

BACTERIAL MICROBIOTA PROFILING IN STOMACHS WITH AND WITHOUT *HELICOBACTER PYLORI*

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SUMMARY

The human gut microbiota has come into focus in the search for component causes of chronic diseases, such as gastrointestinal cancer. Some scientists claim that *Helicobacter pylori* belongs to the normal bacterial flora from the human stomach, despite its associations with gastric ulcers and cancer in adulthood and that it seems to have beneficial effects in early ages. It has been shown that the stomach without *H. pylori* has a different bacterial composition beyond the absence of *H. pylori*. Factors that may shape the composition of the stomach microbiota are antibiotic treatment, diet, stomach acidity and histopathological changes i.e. development of atrophic gastritis in the corpus. Investigations of the bacterial microbiota in healthy human stomachs and changes in the microbiota due to factors mentioned above are necessary to rule out the impact of a well balanced microbiota in a healthy stomach and dysbiosis in gastric disease development. It has been suggested that other bacteria than *H. pylori* may be involved in pre-cancer lesions of the stomach. Next generation sequencing platforms give us a chance to explore the non-*H. pylori* microbiota in the stomach and will hopefully provide biomarkers for future studies of risk individuals.

INTRODUCTION

The human microbiota consists of about 100 trillion microbial cells that outnumber our human cells by 10 to 1 (Savage, 1977). Especially the human oral and gastrointestinal (GI) microbiota have been extensively studied but are yet not fully described. However, it has been established that the microbiota is partly site-specific (Dethlefsen et al., 2007). The human gastro-intestinal tract (GI-tract) consists of several compartments with varying physiological conditions and as a result different mi-

crobiota. On their passage through the GI tract the bacteria will be exposed to peristaltic activity, food particles, gastric-, pancreatic- and bile secretions at different locations of the tract. In the stomach and upper part of the small intestine the low pH, fast peristalsis and high bile concentrations will limit the bacterial colonization and survival (Manson et al., 2008). Further down in the colon the restrictive feature is the anaerobic environment and as a consequence the anaerobic bacteria outnum-

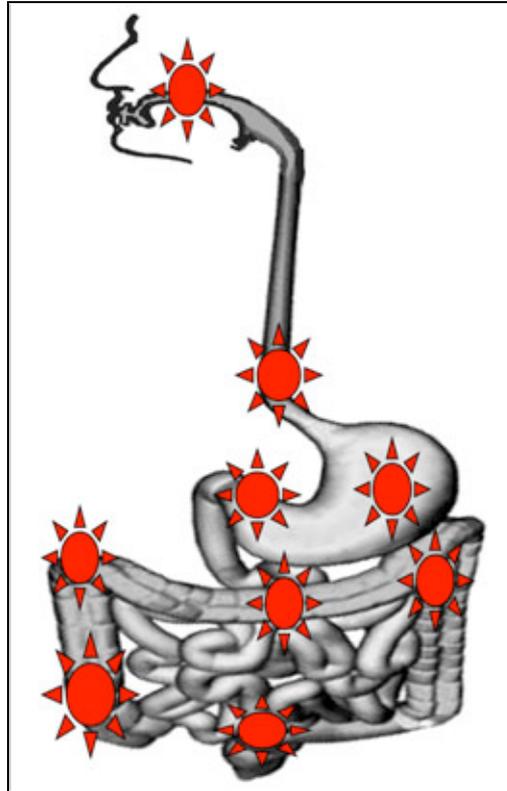


Figure 1: Sampling sites in the human gastrointestinal tract. Upper endoscopy allows sampling from the oesophagus, stomach and duodenum. Lower endoscopy covers the entire large bowel. The small intestine is not possible to sample using traditional endoscopy.

ber the aerobic by 1000:1. The different parts of the GI-tract that are reachable with the endoscope, thus allowing us to obtain biopsy samples from these locations, are shown in Figure 1. Today it is not possible to sample the entire small intestine and consequently the composition of the microbiota in this part of the human GI-tract is considered almost a black box.

Most of the microbiota in the GI-tract is located in the intestinal lumen or in the loose mucus close to the lumen without direct contact to the epithelium (*van der Waaij et al., 2005; Swidsinski et al., 2007a*). The mucus layer (which consists mainly of different mucins) in the stomach and colon is divided into the firmly attached mucus

layer and the loose mucus layer. The mucus layer is a protective barrier that prevents epithelial colonization of microbes and prevents diffusion of damaging chemicals, including gastric acid, to the epithelial surface. Thus, inflammation in the GI-tract is caused either by bacterial penetration of the mucus layer or by a disruption of the same layer.

With new molecular-based methods the information about the microbiota in the GI tract is constantly growing and provides deeper understanding on microbial ecology and the involvement of the microbiota in health and disease. Most studies are based on 16S rRNA gene sequencing methods and the results are descriptive but not functional.

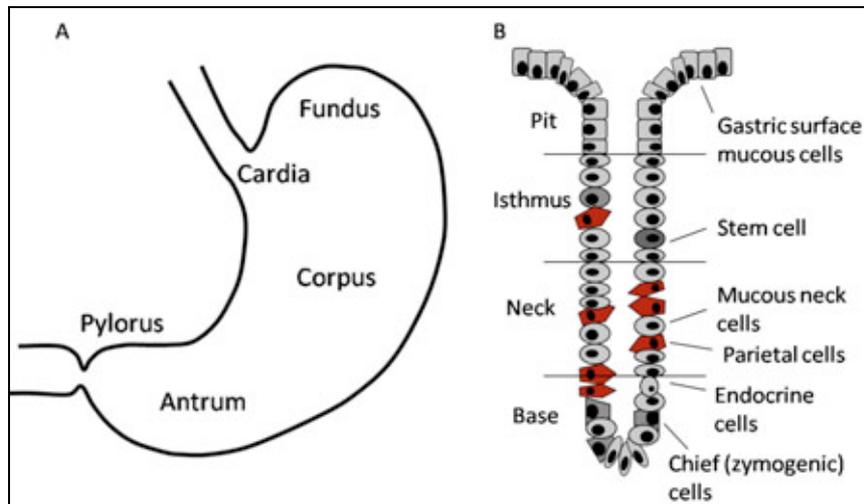


Figure 2. A. Illustration of the human stomach anatomy. B. Schematic view of the gastric unit.

In the near future when sequencing costs will go down, GI tract microbial metagenome studies will probably replace or complement the 16S-based studies. We will then get information about sets of core genes (including functional genes) specific for the gut

microbiota and associations of these genes with disease development. The putative gut-specific genes include genes involved in adhesion of host proteins and harvesting of sugars (Qin et al., 2010).

REVIEW AND DISCUSSION

The oral and oesophageal microbiota

Despite the presence of antimicrobial peptides and the constant flow of saliva, the oral cavity has a very high abundance of microorganisms with 10^9 bacteria per ml saliva and 10^{11} bacteria per gram dental plaque (Aas et al., 2005). The mouth has both soft and hard tissue and different species seem to preferentially colonize either of the tissue types. Great similarities have been found in the oral microbiota in different individuals and these species and genera have been identified as the core microbiome. The most prevalent genera and families in this oral core microbiome are *Streptococcus*, *Corynebacterium*, *Neisseria*, *Rothia*, *Veillonellaceae*, *Heamophilus*, *Actinomy-*

ces, *Granulicatella* and *Prevotella* (Zaura et al., 2009). It would be expected that these genera follow the swallowed saliva down into the stomach. In biopsies obtained from oesophagus, a similar but more sparse biota has been found, with *Streptococcus*, *Rothia*, *Veillonellaceae*, *Granulicatella* and *Prevotella* as the most prevalent genera (Pei et al., 2004).

The gastric microbiota

Anatomy and physiology

A meal can take only minutes to eat, but takes hours to digest, therefore, the main function of the stomach is to store food and send gastric contents ahead with a speed that maximizes digestion in the small intestine. Some di-

gestion of proteins and starch takes place in the stomach. Under fasting conditions the pH in the human gastric lumen is < 2 and is about 5-6 close to the surface epithelial cells. The pH gradient is monitored by the mucus layer that covers the epithelial cells in the stomach. MUC1 is a transmembrane mucin and constitutes the main factor in the firmly attached mucus layer. The other mucins are secreted mainly to the loose mucus and the most secreted is MUC5AC (Corfield et al., 2001).

An adult person produces 2 litres of gastric juice daily. Most of the juice is formed in the tubular glands in the gastric corpus- and fundus-regions. The glands form deep pockets into the gastric wall and comprises of different cell types (Figure 2).

The gastric microbiota in health

Even though the human microbiota along the intestinal tract has been extensively studied, the environment in the stomach has been considered too harsh for most bacteria to thrive and survive in for any length of time and therefore this environment has not been studied to any great extent. However, since the discovery in 1984 of *H. pylori* as the causative agent of peptic ulcers and a risk factor for the development of gastric cancer, this specific bacterium has been extensively studied.

In the normal acidic stomach a sparse cultivable non-*Helicobacter* microbiota has been found dominated by *Veillonella* sp., *Lactobacillus* sp., and *Clostridium* sp. (Zilberstein et al., 2007). A more diverse microbiota has been seen when using 16S rRNA based methods and the main genera found in stomachs except *Helicobacter* have been, *Streptococcus*, *Prevotella*, *Veillonella* and *Rothia* (Figure 3, Li et al., 2009). Whether these other genera belong to a colonizing microbiota or are just swallowed bacteria from the oral

cavity has not yet been determined. The main stomach microbiota consists of the same genus as found in the oral cavity and also in oesophagus biopsies (Keijser et al., 2008). However, it is not a direct reflection of these microbiota. The gastric microbiota is well adapted to the gastric environment and also to environmental changes in their specific stomach.

The gastric microbiota in disease

Helicobacter pylori is a micro-aerophilic, spiral shaped Gram-negative rod and is today the only bacterium considered to colonize the human stomach. *H. pylori* survives in the acidic gastric lumen by production of urease that converts urea to ammonia and carbon dioxide, which neutralizes the microenvironment close to the bacterium. As a result of the urease activity the pH around the bacteria will increase, leading to reduced viscosity and enables *H. pylori* movement through the mucus (Celli et al., 2009). *H. pylori* can adhere to the gastric epithelial cells but the majority (99%) are free-living in the mucus (Falk et al., 2000) and especially in association with mucin MUC5AC, which is the most common mucin in the gastric mucus layer (Van de Bovenkamp et al., 2003). When *H. pylori* is present in the stomach it totally dominates the gastric microbial population in the gastric niche constituting as much as 94% of the total number of sequences in one individual (Figure 3). When the number of *H. pylori* diminishes, for example during atrophy development in the gastric niche, the diversity increases dramatically.

Helicobacter pylori induced atrophic gastritis and gastric cancer

Helicobacter pylori colonizes almost 40% of the population in many western countries. Most individuals

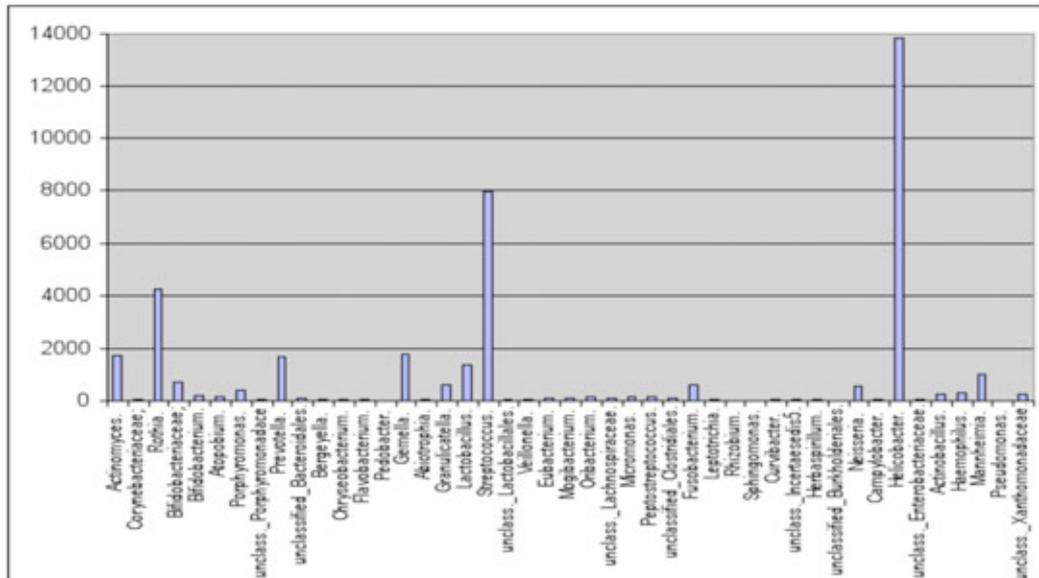


Figure 3: The most prevalent bacteria in the human stomach (number of reads sampled).

with gastritis will have an asymptomatic infection but about 10-20% of the infected individuals will develop ulcers and 1-2% will develop cancer (Kusters et al., 2006). The outcome is depending on the location of infection. Patients with antral-predominant gastritis are predisposed to duodenal ulcer, whereas patients with corpus-predominant gastritis are more likely to develop gastric ulcer, atrophic gastritis, intestinal metaplasia and gastric cancer. The low acid production in corpus-predominant gastritis is due to atrophy development since chronic *H. pylori* induced inflammation during decades can lead to such changes in the gastric mucosa. In corpus-dominated atrophy the acid-producing parietal cells have been changed into more intestinal like non-acid producing cells resulting in intestinal metaplasia and a less acidic stomach. The change in cell types also changes the mucin production in the stomach to the intestinal mucins MUC2 and MUC3 (Babu et al., 2006). Because bacteria often bind to different mucins, this change can affect how

well and which bacteria adhere to the mucus. *H. pylori* has been found to spontaneously disappear in patients with severe corpus-predominant atrophy where the bacteria gradually disappear as the atrophy worsens. The disappearance of *H. pylori* and low acid output due to atrophy opens up for other non-*H. pylori* bacterial populations to invade into this niche (Figure 4).

The gastric microbiota in corpus predominant atrophic gastritis

The atrophic stomach has an increased pH enabling better survival of bacteria than in a normal acidic stomach. In addition a shift can be seen in the most prevalent genera from *Prevotella* to *Streptococcus* in the atrophic stomach. This shift further confirms that the gastric microbiota is not only a reflection of the oral microbiota. The bacteria with the greatest increase in the atrophy group represent the mitis group within the *Streptococcus* spp. In a study where *H. pylori* negative individuals with antral gastri-

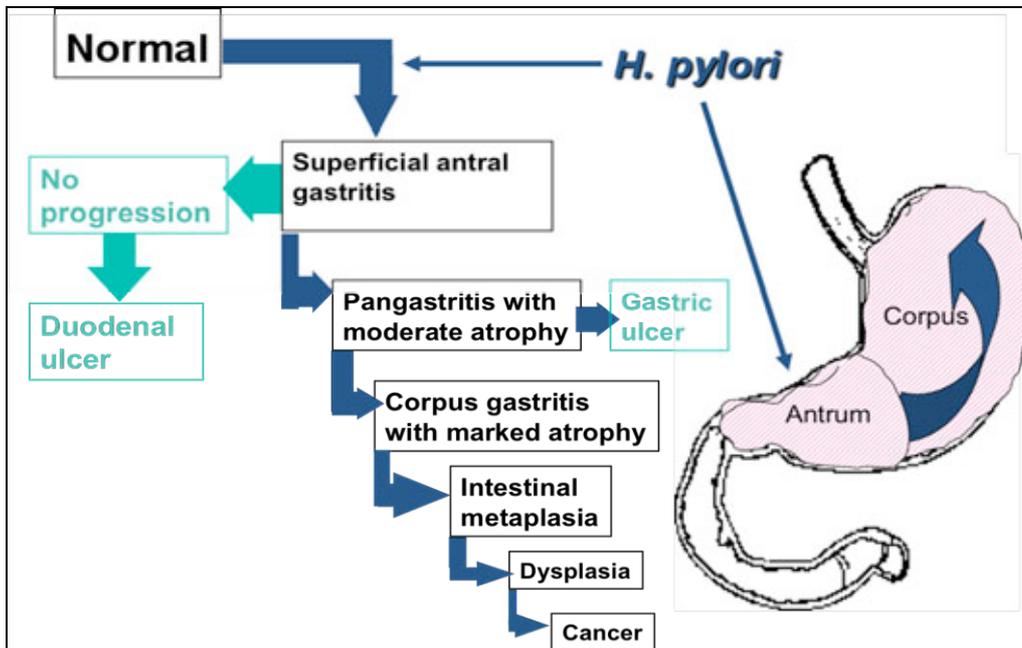


Figure 4: Gastric cancer development and *H. pylori* distribution within the stomach. Curved arrow indicates expansion of the *H. pylori* population into the corpus area when pH increases. Increased pH also allows for non-*H. pylori* bacterial populations to survive in the non-acidic stomach.

tis (where the pH should be expected to be decreased if changed) was compared to non-symptomatic controls (Li et al., 2009). In this study shifts could be observed in the antrum biopsies (in *Prevotella*, *Streptococcus* and *Rothia*) as described for the corpus predominant atrophy stomach.

The gastric microbiota in gastric cancer

As in the atrophic stomach, the pH is increased in gastric cancer and both conditions can also lead to an increased amount of bacteria and increased diversity. In gastric cancer the number of bifidobacteria/lactobacilli, *Veillonella* and streptococci are increased (Sjöstedt et al., 1985). Among the *Streptococcus* especially a group including *S. mitis* and *S. parasanguinis* increases. The changes in the microbiota in individuals with gastric cancer resemble those seen in the atrophic stomach.

The gastric core microbiome

The gastric microbiota is totally dominated by five phyla (*Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*) and each phylum comprises of only a few genera all in high abundance. In the same way as a core microbiome for the oral cavity has been determined (Zaura et al., 2009) it is possible to determine a core microbiome for the healthy human stomach. We determined that, among 13 individuals with no pathology and without dominance of *Helicobacter* sequences, the majority of the bacteria were represented in all individuals (unpublished data). The most prevalent bacterial sequences belonged to *Streptococcus*, *Prevotella* and *Veillonella*. In addition the five most abundant genera from two other studies (Bik et al., 2006; Li et al., 2009) were all among the ten most abundant in our study. These

similarities can be seen regardless that different approaches for DNA extraction, primer design and sequencing were used in the different studies. Taken together this is an indication that the core gastric microbiome is dominated by ten genera. In addition, it has been found that there are no big differences in the microbiota present in the antrum and corpus portion of the stomach (Bik et al., 2006; Li et al., 2009) with the exception of the higher proportion of *Prevotella* in antrum found by Li and colleagues (Li et al., 2009). On the whole, the most prevalent genera in the healthy gastric core microbiota are *Prevotella*, *Streptococcus*, *Veillonella*, *Rothia*, *Haemophilus*, *Actinomyces*, *Fusobacterium*, *Neisseria*, *Porphyromonas* and *Gemella*.

In conclusion, the human gastric microbiota has been investigated and great inter-individual similarities are found in healthy stomachs, with the microbiota dominated by five phyla

and ten genera. In the unhealthy stomach a significant shift is observed in stomachs with corpus atrophy compared to controls, with *Streptococcus* becoming the most abundant genus that may affect disease development. It is however important to understand the relative contributions of the host and environmental factors to the dynamics of the stomach microbiota. High-throughput sequencing platforms will allow us to study the gastric metagenome in the near future and such studies will provide information about the functional repertoire of genes and the expression of these genes. We will also increase our knowledge of metabolic products of active microbial populations (in a healthy compared to a diseased state). Future research in this field will require well-designed prospective epidemiological studies combined with optimized sampling procedures and access to front-line technology platforms.

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