

# The mucosal microbiota in a young child with severe non-*Helicobacter* gastritis

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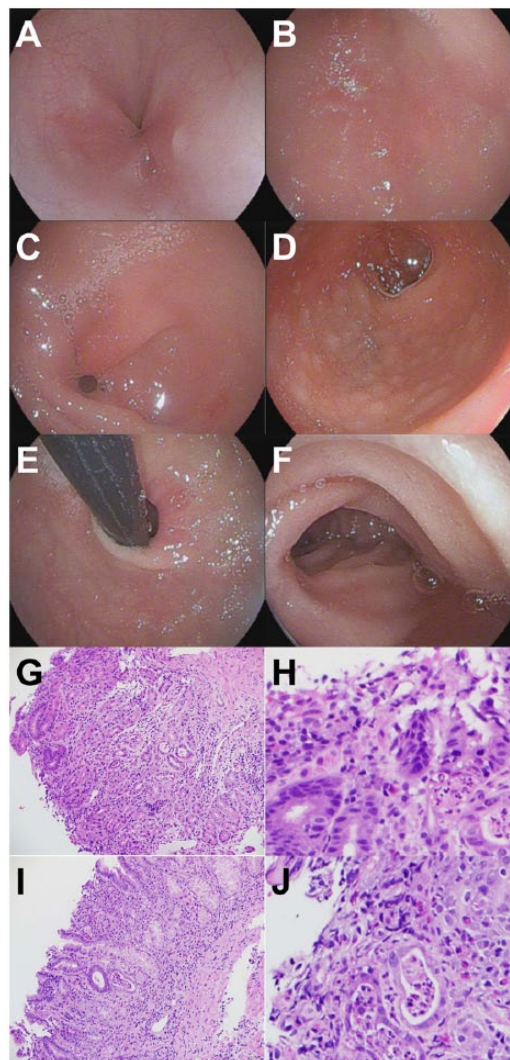
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A 34-month-old boy of Caribbean/Hispanic descent, born abroad and raised in a Houston suburb, following 2 weeks in the Caribbean presented with new-onset painless gagging and vomiting multiple times per day. The emesis contained clear or partially digested food with an occasional bloody tinge. Physical examination and a barium swallow were unremarkable, and trials of ranitidine and proton-pump inhibitor (PPI) were unsuccessful. Esophagogastroduodenoscopy under general anesthesia (Figure 1a–f) revealed mild erythema throughout the distal esophagus, corpus, antrum, and fundus, with prominent antral nodularity and friability. Mild edema was present in the duodenal folds. Biopsies from the fundus (Figure 1g–h) and corpus revealed oxyntic-predominant chronic active gastritis with increased eosinophils. Antral biopsies (Figure 1i–j) revealed severe chronic active gastritis. The esophagus was unremarkable, and duodenal biopsies revealed patchy mild chronic inflammation. Gastrin was focally absent on immunostaining, and there was no evidence of granulomata, giant cells, viral inclusions, or *Helicobacter pylori*.

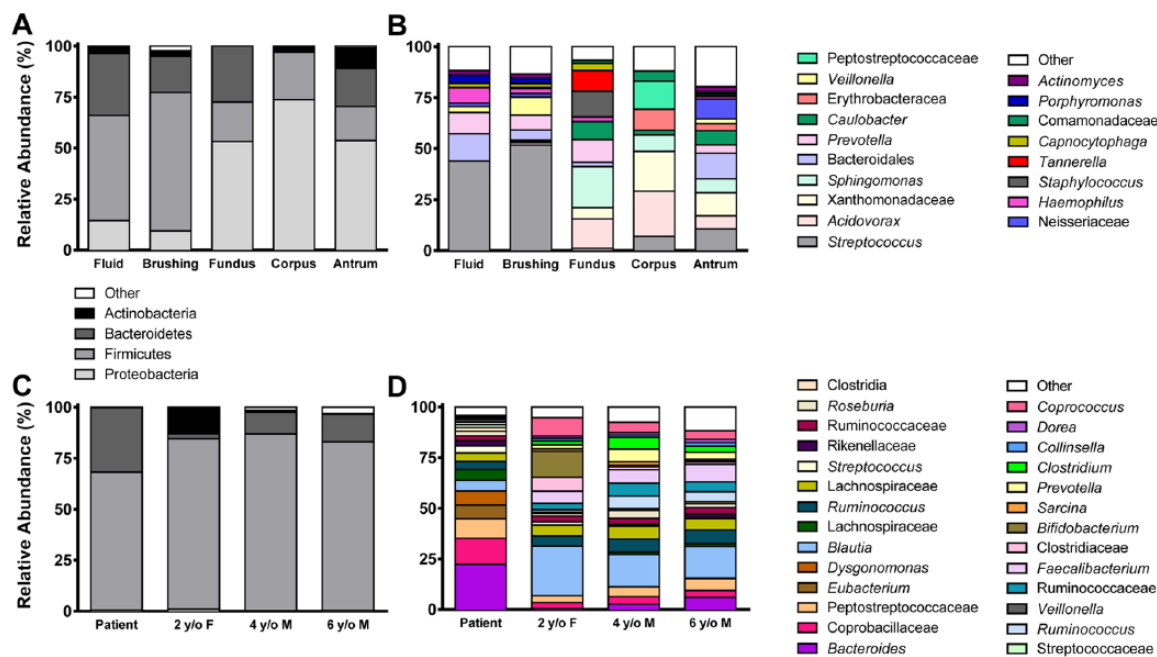
Despite sucralfate and PPI, the child's emesis worsened. Stool studies were significant only for occult blood and elevated calprotectin [533, repeat 638 (reference range 0–120) µg/g]. Antibodies for autoimmune gastritis, celiac disease, inflammatory bowel disease and food allergy were negative. A diagnosis of *H. pylori* gastritis was considered, based on the clinical, endoscopic, and laboratory findings (*H. pylori* IgG positive; IgM, IgA, and stool antigen negative). The child began anti-*Helicobacter* therapy with amoxicillin, metronidazole, clarithromycin and PPI. Vomiting immediately improved but resumed six days after completing the 14-day therapy. He then began 14 days of amoxicillin, levofloxacin, bismuth and PPI, again with immediate improvement. Seven weeks later he resumed intractable vomiting with hematemesis and weight loss, prompting repeat



**Figure 1.** Endoscopic views of: (a) distal esophagus, (b) corpus, (c) antrum with (d) nodularity, (e) fundus, and (f) duodenum. Histological sections of fundus at (g) low and (h) high magnification and of antrum at (i) low and (j) high magnification.

upper endoscopy (unchanged from previous) and colonoscopy (grossly and histologically normal).

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**Figure 2.** Relative abundance of bacteria within gastric fluid, mucosal brushing, and fundal, corpus, and antral biopsies at (a) phylum and (b) operational taxonomic unit (OTU) levels. Fecal bacteria isolated from the patient and three age-similar healthy controls depicted at (c) phylum and (d) genus (when possible) or family levels. Patient's microbiota represent single samples; controls represent average abundances from up to 23 fecal samples per healthy child obtained on separate days over one month as previously reported [Schloss *et al.* 2014].

From this repeat endoscopy, with informed consent, bacterial DNA was isolated from gastric fluid and brushings, mucosal biopsies from fundus, corpus, and antrum, and stool and was sequenced using recently described methods [Schloss *et al.* 2014] (Figure 2). Whereas Firmicutes (primarily *Streptococcus*, likely of oral/nasopharyngeal origin) dominated in aspirated fluid and brushings, the majority of microbes detected in biopsy samples were Proteobacteria, most prominently uncharacterized members of the genera *Acidovorax* and *Sphingomonas* and the family Xanthomonadaceae. Two taxa within the phylum Bacteroidetes, *Prevotella* and an unclassified Bacteroidales family member, were also prominent. In stool, the most abundant genus was *Bacteroides* and the microbes most prevalent in gastric mucosa were absent. No member of *Helicobacter* was present in any sample, as confirmed by genus-specific polymerase chain reaction (PCR), as described previously [Pena *et al.* 2002]. The boy's clinical course immediately improved after starting a 21-day course of quadruple therapy, and he remains asymptomatic one year later. Ethical considerations precluded repeat endoscopy with mucosal sampling from the asymptomatic patient at follow-up or from healthy age-matched controls.

Once thought to be a sterile environment due to the inhospitably low pH, the stomach is now known to harbor a thriving bacterial community of between  $10^1$ – $10^3$  cfu/g mucosa with hundreds of unique taxa identified by next-generation metagenomic sequencing in adults [Andersson *et al.* 2008; Stearns *et al.* 2011; Delgado *et al.* 2013; Bashir *et al.* 2015]. In one study, *H. pylori* DNA was detected only in biopsy samples from which the bacterium was successfully cultured [Dicksved *et al.* 2009]; however, most studies report a surprisingly high prevalence of *Helicobacter* DNA in asymptomatic adults [Bashir *et al.* 2015] and/or in those who tested negative for *H. pylori* by other means [Bik *et al.* 2006; Maldonado-Contreras *et al.* 2011]. Although the presence of *H. pylori* is associated with significant changes to mucosal microbial community composition [Monstein *et al.* 2000; Bik *et al.* 2006; Maldonado-Contreras *et al.* 2011], one study in adults revealed that non-*Helicobacter* antral gastritis was also associated with altered microbial communities, namely increased abundance of Firmicutes primarily within the genus *Streptococcus* [Li *et al.* 2009]. There are no equivalent studies within the pediatric population, although the prevalence of non-*H. pylori* gastritis is believed to be significantly underestimated in children [Kalach *et al.* 2009].

This report is the first characterization of the gastric mucosal microbiota in a child. Although *Prevotella* and *Streptococcus* are among the dominant genera found in most healthy adults, the three most abundant taxa in this boy's gastric mucosa were *Acidovorax*, *Sphingomonas*, and Xanthomonadaceae. These groups all contain potential pathogens and have never been reported in appreciable quantities in a healthy adult stomach. Whether they represent normal mucosal taxa in young children remains to be determined. Given the high inter-individual variability among gastric mucosal microbiota in adults, it is not yet certain whether a healthy 'core' human gastric microbiome exists or whether any non-*Helicobacter* pathogens might cause gastritis. Similarly, high inter-individual community variability is likely to be found within the pediatric population.

Further metagenomic studies will shed additional light on the poorly characterized pathogenesis of non-*H. pylori* gastritis in children. Clear clinical improvement, and eventual cure, with antimicrobial therapy suggests that altered intestinal bacterial populations and/or a specific unidentified pathogen was responsible for this child's presentation. Ultimately, if specific microbes or microbial functions can be causally linked to health or disease, an array of new therapies targeting the gastric mucosal microbiome will be at clinicians' disposal.

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### Conflict of interest statement

The authors declare that there is no conflict of interest.

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