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The role of the gastrointestinal microbiome in *Helicobacter pylori* pathogenesis

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The discovery of *Helicobacter pylori* overturned the conventional dogma that the stomach was a sterile organ and that pH values < 4 were capable of sterilizing the stomach. *H. pylori* are an etiological agent associated with gastritis, hypochlorhydria, duodenal ulcers, and gastric cancer. It is now appreciated that the human stomach supports a bacterial community with possibly 100s of bacterial species that influence stomach homeostasis. Other bacteria colonizing the stomach may also influence *H. pylori*-associated gastric pathogenesis by creating reactive oxygen and nitrogen species and modulating inflammatory responses. In this review, we summarize the available literature concerning the gastric microbiota in humans, mice, and Mongolian gerbils. We also discuss the gastric perturbations, many involving *H. pylori,* that facilitate the colonization by bacteria from other compartments of the gastrointestinal tract, and identify risk factors known to affect gastric homeostasis that contribute to changes in the microbiota.

Introduction

The human microbiota consists of approximately 100 trillion microbial cells that outnumber our human cells by 10 to 1.[1] Through the efforts of the Human Microbiome Project,[2,3] the human oral and fecal microbiota have been more extensively studied than other sites in the gastrointestinal (GI) tract. However, given the recognition that each site of the GI tract has its unique microbiota,[4] it is necessary to further investigate each of these ecosystems to identify their role in health and disease states.

Conventional wisdom espoused the dogma that the stomach was a sterile organ and that pH values < 4 were able to sterilize the stomach,[5] but in the past 30 years with the discovery of *Helicobacter pylori*,[6,7] it is now known that the stomach supports a bacterial community with hundreds of phylotypes (approximate species-level taxa),[8-10] and while pH values <4 prevent bacterial overgrowth, the acidic milieu is not capable of sterilizing the stomach.[11] Data suggest that the microbial density of the stomach is 10^1–10^3 CFU/g.[12-14] The stomach, along with the esophagus and the duodenum, are the least colonized regions of the GI tract, in contrast to the high bacterial counts (10^{10} to 10^{12} CFU/g) observed in the colon.[12-14] The low bacterial densities within this portion of the GI tract are due to the effects of rapid peristalsis, low pH and/or high bile concentration.[15] As *H. pylori* are directly implicated as an etiological agent in several gastric diseases, including gastric atrophy and cancer,[16] it is important to determine the contributions made by other bacteria in gastric health and disease.

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Stomach Anatomy

The human stomach is divided into three anatomic regions: the cardia, the fundus/corpus, and the antrum. The cardia is distal to the gastresophageal junction, and its glands primarily secrete mucus. The fundus/corpus comprises close to 80% of the organ and contains the oxyntic glands. The antrum is proximal to the pyloric sphincter, which separates the stomach from the duodenum, and contains pyloric glands (**Fig. 1**). Both oxyntic and pyloric glands possess mucous neck cells, D cells, and Enterochromaffin cells. They differ as oxyntic glands possess parietal (oxyntic) cells, chief (zymogenic), and enterochromaffin-like cells that produce HCl, pepsinogen, and histamine, respectively. The main feature of pyloric glands is the presence of G cells used to generate gastrin, a key hormone in the regulation of acid secretion.[17]

The role of the stomach is 2-fold. First, the stomach secretes gastric juice, composed mainly of proteolytic enzymes and hydrochloric acid, which provide the environment necessary for denaturing of proteins and facilitates the absorption of nutrients. Second, gastric acid plays a role in suppressing the density of ingested microorganisms and assists in preventing infection by pathogens.[18] The intragastric pH of 1–2 is the primary restrictive component of the stomach, and severely limits bacterial colonization and survival.[15] To prevent damage to the mucosa from HCl and pepsinogen, mucous neck cells throughout the stomach generate mucus that lines the gastric epithelium. While the human gastric lumen has a pH of 1–2, the mucus layer establishes a pH gradient that increases the pH to 6–7 at the surface of the mucosa.[19] This is achieved by unique properties of the mucus which permit acid to flow from parietal cells into crypts which communicate with the lumen, but do not allow acid at pH <4 from penetrating the mucus layer.[19] The mucus layer consists of several mucins, such as MUC1, MUC5AC, MUC5AB, and MUC6, and forms two sublayers, an inner mucus layer that is

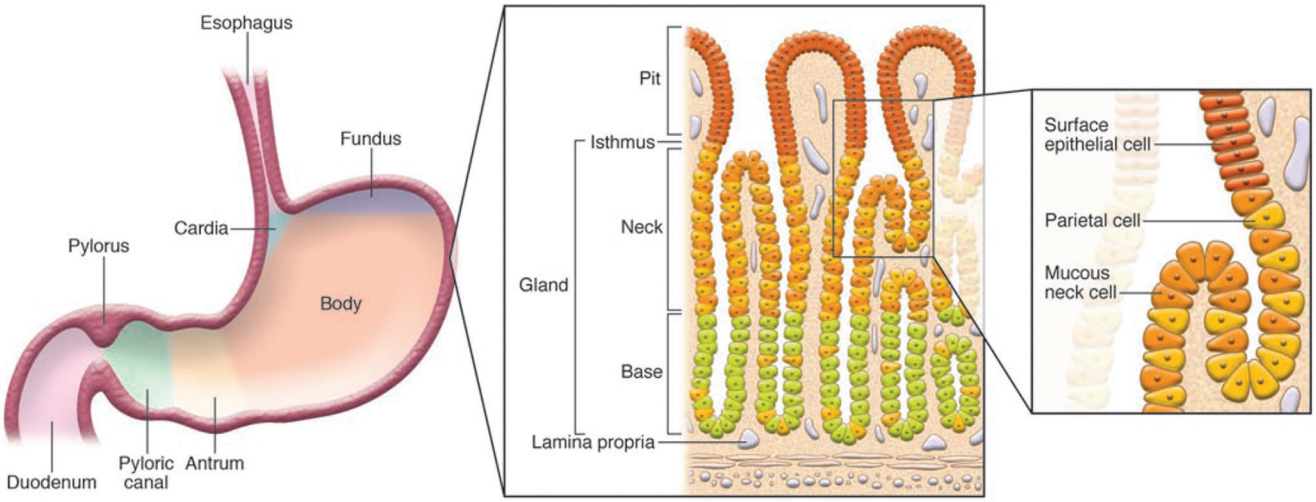

Figure 1. Diagram depicting anatomy of the stomach and histological representation of the oxyntic glands of the body of the stomach. It is these glands, which include parietal cells, that are lost in gastric atrophy. Reproduced with permission from Fox and Wang 2007.[16]

firmly attached to the epithelia and a loose mucus layer interfacing with the lumen.[20,21] In the context of understanding the dynamics of the gastric microbiota, it is necessary to consider the site of isolation, as bacteria (and importantly bacterial DNA) may be isolated from the gastric juice, which is too formidable a barrier for colonization (isolated DNA may reflect transient bacteria), compared with the mucosa, which presents a more hospitable environment for microbial colonization. However, during abnormal or disease states, this balance may be perturbed, leading to bacterial colonization. Reduction of gastric acid secretion, whether by parietal cell loss or drug-induced inhibition, can lead to hypochlorhydria (pH between 4–7) or even achlorhydria (pH 7), and increases the risk of bacterial overgrowth and possible deleterious infections throughout the GI tract.[18]

Gastric Perturbations by *Helicobacter pylori*

Helicobacter pylori are gram-negative bacteria that successfully colonize the human stomach, infecting 50% of the world's population. *H. pylori* are uniquely adapted to colonize the gastric niche. This process has extensively reviewed by others.[22,23] Upon infection, *H. pylori* utilize urease and α-carbonic anhydrase to generate ammonia and HCO_3^{2-} which mitigate the effects of low pH.[24,25] The local increases in pH facilitate the bacteria's passage through acidic gastric fluid and the pH-sensitive mucous layer. Using chemotaxis, the bacteria navigate the pH gradient to their niche near the host epithelium.[26,27] Infection with *H. pylori,* or the closely related pathogen *H. felis*, have been shown to alter the mucus barrier by affecting the expression of mucins *Muc1, Muc4*, and *Muc5b*.[28,29] Once established in the inner mucus layer, *H. pylori* can utilize diverse adhesins (e.g., SabA and BabA) to attach to epithelial cells. Once attached, bacterial effector molecules, both secreted (vacuolating cytotoxin [VacA] and cytotoxin-associated gene A [CagA]) or attached (components of the type IV secretion system [CagL]), modulate gastric epithelial cell behavior leading to loss of cell polarity, release of nutrients and chemokines (e.g., IL-8), and of particular interest for this review, regulation of acid secretion via control of gastrin and H^+/K^+ ATPase.[22,30,31]

In response to *H. pylori* infection, the host mounts an acute inflammatory response characterized by infiltration of neutrophils and mononuclear cells which leads to a chronic, active gastritis. *H. pylori* protect themselves from reactive oxygen and nitrogen species (RONS) via detoxifying enzymes (catalase and superoxide dismutase) and arginase which limits nitric oxide production from immune cells.[22] Furthermore, *H. pylori* lipopolysaccharide (LPS) and flagellin do not elicit strong inflammatory responses, which limit specific immune responses to the bacteria.[22] The ineffectual, acute response leads to the establishment of a chronic inflammatory state. The adaptive immune response to *H. pylori* is mainly mediated by cellular (T cell), rather than humoral (B cell), immunity and is comprised of proinflammatory and regulatory T cell responses.[32] Broadly, proinflammatory T helper 1 (T_H1) and T_H17 cells secrete cytokines (e.g., interleukin-2 (IL-2), IL-17, IL-22, and IFN-γ) that increase proinflammatory cues, and promote both neutrophil recruitment and macrophage activation. T_H1 and T_H17 cells play an important role in controlling *H. pylori* infection, but also mediate infection-associated immunopathology.[33,34] Regulatory T (T_{REG}) cells mediate immune tolerance that allows the persistence of *H. pylori* and minimizes host damage caused by excessive immunopathological T cell responses.[35] A recently proposed mechanism demonstrates how *H. pylori* can modulate both proinflammatory and regulatory T-cell responses via the release of both IL-1β and IL-18, following inflammasome activation.[23] IL-1β promotes the induction of T-box transcription factor (T-bet)-dependent T helper 1 (T_H1) and RAR-related orphan receptor γt (RORγt)-dependent T_H17 cells, and the expression of IFN-γ and IL-17, while IL-18 promotes FOXP3-dependent $CD^{4+}CD2^{5+}$ T_{REG} cells.[34] Therefore, the host's attempts to eradicate *H. pylori*

increase gastric immunopathology (gastritis, epithelial damage such as atrophy and intestinal metaplasia), which alters the gastric compartment and its microbiota, and may subsequently progress to gastric cancer. Due to its role in gastric cancer, *Helicobacter pylori* was one of the first infectious agents recognized by the International Agency for Research on Cancer (IARC) as a class I, or definite, carcinogen.[36]

Gastric Cancer

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide.[37] Both incidence and mortality rates are about twice as high in males as in females.[37] Over 70% of cases occur in developing nations, concentrated in Eastern Asia, Eastern Europe, and Central and South America. In contrast, Australia, Africa, Southern Asia, Western Europe and North America are areas of low risk. Not surprisingly, in 2008, Eastern Asia has the highest mortality rates (28.1 and 13.0 per 100 000 men and women, respectively), while North America has the lowest (2.8 and 1.5 per 100 000 men and women, respectively).[38] The high mortality to incidence ratio is due in part to the lack of clinical symptoms in most cases of early gastric cancer, which makes early detection difficult.[39] Improvements in sanitation (resulting in reduced *H. pylori* infections), nutrition (greater access to fresh food and decreased dietary salt intake), use of endoscopies and antimicrobial eradication of *H. pylori* have contributed to the decrease in gastric cancer rates worldwide.[40]

Approximately 90% of gastric cancers are adenocarcinomas, malignant epithelial tumors that arise from the gastric glandular epithelium.[41] Anatomically, gastric cancers are categorized as proximal and distal. Proximal adenocarcinomas are more similar to esophageal adenocarcinomas and may be associated with the absence of *H. pylori*,[40] while distal adenocarcinomas originate in the antrum and are commonly associated with *H. pylori* infection. Histologically, gastric adenocarcinomas are classified as either diffuse-type or intestinal-type.[42] Diffuse-type tumors are characterized by pan-gastritis but no atrophy, are potentially familial in distribution, are present in younger populations and are found uniformly throughout the world in both men and women.[43,44] Intestinal-type tumors are characterized by a corpus-dominated gastritis with gastric atrophy and intestinal metaplasia, are associated with regions of high gastric cancer risk and *H. pylori* infection, and occur more frequently in elderly men.[16]

Helicobacter pylori, the Gastric Microbiota and Progression to Gastric Cancer

As the host's chronic inflammatory response is incapable of eradicating *H. pylori,* chronic gastritis ensues, which over decades can progress through a series of discrete steps known as the Correa pathway which involve atrophy, intestinal metaplasia, dysplasia, and intestinal-type gastric adenocarcinoma.[16,45] In the context of the stomach, atrophic gastritis is the loss of specialized glandular tissue, such as the oxyntic glands, which impairs acid secretion and the differentiation of gastric progenitor cells.[46-48] The loss of parietal cells, which creates a state of hypochlorhydria (pH >4), facilitates the colonization of the stomach by various bacteria, including those with nitrosating ability which are not regularly cultured from a normal, healthy stomach.[45]

Since the advent of H_2 receptor antagonists (H2RA) in the mid-1970s, there has been an ongoing clinical interest in the microbiota colonizing the stomach. Hypochlorhydria induced by acid suppression is associated with higher levels of gastric nitrites and an increased risk of gastric cancer.[49-51] Chronic H2RA therapy or atrophic gastritis promote overgrowth of nitrosating bacteria that convert nitrite and other nitrogen compounds in gastric juice to produce carcinogenic N-nitroso compounds (NOC).[45] These chemical reactions are favored in hypochlorhydric stomachs where pH > 4 allows the persistence of nitrites by reducing the antioxidant activity of vitamin C, a powerful nitrosation inhibitor.[52,53] The introduction of proton pump inhibitors (PPI) elevated and sustained gastric pH levels even further.[54] Studies found a logarithmic relationship between intragastric pH and median bacterial counts in the gastric juice and mucosa and increased risks for enteric infections and bacterial diarrhea.[55,56] A review of the literature notes that multiple non-*H. pylori* organisms have been isolated from the stomach in hypochlorhydric patients, including *Lactobacillus* spp, *Streptococcus* spp, *Pseudomonas* spp, *Xanthomonas* spp, *Proteus* spp, *Klebsiella* spp, *Neisseria* spp, *Escherichia coli*, and *Campylobacter jejuni*.[54]

Acid suppressive drugs also affect the progression of *H. pylori* pathogenesis. In stomachs with normal or high acid production, *H. pylori* gastritis is limited to the antrum and this pattern is usually associated with the development of duodenal ulcers, and not gastric cancer.[16] In stomachs with lower acid secretion, as caused by acid suppression or atrophy, *H. pylori* shifts to a corpus predominant gastritis, which drives parietal cell loss and is associated with increased gastric cancer risk.[56,57] Furthermore, increased pH may enhance *H. pylori*-induced lesions to the gastric mucosa mediated by RONS.[58] Another harmful effect of acid suppression is the deregulation of gastrin. Both *H. pylori* infection and high pH induce hypergastrinemia (to stimulate parietal cells), but prolonged hypergastrinemia can be deleterious due to gastrin's trophic effects on the oxyntic mucosa, which promotes gastric stem cell proliferation and increase the risk of enterochromaffin-like cell hyperplasia.[56,59] Eradication of *H. pylori* did not lead to full recovery of acid secretion in patients with profound hypochlorhydria but did reduce hypergastrinemia.[60,61] One study indicated that in *H. pylori*–infected patients, the high serum levels of gastrin prior to PPI therapy were associated with the most marked progression in gastric atrophy during acid suppression therapy.[62] At the same time, *H. pylori* has been shown to enhance the acid suppressive effects of both H2RAs and PPIs, as well as increasing the risk of atrophic gastritis, bacterial levels and elevation of N-nitrosamines.[56,63] The presence of both *H. pylori* and non-*H. pylori* bacteria also increased atrophy observed in patients under acid suppressive regimes.[56] Animal studies support the hypothesis that Helicobacter infection might accelerate atrophy in hypergastrinemic individuals or patients undergoing acid suppression therapy.[64-66] In Mongolian gerbils, omeprazole

treatment of *H. pylori* infected animals led to increased neutrophil and lymphoid infiltration, higher corpus atrophy scores and increased adenocarcinomas.[66] *H. felis*–infected hypergastrinemic mice treated with omeprazole manifested a more rapid progression to dysplasia.[64] The pathological changes to the stomach can become so profound that the niche inhabited by *Helicobacter* spp changes, as evidenced by the decline in *Helicobacter* spp colonization levels observed in cases with severe achlorhydria and gastric cancer in humans[16,67] and mice.[68] The loss of *H. pylori* may also facilitate the colonization of other bacterial populations into this niche. As such, gastric atrophy is considered a critical step in the progression to intestinal-type gastric cancer, and is a strong marker of gastric cancer risk.[69]

Methods for Determining the Gastric Microbiota

Given culture conditions have not been established for the majority of microbes colonizing the GI tract, culture-based methods provide an incomplete and biased picture of the biodiversity of intestinal microbiota. Therefore, culture-independent molecular methods based on 16S rRNA genes, such as fluorescent in situ hybridization (FISH),[70] dot-blot hybridization with rRNA-targeted probes,[71] targeted qPCR,[72] traditional or sequence-aided community fingerprinting [including denaturing gradient gel electrophoresis (DGGE),[73] temperature gradient gel electrophoresis (TGGE),[74] and terminal restriction fragment length polymorphism (T-RFLP)[75]], sequencing of cloned 16S rDNA,[74] microarrays (PhyloChip),[76] and next-generation sequencing[77] (NGS) have been used to determine the gut microbiota in diverse regions of the GI tract. While new technologies (e.g., API[78] and MALDI-TOF mass spectrometry[79]) have improved the identification of cultured organisms, culture remains limited by the inability to culture all the organisms of interest, but also by time consuming technical demands. It has been argued that culture-based methods provide the advantage of distinguishing viable microorganisms, which DNA-based assays cannot.[80] However, in the context of decreased bacteriocidal activity in the stomach (e.g., hypochlorhydria), bacteria in transit can also be cultured.

For all technologies dependent on hybridization, amplification, identification or sequencing of the 16S rRNA gene, the quality of DNA extractions is critically important as it may bias the results, due to varying degrees of microbial resistance to processing by enzymes, chaotropic agents, or bead beating.[81] FISH, dot-blot hybridization and qPCR are highly specific and useful techniques when a defined set of organisms are being studied. However, given the need to design and test specific probes for each queried organism and the low-throughput nature of the assays, these techniques are not as useful in surveying large collections of microbes. Newer technologies, such as microarrays/PhyloChip and high-throughput qPCR arrays, easily address the concerns of the low-throughput nature of dot-blot hybridization and qPCR and allow the capacity to query 100s to 1000s of organisms in a single run. Nevertheless, a significant investment has to be made to design and test comprehensive qPCR probe sets or microarrays like the PhyloChip. In the case of the PhyloChip, the array can distinguish >50 000 different operational taxonomic units (OTUs) and incorporates bioinformatic tools to dissect the generated data.[76,82] The high number of detectable OTUs effectively allows most users to use the PhyloChip without a priori knowledge of the sample composition, which is not possible with qPCR or FISH. However, the limitation of the PhyloChip lies in its inability to multiplex samples, which makes it unfeasible for most labs to process more than a few samples.

Techniques that allow unbiased surveillance of the entire microbial community without a priori knowledge of the composition rely on 16S rRNA gene analysis and include community fingerprinting, Sanger-sequencing of 16S rDNA libraries and next generation sequencing (NGS) of 16S rRNA genes. The 16S rRNA gene is homologous in all bacteria, highly conserved in overall structure, not readily transferred between species, and contains 9 variable regions that allow phylogenetic identification of species or the definition of operative taxonomic units (OTUs).[83] Community fingerprinting techniques are capable of surveying unknown microbial communities and are flexible in post-processing. As bands can be analyzed visually on the gel, and subsequently confirmed using PCR or sequencing methodologies, the user can customize the degree of confidence in the assay's results. The drawbacks for these techniques are the low resolution between bands (i.e., multiple organisms can have similar bands) and the high level of expertise needed for execution. Sanger-sequencing of 16S rDNA libraries can be used in conjunction to community fingerprinting methods or direct PCR amplification from the sample. Earlier microbiome studies relied on library sequencing.[71,74] The advent of better sequencing technologies, which process more reads and do not require cloning, and better bioinformatics tools have rendered this technology more obsolete. While not free of biases in PCR amplification, massively parallel sequencing with NGS removes selection biases that could occur with 16S rDNA clonal libraries, provide easier processing, increase sequencing coverage and provide better resolution than other methods. Technically, many of the skills needed for processing NGS samples are familiar to molecular biologists. Its current limitations are primarily complexity of bioinformatic analysis, and secondarily, access to equipment and cost. The secondary concerns are being addressed as the equipment becomes less expensive and more readily available, and the cost of sequencing continues to decrease. The primary obstacle for most labs has been processing millions of relatively short reads effectively, but considerable resources have been allocated to resolve these limitations. Applications like 16S profiling have become quite standard. Briefly, programs take raw data generated by the NGS machine and remove low-quality sequences and further processing, such as trimming barcodes and adaptor sequences, prepare sequences for comparison. Software aligns sequences against reference databases such as SILVA,[84] GREENGENES,[85] or the Ribosomal Database Project (RDP)[86] to identify microbes most closely associated to a given sequence. Currently NGS may be the best method in terms of balancing ease of use, accessibility and cost for microbiome studies. A more complete discussion of methods, DNA isolation and bioinformatic analysis can be found in this review.[81]

The Gastric Microbiota

Despite the declining prevalence of *H. pylori* infection worldwide, *H. pylori* still infects 50% of the world's population.[22] We and others would argue that *H. pylori* are indeed an autochthonous species in the gastric niche.[1,74,87] As such, this review will describe the gastric microbiota in both humans and key rodent models, with the inclusion and absence of *H. pylori*, while evaluating the effects of altered gastric states on the microbiota. In this review, we will include higher and lower taxonomic information, such as phylum and genus, for ease of comparison (**Fig. 2**). For older publications, we have updated classification systems to better reflect current nomenclature. The studies reviewed have been conducted in populations worldwide, but as samples for gastric microbiota analysis are more difficult to obtain than the oral or fecal microbiota samples, many studies rely on patients undergoing an endoscopy. This may bias studies as these subjects may not be reflective of an asymptomatic population. Collections of gastric juices have been used in the past, but may be compromised because the samples also reflect the transient populations of the stomach.

Gastric Microbiota in Humans

The major constituent of the gastric microbiota in more than half of all humans is the Proteobacteria *H. pylori*.[22] As discussed above, the bacteria's effects on the gastric mucosa affect the ecological niches in the stomach, which allow the colonization of other bacteria. *H. pylori* are fastidious, microaerophilic bacteria, which have influenced earlier reports utilizing culture as the primary means of *H. pylori* identification.

Culture-Based Identification of Gastric Microbiota

Historically, the low intragastric pH (pH < 2) of the stomach was considered a barrier to gastric microbial colonization. As such, the stomach was historically considered a sterile organ, and the bacteria present were considered transient species. In a review prior to the discovery of *H. pylori*, the bacteria isolated from the stomach (at > 10^3 CFU/g) included Firmicutes (genera *Lactobacillus*, *Streptococcus*, *Clostridium*, and *Veillonella*), Actinobacteria (genus *Bifidobacterium*), and Proteobactearia (coliforms), and at a lower frequency other bacteria (Firmicutes (genera *Peptostreptococcus* and *Staphylococcus*), Bacteroidetes (genus *Bacteroides*), and Actinobacteria (genus *Actinobacillus*) and yeasts (*Candida* and others).[1] We have summarized several representative studies that use culture-based techniques to assess the microbiota in the stomach in **Table 1**. In the literature using culture methodologies, the most prevalent or abundant phylum, regardless of *H. pylori* status, is Firmicutes, followed by Proteobacteria and Bacteroidetes. Depending on the study, Actinobacteria may be the second or third most prevalent phylum. The most commonly found genera were *Streptococcus, Lactobacillus, Bacteroides,* coliforms, *Staphylococcus, Veilonella*, *Corynebactieum* and *Neisseria*, which may reflect both the interest of the investigators and what bacteria are more easily cultivable.[49,79,88-94] Comparing

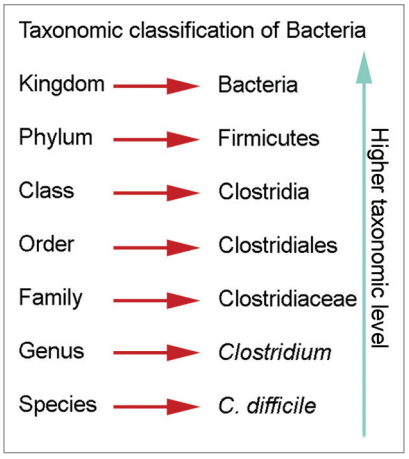

Figure 2. Taxonomic classification of bacteria. Descriptions of the gastric microbiota focus on the levels of phylum and genus.

studies that assess *H. pylori* status, *H. pylori* status did not alter the prevalence ranking with Firmicutes, Proteobacteria and Bacteroidetes being the top three phyla, when the quantification of *H. pylori* was not included.[49,79,88-94] A study that evaluated the effects of gastric cancer found increases in Proteobacteria along with Firmicutes (genera *Veilonella* and *Streptococcus*) and species from the *Bifidobacterium/Lactobacillus* group.[91] Of note, a study using culture and MALDI-TOF mass spectrometry was able to detect a Proteobacteria called *Acinetobacter lwoffii*,[79] which experimentally caused gastritis and hypergastrinemia in mice.[95] These findings suggest that non-*H. pylori* species can promote chronic inflammatory conditions. It is interesting that the literature does have examples of culturable organisms, but the scientific community refused to accept the presence and importance of gastric microbiota. The culture studies also demonstrated the fastidiousness of *H. pylori* and the limitations of culture, as more recent studies, have found that Proteobacteria are the dominant phylum in *H. pylori* infected subjects due to the high levels of gastric *H. pylori*.[9,10]

16s rRNA Based Identification of the Human Gastric Microbiota

Culture-independent studies use a variety of molecular methods (**Table 2**). Of the eight studies reviewed, four different molecular methods were used to survey the human gastric microbiota based on the analysis of a gastric biopsy sample. Three studies utilized NGS technologies,[10,80,96] two studies used Sanger sequencing of a 16S rDNA library,[8,9] two studies used a community fingerprinting method to define a library for Sanger sequencing,[74,75] and one study utilized a PhyloChip.[82] There is considerable variation in the gastric microbiome between individuals at the genus level, and perhaps future standardization of technologies to survey the gastric microbiota will facilitate more robust comparisons.

In the studies surveyed, the most prominent phyla commonly detected in the stomach are Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria

Table 1. Studies analyzing the human gastric microbiota using culture-based methods

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera observed	Genus information	Clinical presentation	Comments
Savage 1977	N/A	N/A	N/A	N/A	Various	N/A	Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and yeasts	9+	Firmicutes (*Lactobacillus*), Firmicutes (*Streptococcus*), Actinobacteria (*Bifidobacterium*), Firmicutes (*Clostridium*), Firmicutes (*Veillonella*), Proteobacteria (Coliforms), other bacteria (Firmicutes (*Peptostreptococcus, Staphylococcus*) Bacteroidetes (*Bacteroides*), and Actinobacteria (*Actinobacillus*)) and yeasts	N/A	Review
Sharma et al. 1984	Gastric juice culture	10	19–26 (mean 22.6)	Male	England	N/A	Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria	9	Actinobacteria (*Corynebacterium*), Firmicutes (*Staphylococcus*), Proteobacteria (*Neisseria*), Firmicutes (*Lactobacillus, Veilonella, Streptococcus,Bacillus*), Bacteroidetes (*Bacteroides*), Proteobacteria (*Acinetobacter*)	Omeprazole daily for 2 weeks on healthy patients	OMP increased bacterial load and nitrite levels but returned to normal after Tx discontinued
Stock-bruegger 1985	N/A	N/A	N/A	N/A	Various	N/A	Firmicutes, Proteobacteria, Bacteroidetes	7	Proteobacteria (Enterobacteriaceae), Firmicutes (Enterococcus), Firmicutes (Streptococcus), Firmicutes (Staphylococcus), Firmicutes (Lactobacillus), Firmicutes (Clostridium), Bacteroidetes (Bacteroides)	Hypochlorhydria	Review
Sjostedt et al. 1985	Gastric juice culture	10	Pool: 28–74	Pool: 39 males, 21 females	Sweden	N/A	Firmicutes, Proteobacteria, Actinobacteria	6	Firmicutes (*Staphylococcus*), Proteobacteria (*Neisseria*), Firmicutes (*Streptococcus*), Actinobacteria/Firmicutes (*Bifidobacterium/Lactobacillus*), Firmicutes (*Veilonella*), Bacteroidetes *(Bacteroides)*	Healthy	Only 1–4 of 10 patients had cultures of each
	Gastric juice culture	10	Pool: 28–74	Pool: 39 males, 21 females	Sweden	N/A	Proteobacteria, Firmicutes, Actinobacteria	9	Firmicutes (*Streptococcus*), Actinobacteria/Firmicutes (*Bifidobacterium/Lactobacillus*) and Firmicutes (*Veilonella*). Also observed Proteobacteria (*Klebsiella, Escherichia, Pseudomonas*), Firmicutes (*Bacillus*), Candida spp	Gastric cancer	2–6 of 10 patients had cultures of each

*N/A, not available; Pos., positive; Neg., negative; #, underlined values are altered by HP infection compared to negative controls

Table 1. Studies analyzing the human gastric microbiota using culture-based methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera observed	Genus information	Clinical presentation	Comments
Thorens et al. 1996	Gastric juice culture	37	20–60 (mean 42)	39 males, 8 females	Switzerland	N/A	Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes	11	Firmicutes (*Streptococcus*), Actinobacteria (*Corynebacterium*), Proteobacteria (*Neisseria*), Actinobacteria (*Micrococcus*), Bacteroidetes (*Bacteroides*), Firmicutes (*Staphylococcus*), Proteobacteria (*Escherichia coli*), Proteobacteria (*Klebsiella*), Actinobacteria (*Rothia* (previously Stomatococcus)), Firmicutes (*Enterococcus, Lactobacillus*)	Patients with routine endoscopy with Omeprazole and Cimetidine	Bacterial overgrowth on acid suppression. Oral and intestinal microbes seen
Adamsson et al. 1999	Culture - Biochemistry, Gas chromatography	30	35–75 (mean 58.5)	14 males, 16 females	Sweden	Pos.	Firmicutes (58%), Proteobacteria (22.2%), Actinobacteria (14.3%), Bacteroidetes (3.8%) and Fusobacterium (1.5%).	12 to 13	Firmicutes (*Streptococcus*), Proteobacteria (*Neisseria*), Proteobacteria (*Haemophilus*), Firmicutes (*Staphylococcus*), Firmicutes (*Peptostreptococcus*), Firmicutes (*Veilonella*), Actinobacteria (*Actinomyces*), Actinobacteria (*Micrococcus*), Bacteroidetes (*Prevotella-Bacteroides*), Proteobacteria (*Enterobacteriaceae*), Yeasts, Firmicutes / Actinobacteria (*Lactobacillus and Bifidobacterium*), Fusobacteria (*Fusobacterium*)	Non-ulcer dyspepsia treated with anti-HP triple therapies (omeprazole, metronidazole and either amoxicillin or clarithromycin)	Data presented prior to antibiotics. HP eradicated.
Mowat et al. 2000	Gastric juice culture	18	HP neg 20–45 (mean 27)	N/A	Scotland	Neg.	Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria	11	Firmicutes (*Streptococcus*), Firmicutes (*Staphylococcus*), Proteobacteria (*Neisseria*), Firmicutes (*Lactobacillus*), Proteobacteria (*Haemophilus*), Bacteroidetes (*Bacteroides*), Firmicutes (*Clostridium*), Firmicutes (*Veilonella*), Proteobacteria (Coliform), Actinobacteria (*Corynebacterium*), Proteobacteria (*Moraxella* (previously *Branhamella*)), Yeast	Healthy	Samples collected during omeprazole treatment (pH 3).

*N/A, not available; Pos., positive; Neg., negative; #, underlined values are altered by HP infection compared to negative controls

Table 1. Studies analyzing the human gastric microbiota using culture-based methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera observed	Genus information	Clinical presentation	Comments
	Gastric juice culture	11	HP pos 21–45 (mean 29)	N/A	Scotland	Pos.	Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria. HP decreased Firmicutes and increases in Proteobacteria/ Bacteroidetes	11	Firmicutes (*Streptococcus*), Proteobacteria (*Neisseria*), Firmicutes (*Staphylococcus*), Bacteroidetes (*Bacteroides*), Firmicutes (*Veilonella*), Proteobacteria (*Haemophilus*), Firmicutes (*Clostridium*), Proteobacteria (HP), Proteobacteria (*Moraxella* (previously *Branhamella*)), Firmicutes (*Lactobacillus*), Actinobacteria (*Corynebacterium*).#	Healthy	Samples collected during omeprazole treatment (pH 8). Nitrosating bacteria found. HP presence was not high.
Kato et al. 2006	Biopsy, Gastric juice culture with qPCR	10	9 to 16	3 males, 7 females	Japan	Pos.	Firmicutes, Actinobacteria, Bacteroidetes	5	Firmicutes (*Lactobacillus*), Actinobacteria (*Bifidobacterium*), Firmicutes (*Eubacterium*), Bacteroidetes (*Bacteroides*), Firmicutes (*Faecalibacterium* (previously *Fusobacteria*) *prausnitzii*)	Children (gastritis, duodenal ulcer, reflux esophagitis, non-ulcer dyspepsia).	Did not culture HP. Only recovered bacteria from 2 children
	Biopsy, Gastric juice culture with qPCR	10	9 to 16	3 males, 7 females	Japan	Neg.		1	Firmicutes (*Lactobacillus*)		Did not culture HP. Only recovered from 1 child with highest pH
	Biopsy, Gastric juice culture with qPCR	10	33–79	4 males, 6 females	Japan	Pos.	Firmicutes, Proteobacteria, Bacteroidetes	9	Firmicutes (*Streptococcus*), Firmicutes (*Bacillus*), Firmicutes (*Veilonella*), Proteobacteria (*Neisseria*), Firmicutes (*Lactobacillus*), Bacteroidetes (*Bacteroides*), Firmicutes (*Peptostreptococcus*), Firmicutes (Staphylococcus)	Adults (gastritis, gastric ulcer, early gastric cancer, gastric adenoma, non-ulcer dyspepsia)	Did not culture HP. Higher bacterial load (100 fold) correlated with higher pH
	Biopsy, Gastric juice culture with qPCR	10	33–79	4 males, 6 females	Japan	Neg.	Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria	9	Firmicutes (*Bacillus*), Firmicutes (*Streptococcus*), Firmicutes (*Staphylococcus*), Proteobacteria (*Neisseria*), Firmicutes (*Veilonella*), Bacteroidetes (*Bacteroides*), Fusobacteria (*Fusobacterium*)	Adults (gastritis, gastric ulcer, early gastric cancer, gastric adenoma, non-ulcer dyspepsia)	Did not culture HP

*N/A, not available; Pos., positive; Neg., negative; #, underlined values are altered by HP infection compared to negative controls

Table 1. Studies analyzing the human gastric microbiota using culture-based methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera observed	Genus information	Clinical presentation	Comments
Zilberstein et al. 2007	Biopsy, Culture	20	Mean age 42	8 males, 12 females	Brazil	N/A	Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, Bacteroidetes	18	Firmicutes (*Lactobacillus*) (41.6%), Firmicutes (*Veilonella*) (41.6%), Firmicutes (*Clostridium*) (33.3%), Actinobacteria (*Corynebacterium)* (25%), Proteobacteria (*Escherichia*) (16%), Bacteroidetes (*Bacteroides*) (16%), Proteobacteria (*Klebsiella*) (16%), Firmicutes (*Peptococcus)* (16%). Other genera observed were Fusobacteria *(Fusobacterium*), Actinobacteria *(Propionibacterium*), Firmicutes *(Peptostreptococcus, Staphylococcus,* and *Streptococcus*), Proteobacteria *(Proteus* and *Enterobacter*) and yeast	Healthy	Found acid resistant strains. Proteobacteria likely from lower bowel
Hu et al. 2012	Biopsy, Culture + MALDI-TOF mass spectrometry	67 samples from 103 patients	81 patients < 50 y old	51 males, 52 females	China	Pos.	Firmicutes, Proteobacteria, Actinobacteria		Proteobacteria (*Neisseria*), Firmicutes (*Streptococcus)*, Actinobacteria (*Rothia*), Firmicutes (*Staphylococcus*)	Upper GI diseases (duodenal ulcers, gastric ulcers, gastritis, dyspepsia, esophagitis)	Detected *Acinetobacter lwoffi*
Delgado et al. 2013	Biopsy, Culture	12	Mean age 60.5	6 males, 6 females	Spain	Neg.	Firmicutes, Actinobacteria, Proteobacteria	9	Actinobacteria (*Propionibacterium*), Firmicutes (*Lactobacillus*), Firmicutes (*Staphylococcus*)	Dyspeptic without PPI	*P. acnes* was surprising but it has shown in other studies (Monstein et al.)

*N/A, not available; Pos., positive; Neg., negative; #, underlined values are altered by HP infection compared to negative controls

Table 2. Studies analyzing the human gastric microbiota using 16S rRNA identification methods

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera (OTUs) observed	Genus information	Clinical status	Comments
Monstein et al. 2000	Biopsy/TTGE/ Sanger-sequenced 16S rDNA library	13 of 22	Pool: male (44–79), female (43–76)	Pool: 10 males, 12 females	Sweden	Pos.	Proteobacteria, Firmicutes, Actinobacteria	11	Proteobacteria (*Pseudomonas*), Firmicutes (*Enterococcus*, *Staphylococcus*, *Streptococcus*), and Actinobacteria (*Rothia* (prev. *Stomatococcus*)). Low abundance Proteobacteria (*Acinetobacter, Brevundimonas, Enterobacter, Helicobacter* (non HP), and *Rhizobium*) and Actinobacteria (*Propionibacterium*)	No gastritis(5) or no clinical gastritis (8)	Gastritis increased HP numbers and reduced detection of other bacteria. Detected other Helicobacters
Bik et al. 2006	Biopsy/Sanger-sequenced 16S rDNA library	23	42–78	22 males, 1 female	USA (13 Caucasian, 5 Hispanic, 5 African-American)	12 of 23 Pos., but 7 of 11 Neg. had counts of HP	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria	128	HP = 72% of reads in Pos. patients, but still 11% in Neg. patients. Non-HP genera in the overall library were Firmicutes (*Streptococcus, Veilonella*), Bacteroidetes (*Prevotella*), Actinobacteria (*Rothia*), Fusobacteria (*Fusobacterium*)	Medically indicated gastroscopies. 8/23 on acid blockers. pH range from 2–7	Only detected *H. pylori* from genus Helicobacter. *H. pylori* is dominant but does not affect underlying structure and diversity. HP samples had relative lack of Bacteroidetes phylotypes
Andersson et al. 2008	Biopsy/454 pyrosequencing of V6 of 16S rRNA amplicons	3	61–76	N/A	Sweden	Neg.	Actinobacteria (46.8%), Firmicutes (29.6%), Bacteroidetes (11.1%), Proteobacteria (10.8%), Fusobacteria (1.1%)	262	Firmicutes (*Streptococcus, Gemella*), Actinobacteria (*Actinomyces*), Bacteroidetes (*Prevotella*)	Healthy	Only 33 of 262 seen in all three Neg. samples. *H. pylori* reduced diversity. Found many throat organisms, so may be transients. Non-throat organisms were Proteobacteria

*N/A, Not available, Pos., positive, Neg., negative, Unk., unknown *H. pylori* status

Table 2. Studies analyzing the human gastric microbiota using 16S rRNA identification methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera (OTUs) observed	Genus information	Clinical status	Comments
		3	61–76	N/A	Sweden	Pos.	Proteobacteria (96%), Firmicutes (1.8%), Actinobacteria (1.1%), Bacteroidetes (0.8%) and Fusobacteria (0.1%)	33	HP 93–97%	Healthy	HP dominates with 93–97% of reads
Dicksved et al. 2009	Biopsy/T-RFLP/Sanger-sequenced 16S rRNA library	10	52–88	8 males, 2 females	Sweden	8 of 10	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria	102	Firmicutes (*Streptococcus, Lactobacillus* and *Veilonella*) and Bacteroidetes:*Prevotella*	Gastric cancer (intestinal- and diffuse-type)	Low abundance of HP. Did not find disease related association from clustering of T-RFLP data from dyspepsia and GC samples
	Biopsy/T-RFLP	5	52–88	2 males, 3 females	Sweden	0 of 5	Firmicutes, Bacteroidetes, Actinobacteria		Firmicutes (*Veilonella, Streptococcus*) and Bacteroidetes (Prevotella)	Dyspepsia	
Li et al. 2009	Biopsy/Sanger-sequenced 16S rDNA library	5	Pool - 40–87	Female	Hong Kong	Neg.	Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Fusobacteria	133	Proteobacteria (*Neisseria*), Bacteroidetes (*Prevotella*), Firmicutes (*Streptococcus*), *Proteobacteria (Haemophilus), Bacteroidetes (Porphyromonas)*	Asymptomatic	Found 1–2 dominant genera per phyla. *Neisseria* and *Haemophilus* higher in asymptomatic patients
		5	Pool - 40–87	Female	Hong Kong	Neg.	Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria	133	Firmicutes (*Streptococcus*), Bacteroidetes (*Prevotella*), Proteobacteria (*Neisseria, Haemophilus*), Bacteroidetes (*Porphyromonas*)	Gastritis	*Streptococcus* higher in gastritis. *Streptococcus* and *Prevotella* levels correspond with Bik et al.

*N/A, Not available, Pos., positive, Neg., negative, Unk., unknown *H. pylori* status

Table 2. Studies analyzing the human gastric microbiota using 16S rRNA identification methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera (OTUs) observed	Genus information	Clinical status	Comments
Maldonado-Contreras et al. 2010	Biopsy/ PhyloChip	4	37–80	2 males, 2 females	4 Amerindians	Neg.	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes	154 (family)		Gastritis	
		8	21–59	4 males, 4 females	6 Amerindians, 2 USA (South Asia and Africa)	Pos.	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes	137 (family)		Dyspepsia, GERD, Gastritis	No differences on taxonomic complexity but HP increases relative abundance of non-Helicobacter bacteria from Proteobacteria, Spirochetes, and Acidobacteria. Decreased abundance of Actinobacteria, Bacteroidetes and Firmicutes.
Stearns et al. 2011	Biopsy/V3 16S rRNA sequencing (Sanger and Illumina) (data for males and females)	4	> 50	2 males, 2 females	Canada	Unk.	Overall antrum Proteobacteria (50.66%), Firmicutes (35.7%), Bacteroidetes (13.47%). Overall corpus Firmicutes (69.0%), Proteobacteria (16.7%), Fusobacteria (8.1%)	23 antrum 24 body		Healthy undergoing gastroscopy	High variability between subjects and between antrum and corpus. Data hard to interpret as they only report phyla and genera present in all 4 samples.

*N/A, Not available, Pos., positive, Neg., negative, Unk., unknown *H. pylori* status

Table 2. Studies analyzing the human gastric microbiota using 16S rRNA identification methods (continued)

Study	Method	# of samples	Age range	Gender	Ethnicity or Location	HP status	Phyla	Genera (OTUs) observed	Genus information	Clinical status	Comments
	Same data (only males)	2	> 50	Male	Canada	Unk.	Antrum: Male (avg) Proteobacteria 99.6%. Corpus Male 1 Proteobacteria (45.0%), Fusobacteria (22.1%), Firmicutes (16.0%), Bacteroidetes (14.9%) and SR1 (2.0%). Corpus Male 2 Firmicutes (*Parvimonas*) (99.44%)		Antrum: Males = 99% Proteobacteria - no genus data (HP?) Corpus: Male 1 = Firmicutes (*Parvimonas*) (99.4%), Male 2 = Proteobacteria (no data) (45%), Fusobacteria (no data) (22.1%), Firmicutes (*Streptococcus, Veilonella*, other) (16%), Bacteroidetes (*Prevotella, Porphyromonas*, other) (14.9%), SR1 (no data) (2%)	Healthy undergoing gastroscopy	n = 2 per site
	Same data (only females)	2	> 50	Female	Canada	Unk.	Female antrum: Firmicutes, Bacteroidetes. Female corpus: Firmicutes.		Antrum: Female = Firmicutes (Streptococcus) (72.6%) and Bacteroidetes (Prevotella) (27.3%). Corpus: Female = Firmicutes [*Veilonella, Streptococcus* and *Oribacterium*] (99.9%)	Healthy undergoing gastroscopy	n = 1 per site
Delgado et al. 2013	Biopsy/454 pyrosequencing of 16S rDNA amplicons	4 sequenced	Mean age 60.5	Pool: 6 males, 6 females	Spain	Neg.	Firmicutes, Proteobacteria, Actinobacteria with small proportions of Deinococcus-Thermus, Bacteroidetes and Gemmatimonadetes	19 core. 69 total	Firmicutes (*Streptococcus, Lactobacillus),* Actinobacteria (*Propionibacterium),* and Proteobacteria (*Methylobacterium)* were dominant. Dominant genera varied by sample.	dyspeptic without PPI	High variability between subjects

*N/A, Not available, Pos., positive, Neg., negative, Unk., unknown *H. pylori* status

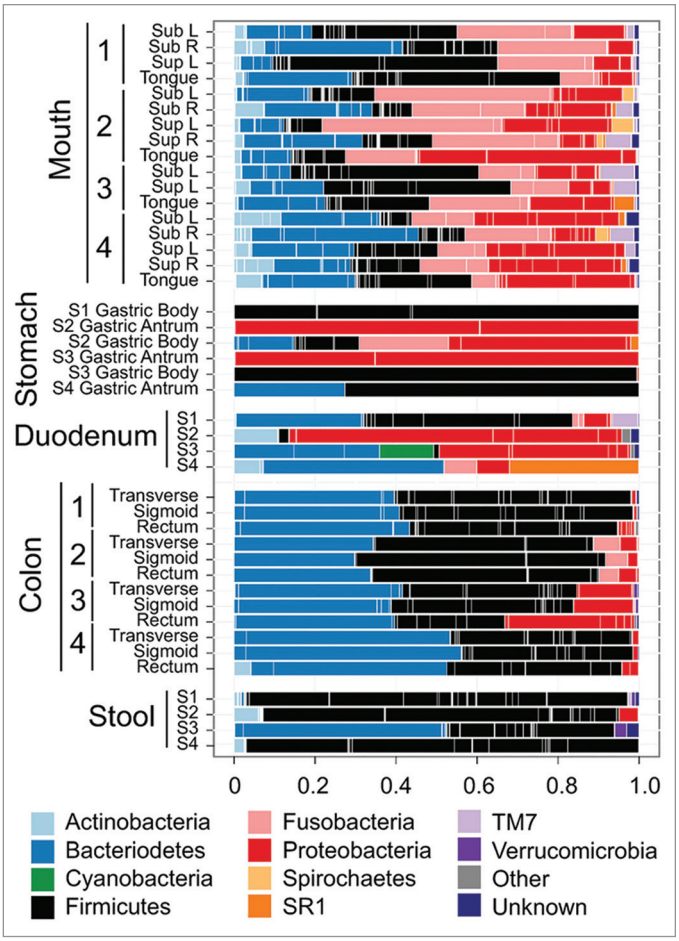

Figure 3. Human microbiota composition in multiple sites of the GI tract, including mouth, stomach, duodenum, colon and stool. Note the high variability between individuals and between the antrum and corpus in the stomach. The stomach microbiota also differs significantly from other sites in the GI tract. Reproduced with permission from Stearns et al. 2011.[96]

(**Table 2**).[9,10,74,75,80,82,96] The most abundant phyla in *H. pylori* positive subjects are Proteobacteria, Firmicutes and Actinobacteria. In the absence of *H. pylori*, the most abundant phyla are Firmicutes, Bacteroidetes and Actinobacteria. In humans, *H. pylori* are by far the most dominant species in the stomach, comprising 72 to 99% of sequencing reads.[9,10] In the absence of *H. pylori*, analysis of the known *H. pylori* negative subjects consistently shows the presence of *Streptococcus* spp, which seems to be the most abundant genus in these subjects.[8,75,80,96] In the gastric microbiota, the non-Helicobacter genera commonly detected are *Streptococcus*, *Prevotella*, *Veillonella*, and *Rothia*.

The effects of *H. pylori* on the gastric microbiota are not fully understood. Numbers of *H. pylori* increase with the onset of gastritis,[74] which may reflect changes in the gastric niche that allow *H. pylori* to outcompete other bacteria and increase *H. pylori* levels.[97] While *H. pylori* made up 72% of reads observed and decreased the overall diversity and evenness of the gastric microbiota, Bik et al. determined that the underlying diversity and richness is higher in *H. pylori* positive samples than *H. pylori* negative samples when *H. pylori* reads are removed.[9] However, the authors did note a relative lack of Bacteroidetes in *H. pylori* infected patients.[9] Other studies disagree, finding a strong effect of *H. pylori* on the composition of the gastric microbiota.[10,82] In one study, *H. pylori* accounted for 93–97% of all reads in the infected stomach, and substantially decreased the diversity as only 33 phylotypes were observed in *H. pylori* positive individuals while 262 phylotypes were observed in *H. pylori* negative subjects.[10] In a separate study, PhyloChip data was analyzed using non-metric multidimensional scaling, and demonstrated that *H. pylori* infection accounted for 28% of the variation seen in the analysis. This was despite the fact that no differences in taxonomic complexity were seen in terms of abundance of different phyla or the numbers of families identified.[82] However, the authors determined that *H. pylori* infection increased the relative abundance of Proteobacteria (non-Helicobacter bacteria), Spirochaetes and Acidobacteria and decreased the relative abundance of Actinobacteria, Bacteroidetes and Firmicutes when compared with *H. pylori* negative individuals.[82]

The effects of the absence of *H. pylori* on the microbiota has also been the focus of other studies.[8,80] An important consideration when evaluating this literature is to note that multiple studies report the ability to detect *H. pylori* sequences at extremely low levels in subject who were *H. pylori* negative by other diagnostic means.[9,74,80,82] This may reflect a host response that led to significant reduction of *H. pylori* or the presence of non-*H. pylori* helicobacters.[22] Li et al. sequenced 16S rRNA clones from *H. pylori*-uninfected patients with gastritis and without gastritis.[8] Asymptomatic patients had a much lower level of Firmicutes (genus *Streptococcus*) but instead had a higher proportion of Proteobacteria (genera *Neisseria* and *Haemophilus*) similar to Monstein et al. who observed more Proteobacteria (genus *Pseudomonas*) in asymptomatic *H. pylori* infected patients.[8,74] Their results share similarity with the Bik et al. study;[9] both groups found that *Streptococcus* spp and *Prevotella* spp accounted for ~40% of reads in *H. pylori*-uninfected subjects presenting with gastric disease.[8] A second study focusing on *H. pylori*-negative subjects demonstrated that the gastric microbiota is extremely variable at lower taxonomic levels; the four subjects sampled shared the same ranking in terms of phyla (Firmicutes, Proteobacteria and Actinobacteria, from most abundant to least). But upon closer inspection, the four subjects had different abundances of each phylum, being more evident at the genus level.[80] While the study identified 69 different genera, a core set of 19 was observed in all four samples with Firmicutes (genus *Streptococcus*), Actinobacteria *(genus Propionibacterium*), Firmicutes (genus *Lactobacillus*) and Proteobacteria (genus *Methylobacterium*) being of importance, in spite of the fact that the dominant genus and proportions varied from sample to sample.[80] The authors also compared their sequencing results from the results of bacterial culture from the same gastric samples and found robust concordance as the four dominant cultivable genera were *Propionibacterium*, *Lactobacillus*, *Streptococcus,* and *Staphylococcus*. While three of the four genera match the sequenced data, the inclusion of *Staphylococcus,* which was not a strong contributor to the sequencing data, reflects bias toward cultivable organisms.

Regarding the uniformity of the microbiota within the stomach, Bik et al. and Li et al. found no differences in the microbiota of the antrum and corpus in their populations, with the exception of decreased *Prevotella* in the antrum of gastritis patients by Li et al.[8,9] In contrast, Stearns et al. documented anatomical differences between subjects and between the antrum and corpus (**Fig. 3**).[96] The antrum was dominated by Proteobacteria, Firmicutes and Bacteroidetes while the corpus microbiota was predominantly Firmicutes, Proteobacteria, Fusobacteria and Bacteroidetes. The focus of the study surveyed the bacterial microbiota along the GI tract, and unfortunately, did not assess stomach data in depth. In their effort to find commonality in the microbiota of the four subjects, the authors have focused on the genera shared between subjects and omitted useful information from supplemental tables. For example looking at the phyla represented in the three antral samples, it is evident that the two male samples were composed of >99% of Proteobacteria, while the single female sample did not have significant amounts of Proteobacteria, as the microbiota was composed of Firmicutes (72.6%) and Bacteroidetes (27.3%) (**Fig. 3**).[96] Hence the representation of a Proteobacteria dominated antrum is misleading. As *Streptococcus* and *Prevotella* were present in all four samples, it was possible to determine that these constitute the two most abundant genera in the female antrum, as seen in other *H. pylori* negative samples in other studies.[8,9] However, the sequence reads corresponding to *Helicobacter* spp were not reported, and it is not possible to determine whether *Helicobacter* spp were in fact the dominant species in the antrum of the male subjects, as noted in other studies.[9,10] Another interesting finding is that Proteobacteria were the most abundant phyla in the corpus of one of the two male subjects, while the corpus of the other male subject is exclusively colonized by Firmicutes of the genus *Parvimonas*.[96] Unfortunately, the sample size is too small and the inter individual variability is too high to determine if there are effects due to gender in this study. Dicksved et al. explored the effect of gastric cancer on *H. pylori* and the gastric microbiota.[75] The authors found few differences in the microbiota of 10 gastric cancer patients and five *H. pylori*-negative dyspeptic controls.[75] While 8 of 10 gastric cancer patients were *H. pylori* positive, the abundances of *H. pylori* were very low, perhaps reflecting the changes in the gastric niche that occur with gastric cancer.[75] The altered stomach was colonized by multiple *Streptococcus* spp, including *S. mitis, S. parasanguinis* and *S. bovis* (currently *S. infantarius* which has been associated with colorectal cancer[98]). However, this study relied on T-RFLP to determine the microbiota, which lacks the resolution to determine subtle shifts in abundance or species composition that may influence gastric pathogenesis.[75] Currently studies are lacking that systematically evaluate the gastric microbiota in clinically defined populations to enable distinguishing differences in microbial numbers or diversity related to atrophy, intestinal metaplasia and gastric cancer (intestinal- vs. diffuse-type cancers). It is noteworthy that three studies found a relationship between increased *Streptococcus* spp and gastric disease.[9,75,82]

While it has been conjectured that the indigenous microbiota might be a reflection of transient bacteria from the mouth and esophagus, three separate studies demonstrated that in spite of high inter-subject variability, the gastric microbiota were distinguishable from microbiota found in the mouth, nose, and distal GI tract.[10,80,96] Comparing the general trends observed in this review with data from other similar sites, the human gastric microbiota is different from the microbiota of the oropharynx,[99] but in the absence of *H. pylori*, the structure and composition most resembles the microbiota reported for the distal esophagus with unique differences due to the makeup of Proteobacteria.[10,100]

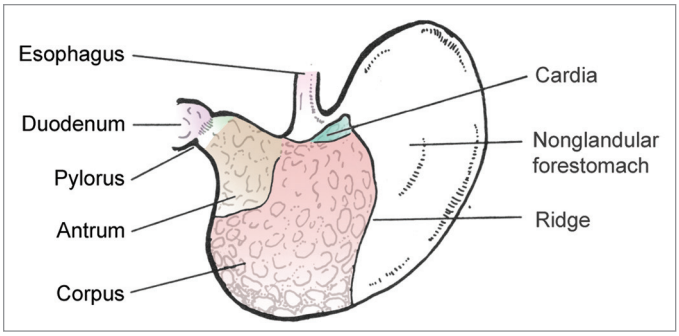

Figure 4. Illustration depicting anatomy of the mouse stomach. The anatomy of the gerbil stomach is similar. The nonglandular forestomach is the site of dense colonization by lactobacilli, which substantially contribute to the differences in the gastric microbiota of humans and rodents.

Gastric Microbiota in Mice and Mongolian Gerbils

When considering the gastric microbiota of rodents in the context of *H. pylori*-induced disease, it is important to recognize several key differences: (1) *H. pylori* is not an autochthonous member of the microbiota and mouse-adapted strains are needed to infect the mouse,[101-103] (2) mice have relatively high intragastric pHs of 3–4,[71,104] while Mongolian gerbils have a pH <2[105], more similar to humans, (3) the gastric anatomy differs between humans and rodents, as there is a considerable non-glandular forestomach composed of squamous epithelium (**Fig. 4**),[106] and (4) transient bacteria in the stomach may be due to coprophagia, which is common in mice but not in Mongolian gerbils.[106] Using culture methods, it has been noted that there is a relatively simple, but indigenous, gastric microbiota in rats and mice, consisting of mainly of Firmicutes (genera *Lactobacillus, Streptococcus, Clostridium, Veilonella*), coliforms from Proteobacteria, anaerobic bacteria like *Bifidobacterium* spp and yeasts.[1,107]

Mice

Using diverse sampling methods, mouse strain backgrounds and vendor sources, the normal gastric microbiota has been shown to be predominantly dominated by Firmicutes (genus *Lactobacillus*) (**Table 3**).[71,77,103,104] Using culture and T-RFLP analysis, *Lactobacillus* represented >99% of the bacteria in the stomach, with the presence of the remaining bacteria (Proteobacteria (genera *Escherichia, Moraxella, Pasteurella, Enterobacter,* and *Actinobacillus*), Firmicutes (genera *Staphylococcus* and *Enterococcus*), and Actinobacteria (genus *Micrococcus*)) at <1%).[103,104] Other studies have *Lactobacillus* spp as the most abundant genus in the gastric microbiota, but detect significant contributions (35–45%) from other bacterial phyla.[71,77]

Table 3. Gastric microbiota studies in mice

Study	Method	# of Samples	Age	Sex	Species	HP status	Phyla (%)	Genus information	HP effect
Kabir et al. 1997	Culture	3	5 wk-old + up to 9 wk infection	Male	BALB/c mice (Japan)	HP infected/no colonization	Firmicutes (99.9%), Proteobacteria (< 0.01%)	Firmicutes (*Lactobacillus*) = 99.9% and Firmicutes (*Enterococcus* and *Staphylococcus*) and Proteobacteria (Enterobacter) = < 0.01%	Blocked by high number of *Lactobacillus* spp
Aebischer et al. 2006	Dot-blot hybridization, then Sanger-sequenced 16S rRNA library	1	6–8 wk old + 2 mo	Female	BALB/c (Germany)	Uninfected		60–90% *Lactobacillus*. 10–40% - other.	
		1	6–8 wk old + 2 mo infection	Female	BALB/c (Germany)	HP P76 infected	Firmicutes, Proteobacteria, Bacteroidetes	Levels shift from uninfected	HP increases Firmicutes (*Clostridium, Eubacterium, Ruminococcus* and *Streptococcus*), Bacteroidetes (*Bacteroides/Prevotella*), Proteobacteria (*Escherichia*). Decreases *Lactobaciillus.* Effect independent of pH/pathology
Tan et al. 2007	Culture and T-RFLP (two sets)	9–10 mice/ group	6–8 wk old + 0.5, 3 or 6 mo	Female	C57BL/6 (Australia)	Uninfected		Firmicutes (*Lactobacillus*) = *99.9%*	
		9–10 mice/ group	6–8 wk old + 0.5, 3 or 6 mo infection	Female	C57BL/6 (Australia)	HP SS1 infected		No change from uninfected	Cultured Proteobacteria (*E. coli, Moraxella osloensis, Actinobacillus muris, Pasteurella spp.*) and Firmicutes(*Enterococcus faecalis, Lactobacillus spp*)
Lofgren et al. 2011	454 pyrosequencing of 16S rDNA amplicons	2	6 wk old + 15 wk	Male	INS-GAS FVB/N (USA)	Uninfected	Firmicutes (55%), Bacteroidetes (25%), Cyanobacteria (15%), Proteobacteria and other (5%)		No *H. pylori* sequences detected. Observed 175 OTUs
		3	6 wk old + 15 wk infection	Male	INS-GAS FVB/N (USA)	HP SS1 infected	Firmicutes (90%), Proteobacteria, Cyanobacteria, Bacteroidetes, and Tenericutes (10%)	Firmicutes (*Lactobacillus*) dominant	Low *H. pylori* reads. Increases OTUs in stomach, cecum and colon. Higher pathology. Observed 235 OTUs

*N/A, Not available

Table 3. Gastric microbiota studies in mice (continued)

Study	Method	# of Samples	Age	Sex	Species	HP status	Phyla (%)	Genus information	HP effect
Rolig et al. 2013	PhyloChip	5	7 wk old + 4 wk	Female	C57BL/6 (Taconic Farms/USA)	Uninfected	Firmicutes (73.4%), Bacteroidetes (11.8%) Verrucomicrobia (8.5%), Proteobacteria (3.2%), Actinobacteria (1.9%) (% of species)	Firmicutes (class Clostridia) 44%	
		4	7 wk old + 4 wk infection	Female	C57BL/6 (Taconic Farms/USA)	HP SS1 infected			Increases Firmicutes (class Clostridia), Proteobacteria (*Helicobacter*), Verrumicrobia. Decreased Firmicutes (class Bacilli), Bacteroidetes, Proteobacteria
Lertpiriyapong et al. 2013	qPCR for 3 ASF species	27	7–11 wk old + 7 mo	13 males, 9 females	INS-GAS FVB/N (USA)	Uninfected	Firmicutes, Bacteroidetes	3 restricted ASF species	
		22	7–11 wk old + 7 mo infection	15 males, 12 females	INS-GAS FVB/N (USA)	HP SS1 infected	Firmicutes, Bacteroidetes, Proteobacteria	3 ASF species + *H. pylori*	Increased Firmicutes (*Lactobacillus*) and decreased Firmicutes (*Clostridium*) and Bacteroidetes (*Bacteroides*). *H. pylori* = 0.55% of bacteria in all mice. Increased pathology ~increased bacterial load

*N/A, Not available

However, while differences in levels of *Lactobacillus* spp prompted further investigation into the gastric microbiota, Rolig et al. found that the dominant phyla Firmicutes (74% of reads) was mainly composed of the class Clostridia (44% of reads) and not *Lactobacillus* spp in their control mice.[76] In studies where *Lactobacillus* spp did not compose >99% of the stomach microbiome, Bacteroidetes was the second most abundant phylum, and significant contributions were made by Cyanobacteria, Verrumicrobia, Proteobacteria and Actinobacteria[76,77] The variability in results was highlighted by Rolig et al., who showed that C57BL/6 mice from different vendors had different levels of two different *Lactobacillus* spp and had different responses to *H. pylori* infection, highlighting the importance of husbandry and the environment on the gastrointestinal microbiota profile.[76] Another possible explanation for the reported variability of *Lactobacillus* spp levels in the stomach is the inclusion of the squamous epithelium forestomach during sectioning of the stomach. In our studies, the squamous forestomach, which plays a limited role in *H. pylori*-induced pathogenesis, is routinely removed during necropsy.[72,77] However, it has been noted that the squamous epithelium is the primary site of colonization of lactobacilli.[108] Further standardization of methodologies is required to compare equivalent data.

Studies have begun to highlight the diversity in the gastric microbiota of mice and their interactions with *H. pylori* and its associated immunopathology. The difficulty in establishing infection in mice[101,103] and the low levels of *H. pylori* in the mouse stomach[72,77] reflect that the bacteria are not autochthonous to the mouse. Kabir et al. studied the effect of the gastric microbiota on *H. pylori* infection and found that multiple strains of *H. pylori* could colonize germ-free (GF) BALB/c mice, but failed to colonize specific-pathogen free (SPF) BALB/c mice when the predominant gastric bacteria were *Lactobacillus* spp.[103] Coinfection of *H. pylori* and *L. salivarius* of GF mice demonstrated that *L. salivarius* alone prevented *H. pylori* colonization of the mouse stomach.[103] This result is similar to the clearance of *H. felis* from SPF C57BL/6 mice, where competition from *Lactobacillus* spp invading the gastric niche was postulated to have contributed to the eradication of *H. felis*.[109]

When infection is achieved, *H. pylori* represent 10–30% of the microbiota in the absence of significant pathology and <5% of the bacteria in stomachs with significant disease.[72,77] The decreasing levels highlights an inverse correlation between *H. pylori* levels and the degree of gastric pathology that is commonly observed in mice.[110] In spite of a relatively small contribution in numbers, *H. pylori* exerts strong effects on the microbiota and overall health of the murine stomach. Using mouse-adapted *H. pylori* SS1, Tan et al. were able to infect C57BL/6 with a *Lactobacillus*-dominated gastric microbiota (> 99%).[104] While the gastritis observed was mild, *H. pylori* were detected for the duration of the study and there was an increased gastric pH to 5, by 6 mo of infection. In spite of conditions associated with bacterial overgrowth, *H. pylori* infection did not cause shifts in bacterial

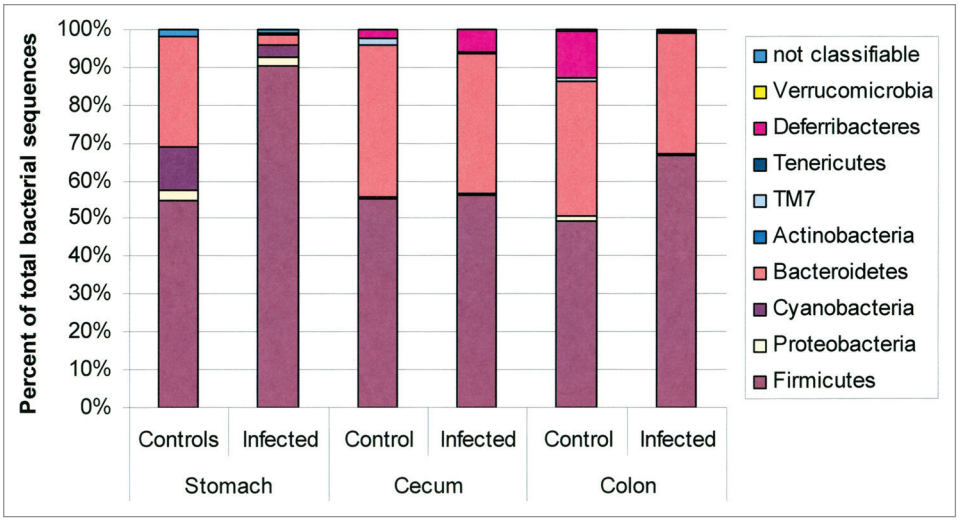

Figure 5. Microbiota composition in stomach, cecum, and colon of *H. pylori*-infected male INS-GAS mice (n = 3, 15 weeks postinfection) vs. uninfected controls (n = 2). Note the significant increase in the relative abundance of *Firmicutes* and decrease of *Bacteroidetes* in the stomachs of *H. pylori*–infected INS-GAS mice ($p < 0.05$), whereas no significant changes were observed in the colon and ceca of *H. pylori*-infected mice. Reproduced with permission from Lofgren et al. 2011.[77]

composition.[104] Rolig et al. also found that uninfected mice and mice with short-term *H. pylori* infection (4 wks) had little effect on the observed phyla (Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria, and Actinobacteria).[76] As seen in the Maldonado-Contreras et al. study,[82] analysis of the PhyloChip data detected specific taxa that varied with *H. pylori* infection. *H. pylori* caused increases in Firmicutes (class Clostridia), Proteobacteria (*Helicobacter hepaticus*) and Verrumicrobia, and was associated with decreases in Firmicutes (class Bacilli), Bacteroidetes and Proteobacteria.[76] More dramatic effects were observed using *H. pylori* P76 in BALB/c mice with a more diverse gastric microbiota.[71] *H. pylori* infected the stomach and increased the colonization of the stomach by lower bowel bacteria (Firmicutes (genera *Clostridia, Eubacterium, Ruminococcus* and *Streptococcus*), Bacteroidetes (genera *Bacteroides/Prevotella*), and Proteobacteria (genus *Escherichia*)), while a dramatic loss of *Lactobacillus* spp was observed (from > 60% in uninfected mice to 10–30% in infected mice). The shifts in the gastric microbiota were independent of significant changes in pH or pathology, implying that *H. pylori* infection may mediate initial alterations in the microbiota in a relatively healthy mouse stomach.[71] However, vaccination against *H. pylori* caused a 100-fold reduction in *H. pylori* levels and abrogated shifts in the stomach microbiome.[71]

While Aebischer et al.[71] and Rolig et al.[76] assessed microbiota changes following acute infections with *H. pylori*, other models are needed to evaluate the overall effects of long-term *H. pylori* infection and its associated histopathological changes on the composition of the stomach microbiota. Of the models reviewed, the hypergastrinemic INS-GAS mice present the most rapid progression to atrophy and gastrointestinal intraepithelial neoplasia (GIN) in response to *H. pylori*,[65,72,111,112] and presents an opportunity to observe changes in the microbiota associated with disease progression. Lofgren et al. demonstrated the importance of the microbiota in *H. pylori*-induced disease, as GF INS-GAS mice mono-associated with *H. pylori* had a delayed progression to neoplasia compared with SPF mice.[77] In uninfected SPF mice, the phyla Firmicutes and Bacteroidetes accounted for >80% of the represented bacteria with a large contribution of Cyanobacteria. *H. pylori* infection increased the percentage of Firmicutes to >90% with a large proportion of *Lactobacillus* spp, which greatly reduced the contribution of Bacteroidetes (**Fig. 5**). Nevertheless, *H. pylori* infection actually increased the number of OTUs detected (235 in infected vs. 175 in uninfected),[77] similar to increases in diversity observed in *H. pylori* infected humans.[10] However, *H. pylori* infection induced no changes in the relative abundance of phyla in the cecum or the colon when compared with uninfected controls.[77] Using a reductionist model consisting of 3 autochthonous murine bacteria, it was demonstrated that a simple gastric microbiota alone accelerated the progression to GIN compared with a GF mouse.[72] Inclusion of *H. pylori* further accelerated the development of GIN in the INS-GAS mice with a restricted Altered Schaedler's Flora (rASF). *H. pylori*-infected SPF INS-GAS mice and *H. pylori*-infected rASF mice developed GIN at similar rates, indicating that the 3 species (ASF356 (*Clostridium* spp), ASF361 (*Lactobacillus murinus*) and ASF519 (*Bacteroides* spp) were able to recapitulate the effects of a complex microbiota.[72] Including *H. pylori*, it was possible to track the concentrations of these 4 species using qPCR to gain insights into the colonization dynamics that might be associated with GIN development. The baseline composition in uninfected rASF was similar between male and female INS-GAS mice with Bacteroides species being dominant, followed by smaller, similar percentages of *Lactobacillus* and *Clostridium* species. As noted by Lofgren et al.,[77] *H. pylori* infection increased the percentage of *Lactobacillus* spp (from ~15% to 65% in males and from 25% to 95% in females). Consequently, decreases were observed in Clostridium (males 25% to 12% and females from 25% to <5%), and Bacteroides (males 60% to 20% and females 50% to <5%).[72] To test the value of the reductionist model, the authors used qPCR to track ASF species within the complex gastric microbiota in SPF males and females. In SPF INS-GAS mice, *H. pylori* infection decreased the levels of *Bacteroides* (ASF519) and *Clostridium* (ASF356), while increased levels were seen in two *Lactobacillus* spp (ASF360 and ASF361) and *Eubacterium plexicaudatum* (ASF492).[72]

Changes in *Lactobacillus* levels after *H. pylori* infection seem contradictory. Decreasing *Lactobacillus* levels in BALB/c and C57BL/6 mice acutely infected with *H. pylori*, but minimal gastric

Table 4. Gastric microbiota studies in Mongolian gerbils

Study	Method	# of Samples	Age	Sex	HP status	Phyla	Genus information	HP effect
Sun et al. 2003	TTGE/NGS-sequenced 16S rDNA library	5	5 wk old + 12 wk	Male	Uninfected	Firmicutes, Proteobacteria	Firmicutes (*Lactobacillus)* (82.4%), and Proteobacteria (*Pseudomonas)*, Proteobacteria (*Acinetobacter*), Firmicutes (*Bacillus*, *Enterococcus*, *Paenibacillus*) (17.6%)	No info on levels, just identification
		5	5 wk old + 12 wk infection	Male	HP ATCC 43504	Firmicutes, Proteobacteria, Actinobacteria	Firmicutes (*Lactobacillus)* (51.7%), Proteobacteria (*Helicobacter pylori)* (37.1%) and Proteobacteria (*Pseudomonas*, *Acinetobacter), Actinobacteria (*Corynebacterium), Firmicutes (*Enterococcus*, and *Staphylococcus*) (11.2%)	Severe chronic active gastritis
Zaman et al. 2010	Culture + API	1	5 wk old + 8 wk infection	Female	H pylori TK1042 (low detection)	Firmicutes, Proteobacteria, Actinobacteria	Firmicutes (*Lactobacillus*), Proteobacteria (*Escherichia, Kluyvera*), Actinobacteria (*Bifidobacterium, Actinomyces*)	Based on culture of single gerbil. Altered obligate anaerobe composition.
		1	5 wk old + 8 wk infection	Female	H pylori TK1402 (high detection)	Firmicutes, Proteobacteria, Actinobacteria	Firmicutes (*Eubacterium)*, Proteobacteria (*Escherichia, Kluyvera*), Actinobacteria (*Bifidobacterium, Actinomyces*)	
Yin et al. 2011	Culture	8	6–8 wk old + 12 wk	Male/ Female	Uninfected	Firmicutes, Actinobacteria	Firmicutes(*Lactobacillus*), Actinobacteria (*Bifidobacterium*)	
		3	6–8 wk old + 12 wk infection	Male/ Female	HP J99	Firmicutes, Actinobacteria, Bacteroidetes	Observed new species and shifts in microbiota	Observed Bacteroidetes (*Bacteroides*) and Firmicutes (*Enterococcus*). Trend of increasing Actinobacteria (*Bifidobacterium*). Increased gastritis
Osaki et al. 2012	qPCR	5	8 wk old + 1 y	Male	Uninfected	Firmicutes, Actinobacteria	Actinobacteria (*Atopobium* spp, *Bifidobacterium* spp), Firmicutes (*Clostridium coccoides*, *C. leptum*, *Enterococcus* spp and *Lactobacillus* spp)	
		5	8 wk old + 1 y infection	Male	HP positive	Firmicutes, Actinobacteria	Actinobacteria (*Atopobium* spp, *Bifidobacterium* spp), Firmicutes (*Clostridium coccoides*, *C. leptum*, *Enterococcus* spp and *Lactobacillus* spp)	vs. uninfected - lower *Clostridium coccoides*
		6	8 wk old + 1 y infection	Male	HP negative	Firmicutes, Actinobacteria, Bacteroidetes	Actinobacteria (*Atopobium, Bifidobacterium*), Firmicutes (*Clostridium, Enterococcus, Lactobacillus*, *Eubacterium*) and Bacteroidetes(*Prevotella*)	vs. uninfected -lower *Clostridium* and *Bifidobacterium*. Higher *Atopobium*

*N/A, Not available

pathology[71,76] contrast to the increasing *Lactobacillus* levels noted in INS-GAS FVB/n mice after long-term *H. pylori* infection associated with gastritis and atrophy.[72,77] While husbandry conditions and strain background are important factors to consider, the fluctuation in lactobacilli may reflect temporal dynamics dependent on pathology. Increased pathology with increasing gastric atrophy after long-term infection has been associated with higher gastric, bacterial levels.[72] Therefore, severe gastric lesions may facilitate colonization by lower bowel bacteria.

Another topic of interest is the role of antibiotics on *H. pylori*-induced disease and its effect on other gastric microbiota. Rolig et al. demonstrated that antibiotic treatment of mice altered the levels of 4400 OTUs in the stomachs of treated mice compared with untreated mice. These changes in the gastric microbiota reduced the severity of gastritis following subsequent *H. pylori* infection.[76] Similarly, antibiotic therapy combined with Sulindac significantly delayed the normal progression of gastric pathology observed in uninfected INS-GAS mice, which like Lofgren et al., demonstrates that other microorganisms, whether in the stomach or elsewhere, may contribute to the severity of gastritis.[77,113]

Mongolian gerbils

The Mongolian gerbil is a useful model of *H. pylori* pathogenesis. Favorable attributes of the model include a low intragastric pH,[105] and a male-predominant predisposition to increased susceptibility to progressive *H. pylori* gastritis.[66,78,114-119] Limitations of the model include lack of immunologic reagents and lack of availability of inbred strains. In spite of its importance in *H. pylori* research, there have been no NGS studies that directly assess the abundance and structure of the gastric microbiota in gerbils. The phyla commonly observed in current, published studies are Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes, with the first two being present in all studies (**Table 4**).[78,117-119] The gastric microbiota of uninfected Mongolian gerbils are dominated by the genus *Lactobacillus* similar to findings in mice.[117-119] Lactobacilli are prevalent in gerbil stomachs like the mouse, due to their colonization of the non-glandular forestomach.[108]

Three studies examined the effects of short, 8–12 wk *H. pylori* infections on the gastric microbiota, and observed few changes in the gastric phyla but documented changes in less abundant gastric genera.[78,118,119] In gerbils with *H. pylori* infection, two groups saw decreases in lactobacilli, but both had small numbers and depended on culture for identification.[78,119] The most comprehensive study utilized TTGE and NGS to sequence a 16S rRNA clone library, and found a decrease in *Lactobacillus* diversity with *H. pylori* infection and noted changes in some of the least abundant species.[118] Osaki et al. used qPCR to monitor 15 species of interest after 1 y of CagA+ *H. pylori* TK1402 infection.[117] In their control gerbils, *Lactobacillus* was the most abundant genus, followed closely by *Enterococcus,* and finally equal levels of *Atopobium* spp and *Clostridium* spp The *H. pylori* infection efficacy was 45%. *H. pylori* positive gerbils had lower levels of *Clostridium coccoides* compared with controls. *H. pylori*-infected but negative animals, had lower levels of *Clostridium coccoides* and *C. leptum*, as well as *Bifidobacterium* spp The *H. pylori* negative group had increased levels of Actinobacteria:*Atopobium* and were the only group with detectable levels of the Firmicutes species *Eubacterium cylindroides* and the Bacteroidetes genus *Prevotella*.[117] It is possible that the gerbils that cleared *H. pylori* had increased gastric damage that led to altering the gastric niche.

Highlighted differences between rodent and human gastric microbiota

The two major differences between the rodent and human gastric microbiota are (1) the prevalence and abundance of *H. pylori* in the human stomach, and (2) the effect of the non-glandular stomach on the bacterial species composition in the rodent stomach. The phyla observed in *H. pylori* positive humans in order of abundance are Proteobacteria, Firmicutes and Actinobacteria. Proteobacteria are otherwise not the main phyla in any reviewed study, whether in rodents or *H. pylori*-uninfected humans. In the absence of *H. pylori*, the most abundant phyla in humans are Firmicutes, Bacteroidetes and Actinobacteria, which arguably resembles the structure observed in the normal mouse (Firmicutes, Bacteroidetes, and varying contributions by Cyanobacteria, Verrumicrobia, Proteobacteria, and Actinobacteria). However, in humans, the main contributors to these phyla are the genera *Streptococcus* and *Prevotella*, which are not abundant in either mice or gerbils. Instead, *Lactobacillus* colonize the squamous epithelium (with subsequent spillover effect and presence in samples of glandular stomach) and can often outcompete all other species.

Host and Environmental Factors that Promote *H. pylori* Pathogenesis and Influence the Gastric Microbiota

Having discussed the important role of pH in maintaining stomach function and the studies evaluating changes in the gastric microbiota in the context of *H. pylori* pathogenesis, this section will evaluate other factors that affect *H. pylori* pathogenesis and have the potential to perturb the microbiota.

Cytokine gene polymorphisms

Cytokine gene polymorphisms in *IL-1*β and *IL-8* have been associated with increases in gastric cancer risk. IL-1β is a proinflammatory cytokine that can inhibit acid secretion in the stomach. *H. pylori*–infected individuals with polymorphisms in the *IL-1*β gene or the *IL-1* receptor antagonist have an increased risk of developing gastric atrophy and gastric cancer.[120,121] In both mice and humans, overexpression of IL-1β has been linked to increased risk of gastric cancer.[120,122] Another proinflammatory cytokine, IL-8, is involved in the recruitment and activation of neutrophils. A polymorphism in the IL-8 promoter region that increases IL-8 levels, is associated with increased gastric cancer risk,[123,124] and stomach cancers with high levels of IL-8 levels have a poor prognosis.[125] Recent clinical and epidemiological studies link increased mRNA and serum levels of CXCL1, another cytokine in the CXC chemokine family to which IL-8 belongs, to gastric cancer.[126-128] In mouse models of gastric cancer, CXCL1 and transgenic IL-8 are upregulated by Helicobacter infection and increased CXCL1 expression correlates with dysplasia scores.[112,129,130] It is not well understood if changes in systemic inflammatory status are produced by changes in microbiota composition or if they assist in shaping the composition of the microbiome in diverse sites of the body. However, recent studies have shown a correlation between

detrimental changes in the fecal microbiota composition and increases in proinflammatory cytokines that lead to disease. Biagi et al. correlated the proliferation of Proteobacteria and reduction in Firmicutes and Bacteroidetes with increases in IL-6 and IL-8.[131] The symbiont *Bacteroides fragilis* expressing polysaccharide A can suppress proinflammatory IL-17 production induced by *Helicobacter hepaticus,* a bacterium with pathogenic potential.[132] The loss of a member of the Firmicutes, *Faecalibacterium prausnitzii*, is linked with higher risk of recurrence of Crohn Disease (CD) in humans.[133] Experiments in vivo demonstrated that *F. prausnitzii* is protective in a chemically induced colitis model due to its antiinflammatory effects which block NF-κB activation and IL-8 production.[133] Similarly, intestinal commensals, specifically segmented filamentous bacteria (SFB), have been implicated with the regulation of gut immune maturation and the production of IL-17.[134]

Age

The fecal microbiota experiences dramatic changes from birth to death. From birth to the first three years of life, a less complex microbiota increases in diversity and stabilizes, culminating in a stable and species-rich state throughout adulthood that ultimately declines in symbionts in old age.[131,135-138] These observations were robust and independent of geography in three populations worldwide.[135] As noted previously, the incidence of intestinal-type gastric adenocarcinomas associated with *H. pylori* increases with age, with a peak incidence in the eighth decade of life.[139] The association of disease with increasing age fits into the Correa model of *H. pylori*-induced gastric pathogenesis and changes in acid secretion that are common to aging, as described above. The cumulative lifetime exposure to RONS, proinflammatory cytokines and tissue damage promoted by *H. pylori* are coupled in old age with a decreasing capacity to deal with antigens (immunosenescence) and reduced ability to control inflammatory responses (inflammaging). Immunosenescence, the overall decline of immunity associated with age, is demonstrated in impaired antigen presentation, reduced cytotoxic function, accumulation of effector T cells, decreased output of naive T cells and reduced B cell production in the elderly.[140] Inflammaging is a process illustrative of *H. pylori* infection, in which chronic inflammation over time can overwhelm the body's repair capacity and promotes host damage and disease.[141] The aging of the immune system increases the susceptibility to pathologies associated with inflammation, such as cardiovascular disease, autoreactivity and microbial infections.[140] These processes have been shown to affect the microbiome of the elderly, for example changes in the composition of Firmicutes (leading to inflammation), and increases in the proportion of Bacteroidetes.[131,136] Decreases or shifts in beneficial *Clostridium* clusters XIVa and IV, which include several butyrate producers, have also been observed in many elderly individuals.[131,136,142,143] Greater proportions of Enterobacteriaceae were found in elderly volunteers independent of geographic location in Europe, but the importance of this finding remains unresolved.[144]

Gender

Irrespective of location and ethnicity, males are twice as likely as females to develop gastric cancer.[139] The pattern of the M/F incidence of gastric cancer is a global phenomenon, equally seen in populations with high and low risk for gastric cancer. This remains one of the unresolved epidemiological questions given that the sexual dimorphism has not been explained by putative risk factors such as smoking, alcohol and obesity.[145] Importantly, epidemiological evidence points to the protective role of female hormones[146] and this variable is now being studied using in vivo models.[111,112] However, the differences in gastric microbiota between males and females is not well established. Data from the Human Microbiome project found a low degree of correlation between the microbiome and gender,[2] and a study of European, American and Japanese subjects identified three distinct phylogenetic classifications of gut microbiota (enterotypes) in all their subjects but found no strong correlation to gender.[147] However, examining functional biomarkers, five functional modules differentiated males and females, indicating that biomarkers derived from metagenomics may be more informative than phylogenetic biomarkers.[147] In contrast a European study noted strong gender effects with males having higher levels of the *Bacteroides-Prevotella* group than females.[144] Two recent studies have also demonstrated that sex exerts an effect in murine models.[72,148] Non-obese diabetic (NOD) mice develop type 1 diabetes with higher incidence in females. However, development of type 1 diabetes is prevented in GF female NOD mice and GF female NOD mice that receive a microbiota transplant from a male NOD mouse.[148] In the INS-GAS model of gastric cancer, male mice develop gastric cancer while female mice develop cancer at a much lower frequency.[111] The GI microbiota contributes to pathogenesis as GF INS-GAS mice have a delayed onset of cancer.[77] A restricted microbiota consisting of *H. pylori* and three species of the 8 species of Altered Schaedler's Flora was able to promote gastric cancer in male mice, which exhibited a higher bacterial colonization levels and different distribution of bacteria compared with female mice, highlighting that differences in microbiota between males and females can affect *H. pylori*-associated pathogenesis.[72]

Ethnicity/geography

The incidence of gastric cancer varies in different parts of the world with the highest incidence rates documented in Eastern Asia, Eastern Europe, and South America, while North America and Africa show the lowest recorded rates.[37] In the United States between 2003–2007, gastric cancer mortality rates followed ethnic divisions as mortality rates were highest among African-Americans, followed by Asian/Pacific Islanders, Native Americans, Hispanics, and Caucasians.[149] The gastric cancer data are reflective of the prevalence of *H. pylori* which exhibits geographical variation with 80–90% prevalence in various developing countries and less than 40% prevalence in industrialized countries.[22] However, racial differences alone do not explain gastric cancer rates, as migrants from high-risk regions have a decreased gastric cancer risk when they relocate to a lower risk area.[150]

Similarly, many studies evaluating the fecal microbiota between different geographic locations or different ethnic groups have found large variation in specific bacterial groups (but not at the phyla level) between these populations.[2,135,144,151] There has

been considerable interest in the variation between *Prevotella* spp and *Bacteroides* spp as they exhibit considerable variation between populations.[135,144,151] However, others have classified fecal microbiomes into three enterotypes with little correlation between gut microbiomes and nationality.[147] Further studies are necessary to separate the effects of ethnicity/geography from diet (and other variables) as they may be proxies for each other, as diet has been previously shown to play an important role in defining the composition of the microbiome.[152]

Diet

In the Correa model, it is postulated that diet plays a role in the progression of gastric cancer initiated by *H. pylori*. Excessive salt and nitrates may promote inflammation, while deficiencies in ascorbic acid or low intake of fresh fruits and vegetables, may decrease the stomach's ability to deal with inflammation.[45,153] While vitamin C supplementation has shown no protective effects,[154] high incidences of gastric cancer have been associated to countries with high salt intake.[155] Animal models assessing the role of high salt in *H. pylori* gastric pathology have yielded mixed results.[115,156] However, a recent study has demonstrated that salt upregulates the expression of *H. pylori CagA*.[157] As the effects of salt may not be independent of CagA, studies assessing the effect of salt using the mouse-adapted *H. pylori* strain SS1, which lacks a functional CagA,[102] may have to be interpreted with this variable in mind. Indeed, in a subsequent study, high salt diets promoted gastric adenocarcinomas in Mongolian gerbils infected with *H. pylori* strains with a functional CagA, but not with *H. pylori* lacking CagA.[114] Iron is another important dietary factor, as iron deficiency has been associated with the presence of *H. pylori*[158,159] and hypochlorhydria.[49,56] Animal models have demonstrated that Helicobacter infection results in iron deficiency,[158] while reduced dietary iron coupled with *H. pylori* infection promoted gastric cancer.[116] Interestingly, reduced serum ferritin levels in patients were associated with *H. pylori* isolates that induced more robust proinflammatory responses in vitro.[116] The contributions of diet to *H. pylori* pathogenesis are reviewed in greater detail by Peek and Cover.[160]

Diet is also believed to strongly influence the microbiota.[143,152] In an elderly population, the quality and diversity of the diet correlated with microbial diversity, as well as changes in frailty, inflammation and altered abundances of short chain fatty acids (SCFAs) producing bacteria.[143] The oldest and frailest subjects had the fewest copies of genes involved in SCFA production in their fecal metagenomes, and in general had increased *Bacteroides* spp, *Parabacteroides* spp or *Alistipes* spp coupled with a loss of *Prevotella* spp[143] Diets rich in carbohydrates and polysaccharides result in increases in *Prevotella* while diets rich in protein and animal fat promote *Bacteroides*,[151,152] and these genera seem to mutually exclude each other and define stable enterotypes.[135,147] Although, *Prevotella* spp are associated with "healthier" diets and lifestyles, it is worth noting that the family *Prevotellaceae* has also been associated with the development of inflammatory bowel disease and periodontal disease.[13]

Animal studies have also demonstrated the strong effects of diet on the microbiome. Obese mice have a greater capacity to harvest energy from their diet compared with lean mice, and transplantation of the "obese" and "lean" microbiota to GF mice led to higher levels of body fat in the first group.[161] As in *H. pylori* pathogenesis, iron plays an important role in the health of the lower bowel. Depletion of luminal iron in a mouse model of Crohn Disease-like ileitis increased the numbers of *Bifidobacterium, Succinivibrio, Clostridium* and *Turicibacter*, while numbers of potentially pathogenic *Desulfovibrio* spp were decreased.[162] In a chemically induced model of colitis, decreases in Firmicutes and increases in Bacteroidetes and the family *Enterobacteriaceae* were noted, but these effects were prevented with ferric iron supplementation.[163]

Other infections

Infections, both clinical and subclinical, have a great impact on *H. pylori* pathogenesis, as well as the microbiota. Infections by parasites and other non-gastric helicobacters have been shown to modulate *H. pylori* pathogenesis. Coinfection of mice with the nematode *Heligmosomoides polygyrus* and *H. felis* induced an antiinflammatory T_H2 response, and concomitant reduction in proinflammatory T_H1 immune responses normally induced by *H. felis*.[164] The development of gastric atrophy was also significantly reduced.[164] The opposite effect was observed by modulating the host's response using a parasite that induces a strong T_H1 response, such as *Toxoplasma gondii*. Coinfection with *T. gondii* exacerbated *H. felis* infection leading to increased morbidity.[165] Similarly, intestinal helminths had a higher prevalence in humans residing in low gastric cancer risk areas in Colombia.[166] In a Chinese population, individuals coinfected with *H. pylori* and *Schistosoma japonicum* were protected from atrophy.[167] Bacteria that colonize the lower bowel in mice were able to both enhance and minimize *H. pylori* pathogenesis by modulating the T_H1, T_H17 and T_{REG} responses.[168,169] These effects were highly species-specific, as coinfections with either *H. bilis* or *H. muridarum* reduced *H. pylori*-induced gastric pathology and induced a T_H2 immune response, while coinfection with *H. hepaticus* enhanced the proinflammatory T_H17 response.[168,169] Similarly, parasite infections and Helicobacter infections have been shown to shift the composition of intestinal bacteria, and increase the diversity of the gastric microbiota.[71,170-173] *H. polygyrus* infection significantly elevated the numbers of lactobacilli in the ileum of infected mice,[173] which is of interest as *Lactobacillus* spp have been reported to attenuate *H. pylori* gastritis in mice.[174] We have conducted studies that show that *Helicobacter* spp infection in mice directly affects the levels of bacteria in mice with restricted microbiota,[171,172] but further studies are necessary to evaluate changes in complex microbiota that may further modulate the immunomodulatory effects of subclinical *Helicobacter* spp infections. The idea that infection of other compartments of the GI tract may directly affect gastric pathogenesis raises the question of whether direct influence of the gastric microbiota is necessary to influence disease outcome. Conversely, *H. pylori* infection of the stomach has been implicated with decreased incidence of inflammatory bowel disease (IBD) and esophageal cancer,[87,175] implying that infections of the stomach can alter the severity of disease of both the upper and lower GI tract.

Concluding Remarks

The role of the bacteria in the development of gastritis, ulcers and cancer has generated considerable debate. The origins of these hypotheses were the observation that bacterial overgrowth occurred in hypochlorhydric stomachs. *H. pylori* infection is a major cause of hypochlorhydria and has a major role in the progression from gastritis to atrophy and finally to gastric cancer. *H. pylori* and the associated changes in the stomach alter the ecological niche inhabited by the gastric microbiota. However, the gastric microbiota also competes, as observed in rodents and older people, with *H. pylori* for a gastric niche, and may play an important role in the progression of disease. More studies involving the microbiota-host-environment interactions are needed to fully understand the role of gastric bacteria in human health and disease.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed

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