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Eradication of *Helicobacter pylori* in Children Restores the Structure of the Gastric Bacterial Community to That of Non-infected Children

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Keywords

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Introduction

H. pylori alters the gastric microbial composition and causes reduced gastric inflammatory disease in children in Chile[1–3]. These findings suggest that *H. pylori*-associated gastric microbiota may influence the pathogenesis of *H. pylori* infection beginning in childhood, at least in Chile. Here we characterized the gastric microbiota in *H. pylori*-infected children in an entirely different geographic region of South America, namely Venezuela, where *H. pylori* also is endemic[4], and determined the impact of *H. pylori* eradication on bacterial community structure.

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Conflict of interest. The authors declare no conflict of interest.

Methods

Sixteen children (≤13 years) with nausea and abdominal discomfort without antibiotic or antacid therapy during the preceding month underwent endoscopic antral biopsy for *H. pylori* urease testing and histology (both required positive for infection) and microbiota analysis. Eleven subjects were infected and treated with amoxicillin, clarithromycin and omeprazole for 14 days. Two months later, the children were re-biopsied for *H. pylori* testing and microbiota analysis. Biopsy DNA was isolated, the V4 region of the 16S rRNA bacterial gene was amplified by PCR, and 250 base single end reads were sequenced and analyzed, as previously described[3].

Results

The overall gastric bacterial community structure (β-diversity) in the *H. pylori*-infected children was different from that of non-infected children using unweighted UniFrac (*P*=.002) (Figure 1A), weighted UniFrac (*P*=.005) and Bray-Curtis analyses (*P*=.006). This finding suggested that *H. pylori* impacted the gastric commensal bacterial composition in infected children and prompted us to determine whether difference in the abundance of taxa with a prevalence >1% correlated with difference in the bacterial communities between infected and non-infected children. The relative abundance of taxa in the *H. pylori*-infected children was significantly reduced at the class (3 taxa), order (4 taxa), family (5 taxa) and genera (5 taxa) levels compared with taxa harbored by non-infected children (*P*=.01-.04), as determined by the Kruskal-Wallis test (Figure 1B). Since reduced gut microbial diversity is associated with certain disease processes, we determined the relative phylotype abundances (α-diversity) for the gastric microbiota. *H. pylori*-infected children harbored significantly less diverse gastric bacterial communities compared with non-infected children by both Shannon (*P*=.008) and Simpson (*P*=.02) indices (Figure 1C). These findings suggested that *H. pylori* impacted the gastric microbial community structure of children residing in this northern region of South America.

To substantiate these findings, we next analyzed the gastric microbial composition in *H. pylori*-infected children whose *H. pylori* was successfully eradicated by antibiotic treatment (7 of the 11 treated children). The bacterial community structure after *H. pylori* clearance was not significantly different from that of non-infected children (β-diversity) based on unweighted UniFrac analysis (*P*=.107), as well as weighted UniFrac analysis (*P*=.439) and Bray-Curtis analysis (*P*=.260). Overall, the relative proportion of the 10 most abundant bacterial taxa after *H. pylori* eradication did not differ significantly from that of non-infected children (Kruskal-Wallis test; *P*>0.5) (Figure 1D). Kocuria, an environmental Gram-positive bacteria that may inhabit skin and mucus membranes, is typically amoxicillin-resistant, and may cause systemic infection in immunocompromised hosts, dominated the microbiota in a single child. Finally, gastric bacterial diversity increased significantly after clearance of *H. pylori,* based on comparisons of Shannon and Simpson indices (both *P*=.04) (Figure 1E), reflecting the more diverse bacterial composition after *H. pylori* eradication. Thus, eradication of *H. pylori* in infected Venezuelan children was associated with restoration of the gastric microbiota to the community structure of non-infected children.

Discussion

This first characterization of the gastric microbiota in Venezuelan children is important because the microbial composition associated with *H. pylori* infection may impact the risk for gastric disease sequelae, as shown for adults in mountainous Pacific South America[5]. Further, in countries with a high prevalence of *H. pylori,* gastric cancer in adults is strongly related to acquiring *H. pylori* in childhood. However, children have reduced levels of *H. pylori*-associated gastric inflammation, the key risk factor for gastric cancer, compared with infected adults[1,2]. Indeed, gastric cancer in children is exceptionally rare, although *H. pylori* is typically acquired in early childhood. That *H. pylori* infection in children is associated with an altered gastric microbiome, as we previously reported for Chilean children[3] and confirmed here, raises the possibility that the gastric microbiota in infected children may contribute to mucosal changes such as increased regulatory T-cell responses in infected children[1–3]. In a mouse model, *H. pylori* accelerates the pathogenesis of gastric cancer [6] and has more impact on the abundance of specific bacterial taxa in younger than older mice[7]. In addition, *H. pylori* infection in neonatal, but not adult, mice promotes tolerance to *H. pylori* and protects against preneoplastic lesions[8].

The complex consortia of generally non-cultivatable bacteria colonizing the stomach requires a molecular approach to identify these microbial communities. Using 16S rDNA sequencing, we show that *H. pylori* clearance restores the gastric microbial composition to the community structure of non-infected children, suggesting *H. pylori* eradication impacts the associated microbiota. Thus, should the gastric microbial composition altered by *H. pylori* contribute to *H. pylori*-associated disease, eradication of *H. pylori* with restoration of the microbial community structure to that of non-infected children may modify infection sequelae. Notably, the class, order and family of bacterial taxa impacted by the presence of *H. pylori* in the Venezuelan children is different from the taxa reported to be impacted by *H. pylori* in Chilean children[3], except for reduced abundance of the acid tolerant order Lactobacillales in both populations. The more prevalent bacterial genera (>1% abundance) associated with *H. pylori* also did not overlap in the Venezuelan and Chilean children. The lack of concordance in the gastric microbial community structure among children in these two populations may reflect the impact of different diets, nutritional status and geographic location. However, both studies demonstrate that the presence of *H. pylori* substantially alters the gastric microbiome in children. While acknowledging the small size of our study population, we provide a starting point for future studies on the clinical impact of restoring the microbiota to that of non-infected children. Thus, characterization of the gastric microbiota in pediatric *H. pylori* infection in endemic regions of the world may provide insight into the role of the microbiome in disease pathogenesis.

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Abbreviations

rDNA	ribosomal DNA
PCoA	principal coordinate analysis
QIIME	quantitative insight into microbial ecology
OTU	operational taxonomic unit
PERMANOVA	permutational multivariate analysis of variance

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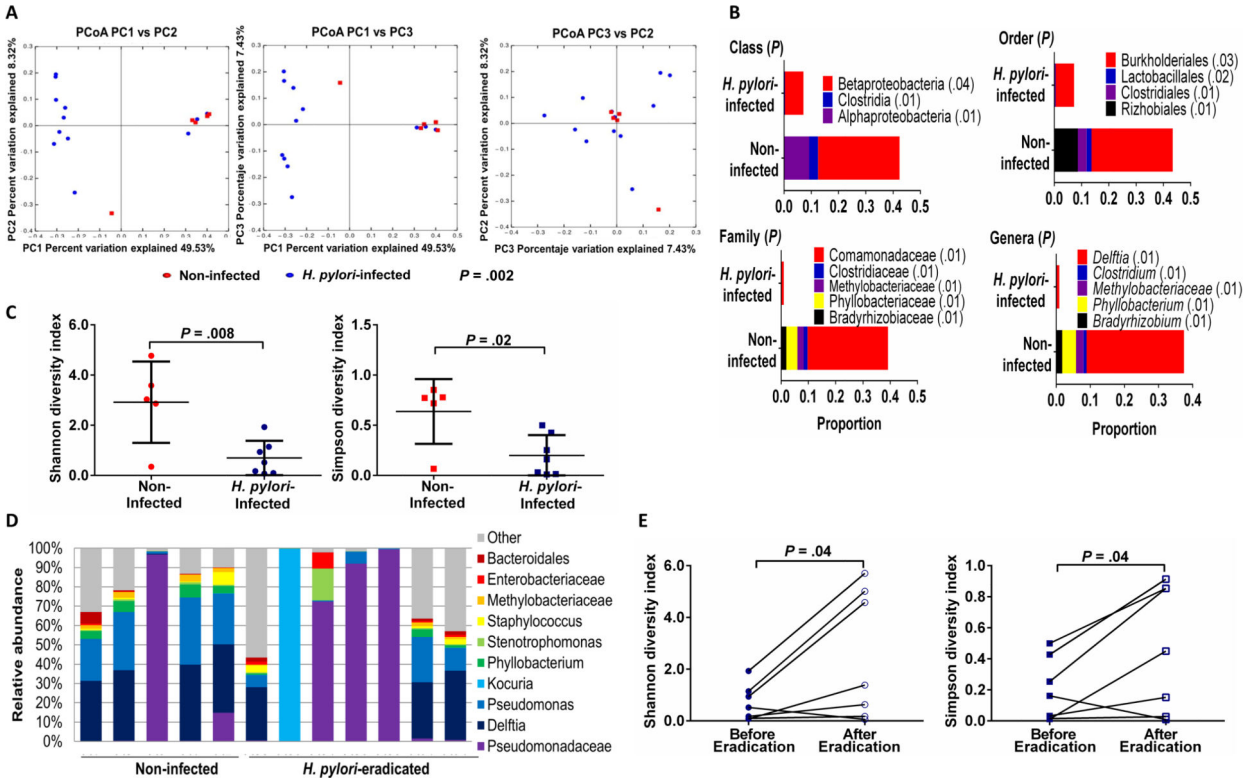

Figure 1.

Gastric microbiota in Venezuelan children is modified by *H. pylori* infection and reverts to that of non-infected children after *H. pylori* eradication. ***(A)*** β-diversity of the gastric microbiota in *H. pylori*-infected (n=11) and non-infected children (n=5) determined by permutational multivariate analysis of variance (PERMANOVA) and illustrated by unweighted UniFrac distances with each dot representing one subject in the principal coordinate analysis (PCoA) presented in 2-dimensional plots. *P* value determined by PERMANOVA test in QIIME. ***(B)*** Taxa abundances (when >1% of total bacterial DNA) at the class, order, family and genera levels among *H. pylori*-infected and non-infected children. *P* values determined by Kruskal-Wallis test with multiple comparison correction and false discovery rate analysis. When a genus could not be assigned, family is listed. ***(C)*** α-diversity of the gastric microbiota in *H. pylori*-infected (n=11) and non-infected children (n=5) using Shannon and Simpson indices. *P* values determined by Student's *t*-test. ***(D)*** Taxa abundances of the 10 most abundant gastric bacteria (when >1% of total bacterial DNA) in children without *H. pylori* (5) and children in whom *H. pylori* was eradicated (n=7). *P*>.05, Kruskal-Wallis test. ***(E)*** α-diversity of the microbiota in *H. pylori*-infected children before (n=11) and after successful *H. pylori* eradication (n=7) using Shannon and Simpson indices. *P* values determined by Student's *t*-test.