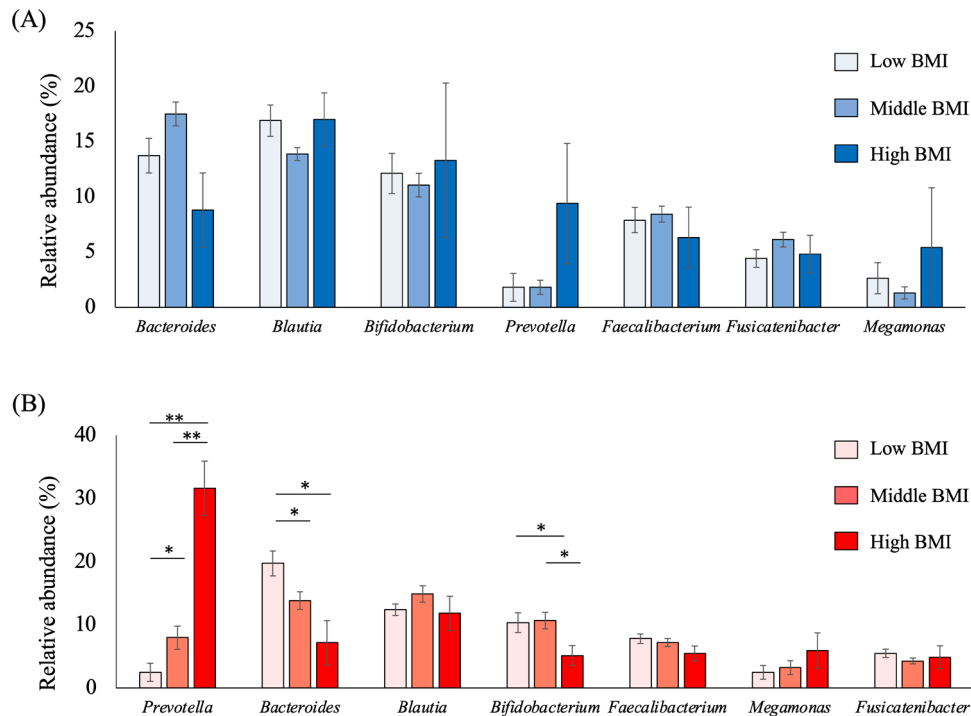
*Collinsella* genus ( $p < 0.05$ ) were significantly higher in the *H. pylori* group than in the control group. The relative abundance of the *Subdoligranulum* genus was significantly higher in the control group than in the *H. pylori* group ( $p < 0.01$ ). At the phylum level, the ratio of the *Firmicutes* to the *Bacteroides* phyla (F/B ratio) showed no significant difference between the two groups (the control group  $4.19 \pm 3.27$  vs the *H. pylori* group  $4.87 \pm 12.04$ ,  $p = 0.63$ ).

### BMI and the relative abundances within the bacterial composition at the genus level

In the control and *H. pylori* groups, the intestinal microbiota was evaluated in association with the BMI. **Figure 5** shows the seven main bacterial types in the intestinal microbiota at the genus level for the control and the *H. pylori* groups, categorised by the BMI. For the control group, these included *Bacteroides*, *Blautia*, *Bifidobacterium*, *Prevotella*, *Faecalibacterium*, *Fusicatenibacter* and *Megamonas* and for the *H. pylori* group *Prevotella*, *Bacteroides*, *Blautia*, *Bifidobacterium*, *Faecalibacterium*, *Megamonas* and *Fusicatenibacter*. In the *H. pylori* group, the relative abundance of the *Prevotella* genus was significantly higher in the high BMI group compared with the middle and low BMI groups (both  $p < 0.01$ ). Furthermore, the relative abundance of the *Prevotella* genus in the middle BMI group was higher than that in the low BMI group ( $p < 0.05$ ). The relative abundances of *Bacteroides* and *Bifidobacterium* were significantly lower in the high BMI group compared with the other two groups (both  $p < 0.05$ ). In the *H. pylori* group, the BMI did not affect the relative abundances of *Blautia*, *Faecalibacterium*, *Megamonas* and *Fusicatenibacter*. In the control group, the relative abundance of the *Prevotella* genus was not significantly higher in the high BMI group compared with the middle and low BMI groups, whereas the relative abundance of the *Prevotella* genus significantly and proportionately increased with an increasing BMI in the *H. pylori* group (low BMI vs high BMI:  $p < 0.001$ , middle BMI vs high BMI:  $p < 0.001$ ) (**figure 6**). At the



**Figure 3** Beta diversity of the 16S rRNA/DNA/amplicon sequence data (control group vs *Helicobacter pylori* group). PC, principal coordinate; PERMDISP, permutational analysis of multivariate dispersions.



**Figure 5** The seven main bacterial types present in the intestinal microbiota at the genus level for the control group (A) and the *Helicobacter pylori* group (B) in association with body mass index (BMI) (\* $p < 0.05$ ; \*\* $p < 0.01$ ). Low: BMI  $< 15 \text{ kg/m}^2$ ; middle: BMI  $15\text{--}25 \text{ kg/m}^2$ ; high: BMI  $> 25 \text{ kg/m}^2$ .

phylum level, we observed no significant differences in the F/B ratio among the three BMI categories in the control groups. However, a significant difference could be detected between the high and middle BMI categories in the *H. pylori* group (figure 7). The *Subdoligranulum* genus had a lower relative abundance in the high BMI category than in the low BMI group, although this trend was observed not only in the *H. pylori* group, but also in the control group (figure 8). The *Collinsella* genus was not associated with the BMI regardless of *H. pylori* infection status (figure 9).

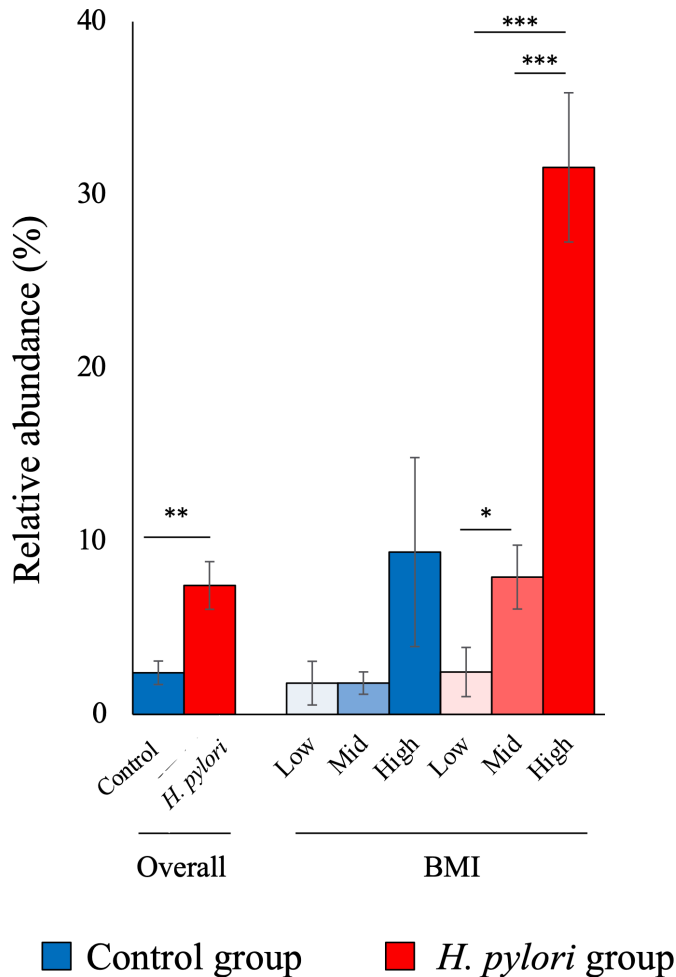
## DISCUSSION

The present study revealed two clinically important results: (1) *H. pylori* infection significantly affected the intestinal microbiota of adolescents aged 14 or 15 years, as determined for Japanese junior high school students; (2) an increase in the relative abundance of the *Prevotella* genus in *H. pylori*-infected adolescents was concomitant with a gain in BMI.

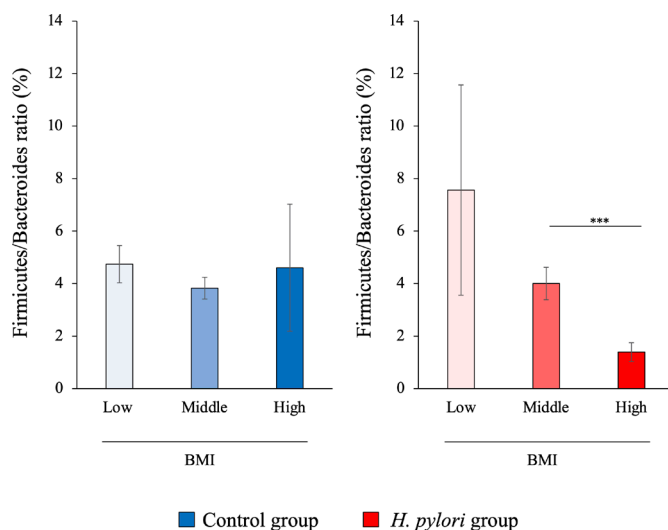
Most reports of the effects of *H. pylori* on the intestinal microbiota based on the analysis of faeces samples were in adults and data were lacking for children.<sup>25 26</sup> The present study showed a difference in the intestinal microbiota between *H. pylori*-infected and non-infected adolescents based on faeces specimens. Alpha diversity, bacterial richness and variance all showed greater diversity in *H. pylori*-infected students than in controls (figure 2). A previous study showed that the diversity of the gastric microbiota in adolescents was enhanced by *H.*

*pylori* infection.<sup>13</sup> Studies of the relationship between the intestinal microbiota and *H. pylori* infection are limited. One study reported a decrease in the *Firmicutes* genus in the human duodenal mucosa during *H. pylori* infection.<sup>27</sup> In the *H. pylori* infection model of Mongolian gerbils, the abundances of the *Bacteroides* and *Enterococcus* genera were increased in the duodenal mucosa.<sup>28</sup> In adults, *H. pylori* infection<sup>17</sup> reportedly reduced intestinal microbiota diversity and our results were in good agreement with these previous reports (figures 2 and 3). The human gut microbiota has been reported to form by the age of 3 years,<sup>29</sup> so it may be that there is no difference in the effects of *H. pylori* infection on the intestinal microbiota between adolescents and adults.

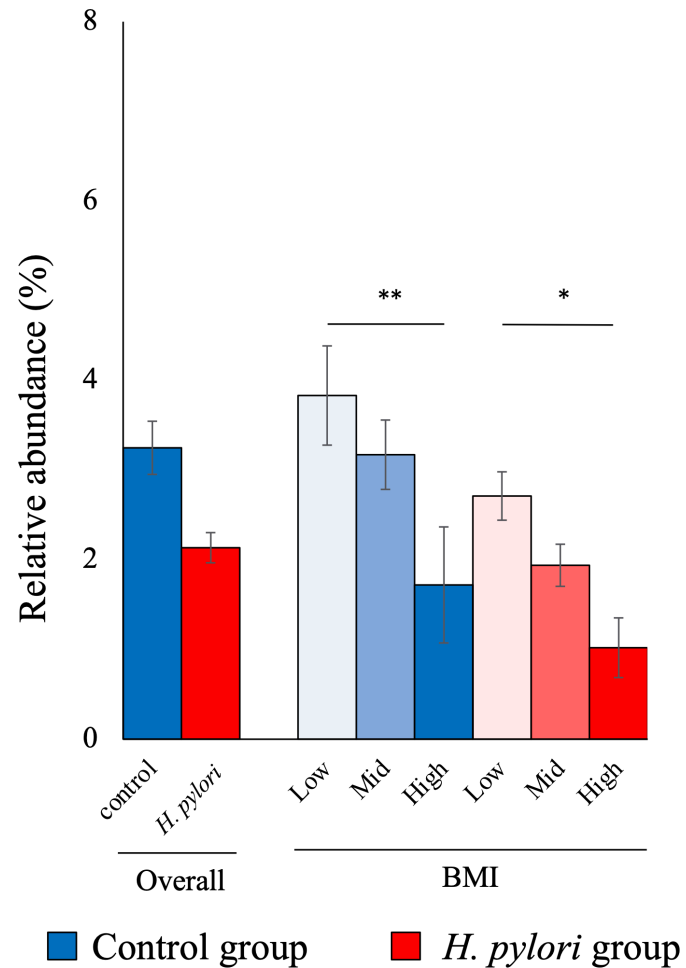
It is known that infection with *H. pylori* reduces gastric acid secretion in children.<sup>30 31</sup> It was further suggested that a decrease in gastric acid secretion due to *H. pylori* infection may affect the intestinal flora of adolescents with *H. pylori* infection. In addition, a decrease in gastric acid secretion caused by *H. pylori* infection may allow a wide variety of bacteria in the oral cavity to more easily pass through the stomach and reach the lower gastrointestinal tract, thereby affecting the intestinal flora in faeces. The inhibitory effect of PPIs on gastric acid secretion affects the composition of the intestinal flora. Administration of PPIs causes an increase in the indigenous bacteria, the *Streptococcus* and *Lactobacillus* genus in the intestine, which is thought to be due to the oral bacteria reaching the intestine to suppress gastric acid secretion.<sup>32 33</sup> This might explain the result of the present study that alpha



**Figure 6** Relative abundance of the *Prevotella* genus in relation to the body mass index (BMI) category in the *Helicobacter pylori* and control groups (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ). Low: BMI  $< 15 \text{ kg/m}^2$ ; mid BMI  $15\text{--}25 \text{ kg/m}^2$ ; high BMI  $> 25 \text{ kg/m}^2$ .



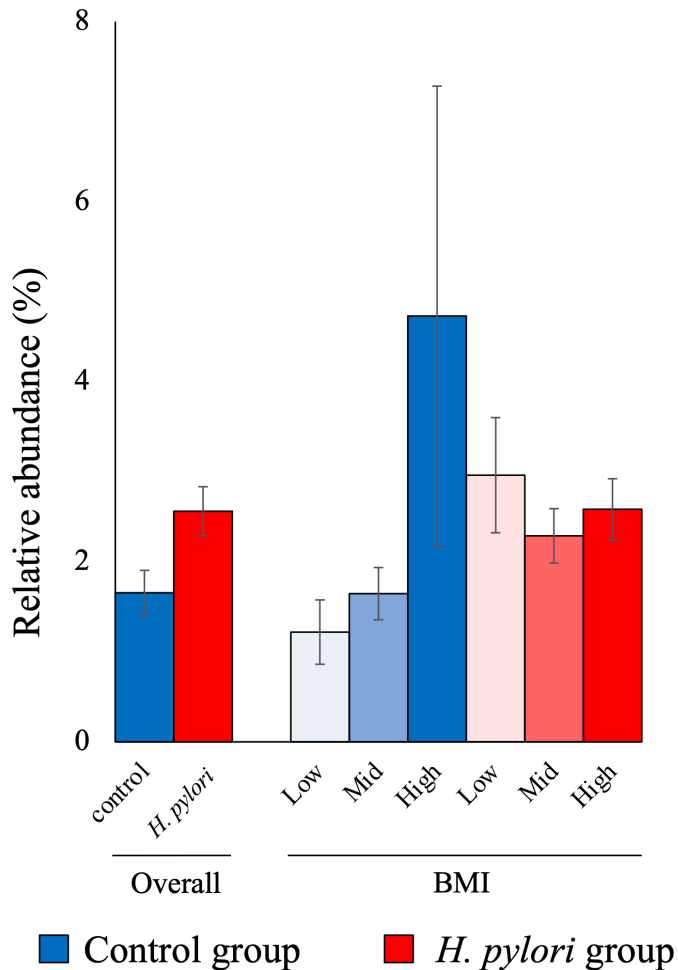
**Figure 7** The ratio of the *Firmicutes* phylum to the *Bacteroides* phylum in relation to the body mass index (BMI) category in the *Helicobacter pylori* and control groups (\*\*\* $p < 0.001$ ). Low: BMI  $< 15 \text{ kg/m}^2$ ; middle: BMI  $15\text{--}25 \text{ kg/m}^2$ ; high: BMI  $> 25 \text{ kg/m}^2$ .



**Figure 8** Relative abundance of the *Subdoligranulum* genus in relation to the body mass index (BMI) category in the *Helicobacter pylori* and control groups (\* $p < 0.05$ ; \*\* $p < 0.01$ ). Low: BMI  $< 15 \text{ kg/m}^2$ ; mid: BMI  $15\text{--}25 \text{ kg/m}^2$ ; high: BMI  $> 25 \text{ kg/m}^2$ .

diversity of the faecal intestinal microbiota was increased in students with *H. pylori* infection. As suggested by the present study, *H. pylori* infection might be a factor that disturbs the intestinal microbiota in adolescents. *H. pylori* infection is involved in the alterations of gut microbiota composition and diversity, which can lead to changes in production level and physiological regulation of the gut metabolic hormones released from the host endocrine system.<sup>34</sup> The mechanisms and clinical importance of the effect of *H. pylori* warrant further investigation.

The *Prevotella* genus increased in abundance during *H. pylori* infection, and this increase was found to be concomitant with a rise in BMI in the present study. A previous report indicated that the *Prevotella* genus was elevated in abundance in school-age children infected with *H. pylori*.<sup>35</sup> This was an epidemiological study, and unfortunately, it is completely unknown why at this time the *Prevotella* genus is elevated in school-age children infected with *H. pylori*. The *Bacteroides* and *Bifidobacterium* genera are dominant among the intestinal microbiota in Japanese children.<sup>36</sup> A previous study showed that the prevalence rate of the *Prevotella* genus in the intestinal microbiota was higher in



**Figure 9** Relative abundance of the *Collinsella* genus in relation to the body mass index (BMI) category in the *Helicobacter pylori* and control groups. Low: BMI <15 kg/m<sup>2</sup>; mid: BMI 15–25 kg/m<sup>2</sup>; high: BMI >25 kg/m<sup>2</sup>.

subjects who consumed carbohydrates more frequently,<sup>37</sup> which suggests that the *Prevotella* genus is closely related to eating habits. In the present study, it is not possible to determine whether infection with *H. pylori* affected the diet and resulted in an increase in the *Prevotella* genus, or whether infection with *H. pylori* increased the *Prevotella* genus and affected the diet, and caused an increase in BMI. In general, the F/B ratio has been found to increase with obesity.<sup>38</sup> The *Prevotella* genus belongs to the *Bacteroides* phylum, the present study thus exhibited inconsistency. At the moment, it is currently difficult to associate the F/B ratio with a determined health status and, more specifically, to consider it as a hallmark of obesity.<sup>39</sup> In the future, regarding the relationship between *H. pylori* and the *Prevotella* genus and BMI, it is necessary to analyse the intestinal flora in early childhood, including the history of eating habits from early childhood.

A correlation between *H. pylori* infection and the onset of diabetes has been reported in epidemiology studies,<sup>40,41</sup> but the reason for this remains unknown. Meanwhile, the prevalence of the *Prevotella* genus increased in patients with obesity,<sup>42,43</sup> non-alcoholic steatohepatitis,<sup>44</sup>

hyperlipidaemia,<sup>45</sup> and even in gestational diabetes, which is considered as a diabetes mellitus preliminary group.<sup>46</sup> The *Prevotella* genus is considered to contribute to hyperglycaemia and insulin resistance.<sup>43,47,48</sup> In the present study, an increase in the relative abundance of the *Prevotella* genus was observed in *H. pylori*-infected children with an increased BMI (figures 5 and 6). *H. pylori* infection in children with an elevated BMI without diabetes mellitus, caused an increase in the prevalence of the *Prevotella* genus (figures 5 and 6) and, as a result, insulin resistance increased, which may predispose individuals to diabetes mellitus. In fact, it is thought that the increase in *Prevotella* genus may be involved in the process of developing abnormal glucose metabolism as a result of obesity.<sup>49,50</sup>

The *Subdoligranulum* genus showed a lower relative abundance in the high BMI category than in the low BMI group, but this trend was seen not only in the *H. pylori* group, but also in the control group (figure 7). The *Collinsella* genus was not associated with BMI regardless of *H. pylori* infection status (figure 8). It has been reported that the *Subdoligranulum* genus is less prevalent among type 2 diabetes patients compared with their non-diabetic counterparts,<sup>51</sup> and a negative correlation with insulin resistance has been shown.<sup>52</sup> An increase in the *Collinsella* genus is reportedly associated with increased insulin, triglyceride and very-low-density lipoprotein levels,<sup>53</sup> and is associated with type 2 diabetes.<sup>54</sup> In our study, of the three genera (*Prevotella*, *Subdoligranulum* and *Collinsella*) that showed significant differences in relative abundance between the *H. pylori* and control groups, the *Prevotella* genus showed the most significant correlation between *H. pylori* infection status and BMI. The *Prevotella* genus was the only genus that showed an association with BMI in the *H. pylori* group, but not the control group.

There are several limitations to the present study. (1) In the selection of subjects in both groups, false negative results by using the urinary antibody in the control group and false positive results by using stool antigen in the *H. pylori* group could not be completely eliminated. (2) The present study evaluated faeces specimens, the microbiota of which may be different from the mucosal-associated microbiota. (3) The effect of eradication of *H. pylori* on the intestinal microbiota could be important,<sup>55</sup> and we plan to investigate this in the future. (4) There was a difference in preschool status between the two groups (table 1), and it could not be completely ruled out that this could have affected the intestinal microbiota.

## CONCLUSION

The present study shows that the intestinal microbiota is significantly affected by *H. pylori* infection in junior high school third grade students in Saga Prefecture, Japan. Furthermore, the relative abundance of the *Prevotella* genus was increased concomitantly with a rise in BMI in *H. pylori*-infected students.

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**Data availability statement** Data are available upon reasonable request. The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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