The uninvited guests of our microbiome: *Helicobacter pylori* and Epstein-Barr virus and their role in gastric cancerogenesis

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Abstract

It is well established that human body is an ecosystem for numerous microorganisms: bacteria, fungi, eukaryotic parasites, and viruses. They form a “microbiome” that under conditions of homeostasis remains in a friendly mutual relationship with the host. However, the composition and diversity of this microbe community is dynamic and can be changed under the influence of environmental factors, such as diet, antibiotic therapy, lifestyle, and the host's genotype and immunity. The result of gut microbiome dysbiosis can lead even to cancer. The aim of this review is the description of the healthy gastrointestinal microbiome and the role of two infectious agents: Gram-negative bacteria *Helicobacter pylori* and Epstein-Barr virus in the development of gastric cancer in terms of gut dysbiosis.

H. pylori is the most important pathogen of gastric microbiome with clear impact on its diversity. Coinfection with Epstein-Barr virus causes chronic gastritis, and the inflammatory process is significantly increased. The process of carcinogenesis begins with chronic inflammation that causes atrophic gastritis, intestinal metaplasia, dysplasia, and finally cancer. It has been proven that chronic inflammatory infection caused by infectious agents increases the risk of stomach cancer. Molecular methods that are progressively used to explore the human microbiome provide hope that this knowledge will be used for future diagnoses and therapy in the state of its dysbiosis and in cases of gastric cancer.

Keywords

gastric microbiota • *Helicobacter pylori* • EBV • gastric cancer • PPI

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What is a microbiome, and what are the effects of gastric microbiome dysbiosis?

Different parts of human body are inhabited by diverse microorganisms, which constitute a community described as microbiota, and the collection of genomes of distinct microbiota is called the microbiome. The microbiome is dominated by bacteria, but it also includes other microorganisms, such as archaea, fungi, viruses, and sometimes eukaryotic parasites.

The foundation for learning about the human microbiome was created by the Human Microbiome Project, implemented in 2007, aimed at sequencing the complete microbiome from 250 people and examining the interaction between microorganisms and the human body [1]. Although we are unable to culture most of the microbes inhabiting the human body, the development of molecular techniques has allowed us to better understand the human microbiome, primarily by sequencing 16S mRNA.

The microbiome is extremely important from the point of view of metabolic processes, absorption of nutrients, and defense against pathogens. Microorganisms are not homogeneously distributed in the human body; in some places there are a lot of them, and in others they are present in very small amounts, depending on the local environment (biology of the area of the body /organ). Additionally, they can vary greatly at different sites within the same organ. The differences in composition as well as the density of microbiome correlates to an individual's health status, genotype, diet, and hygiene. Generally, the ratio of bacteria to human cells was thought previously to be 10:1 or even 100:1, but recently the ratio of bacteria to human cells has been updated to be closer to 1:1, which does not change the biological significance of the microbiome [2].

Within the gastrointestinal tract, there is a large quantitative and qualitative diversity of the microbial community, which is associated with the difference in the structure of the individual parts of the digestive tract. It is related to, among other things, oxygen conditions, pH, and intestinal peristalsis. In the initial segment of the small intestine (duodenum and jejunum), few

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microorganisms can be found, due to the presence of bile and pancreatic juice, low pH, and strong intestinal peristalsis. However, in the final segment of the small intestine, the ileum, the pH is higher (close to neutral), and it is inhabited by numerous, mostly relatively anaerobic bacteria. The large intestine, with slower peristalsis and pH closer to alkaline, has optimal conditions for the growth of most bacteria. Here is the most numerous group of microbes in the human body. In the stomach and small intestines a large percentage of microbiome is formed by facultatively aerobic microorganisms and in the large intestine by anaerobic taxa [3].

The stomach environment appears to be extremely unfavorable to most bacteria. Due to the low pH prevailing in the stomach, this organ was considered for a long time to be sterile. In 1982 the discovery of *Helicobacter pylori* from mucosa of patients suffering from peptic ulcer disease [4] changed the general opinion and motivated further search of other microorganisms inhabiting the stomach. The rapid flow of gastric contents in the upper gastrointestinal tract reduces the growth of microorganisms; in addition, secretory activity of the stomach and duodenum results in the extinction of most bacteria. It is assumed that there are about 10^1 – 10^3 CFU/g in the stomach.

Despite an increasing amount of research on the human microbiome, most information concerns bacteria. The mycobiome has been shown to represent a large group of eukaryotic organisms. Fungi are a natural part of the microorganism community found in the human body, and although they make up a smaller proportion of the microbiota, their relationships with other microbes and the human body are not negligible. Most of the research work on the occurrence of fungi in the human body, their interaction with bacteria, and the effect of mycobiome dysbiosis on the occurrence of the disease state concerns the intestines [5, 6]. Colonization of the human stomach by fungi can be favored by older age, malnutrition, H2-blocker therapy, the use of broad-spectrum antibiotics, and PPI [7]. The quantity of fungi in the human stomach is defined as 0 – 10^2 . Research using fungal DNA co-sequencing gastric juice analysis only allowed the identification of two genera of fungi *Candida* and *Phialemonium* [8].

Little research work is focused on the viruses that make up the microbiome. *Retroviridae*, often classified as human endogenous retroviruses (HERVs), predominate in the microbiome of the stomach. *Herpesviridae* are another group of viruses in high abundance, including Epstein-Barr virus (EBV), found in gastric mucosa. Also, bacteriophages of *Staphylococcus*, *Burkholderia*, and *Enterobacteria* were identified in the gastric microbiome [9].

Interactions between the microbiome and the human body are being increasingly studied, as well as the impact of changes in the composition of the microbiota on human

health and disease. Studies on gnotobiotic animals have shown a very important role of microbiota in the organism's development and host physiology [10]. A kind of balance should be kept between microorganisms and the host, and any sudden disturbance in the composition of the microbiota—a condition called dysbiosis—can be associated with the occurrence of disease symptoms, sometimes even with the development of cancerous lesions. The process of carcinogenesis may be affected by the disturbance of signals stimulating and inhibiting the development of inflammation, the cytotoxic effect, excessive proliferation of epithelial cells, and the production by microorganisms of toxic, intermediate products of metabolism that can cause damage to epithelial cells [10]. The change in the structure of the gastrointestinal microbiome may be the result of many factors, including diet, stress, medicine (mainly antibiotics), and smoking.

Changes in the gastric mucosa, such as ulcers, promote the growth of *Candida albicans*. Gastric ulcers associated with the colonization of *Candida albicans* are well documented in the literature [11]. However, it is difficult to say whether *Candida* is an etiological factor causing ulcers or whether its presence in the stomach is a secondary effect. Also, the co-occurrence of *H. pylori* and *C. albicans* may act synergistically in the development of peptic ulcers [7, 8]. Karczewska et al. [12] showed that 66.7% of patients with gastric disorders with *Candida* in the mucosa were also colonized by *H. pylori*. There have also been studies confirming the presence of *H. pylori* inside *Candida* cells, its ability to survive in *Candida* vacuoles and the possibility of expressing *H. pylori*-specific proteins, e.g., VacA, urease or peroxiredoxin. The relationship between *H. pylori* and *Candida* provides bacteria protection against adverse gastric conditions, including low pH, the presence of antibiotics, and also protects with nutrients [8, 13]. It has been also shown that growth of *Lactobacillus* can counteract colonization of the stomach by *Candida* by producing hydrogen peroxide and organic acids.

The etiopathogenesis of many cancers is still not well understood. It is estimated that about 15%–20% of all cancers can be associated with the presence of an infectious agent. Infectious agents affect cell growth, destabilize the host's immune system, and lead to changes in cells resulting in long-term infection [14]. Increasing attention is being paid to the association of inflammation with the change in the composition of the microbiota and the development of cancer [15]. Infections cause chronic inflammation, which can lead to carcinogenesis [16, 17]. So the tumor formation process may be caused by the presence of an infection caused by some viruses, bacteria, and even parasites.

Gastric cancer is one of the most common cancers in the world with the highest mortality rate. Most often, it is diagnosed at an advanced stage, which results in poor prognosis [18, 19]. Gastric cancer is fifth among the most frequently diagnosed

cancers and third in terms of mortality in the world [20]. Cases of stomach cancer are most commonly reported in East Asian countries, including Korea, Japan, and China, where about half of all new cases are diagnosed, over four times higher than in Western Europe; in Korea, for example, gastric cancer cases have a frequency of 33.8 per 100,000 [21]. This type of cancer is characterized by a relatively low five-year relative survival—about 32% (2010–2016) [22].

Gastric cancer can be divided into subtypes by specific epidemiology and risk factors and recently also by molecular profiles. The Cancer Genome Atlas (TCGA) [23] distinguished four subtypes of gastric cancer taking into account the underlying mechanisms: Epstein-Barr virus (EBV)-positive, tumors with microsatellite instability, and that which is genomically stable but has chromosomal instability. Noteworthy is the separate subtype of gastric cancer associated with EBV infection, which means the importance of external pathogens in the disease.

Normal gastric microbiota

The microbiota of the stomach and duodenum shows more dynamic composition than the intestinal microbiota. From the microbiota studies of healthy people, it was found that gastric microbiota shows high interindividual diversity as compared with the duodenum or any segment of the intestine that shows the least dissimilarity [24].

Five phyla have been identified in the stomach: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, and *Proteobacteria*, while *Prevotella*, *Streptococcus*, *Veillonella*, *Rothia*, and *Haemophilus* are the most common at the genera level [25]. There is no clear answer as to whether the stomach is uniformly inhabited by the same bacteria. According to some authors, there is no difference in microbiota between the antrum and the corpus, only in case of gastritis a decrease in *Prevotella* is observed in the antrum; while other authors distinguish the dominance of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* in the antrum and *Firmicutes*, *Proteobacteria*, *Fusobacteria*, and *Bacteroidetes* in the corpus [26].

Many bacteria have been detected in gastric fluid, such as *Streptococcus*, *Neisseria*, and *Lactobacillus*, but it is difficult to determine whether they belong to transient microbiome or colonize the stomach. Microorganisms travel through the gastrointestinal tract from the mouth; as a result, more and more research is focusing on the gastric mucosa [27]. Studies using methods from molecular biology have confirmed the presence of previously isolated bacteria by classical methods (*Lactobacillus*, *Streptococcus*, *Veillonella*, *Escherichia*) but have also discovered the presence of bacteria not previously included in the microbiota of the stomach: *Prevotella*,

Caulobacter, *Actinobacillus*, *Corynebacterium*, *Rothia*, *Gemella*, *Leptotrichia*, and *Deinococcus* [28].

The effect of *H. pylori* on the composition of the stomach microbiota has been followed in many studies. In the initial period of colonization by *H. pylori*, in response to inflammation, the secretion of hydrochloric acid increases as a result of stimulation of gastrin secreting G cells. However, in the chronic phase, degenerative changes of the epithelium and glands of the mucosa occur, which secondarily results in mucosal atrophy and acidosis; therefore, this process leads to an increase in pH, facilitating the colonization of other microorganisms [14, 29, 30]. After colonization of the stomach by *H. pylori*, it becomes the dominant species in the stomach microbiota [31].

According to some researchers, there is a higher bacterial diversity in *H. pylori*-negative patients, while others report no changes related to the status of *H. pylori*. Brawner et al. [32] observed differences in the change in microbiota related to the status of *H. pylori* only in children: in those who were *H. pylori*-positive there was a greater variety and abundance of non-*Helicobacter*, *Proteobacteria*, and there was a lower quantity of *Firmicutes* than in adult patients. The results of other studies confirm the differences in the composition of the microbiota between *H. pylori*-negative and *H. pylori*-positive persons. In *H. pylori*-negative patients *Firmicutes* dominates, followed by *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria* in last place, and in *H. pylori*-positive patients *Bacteroidetes* significantly dominates, whereas the others are in low abundance. It follows that *H. pylori*-negative people had more complex and highly diverse microbiota [33].

A relationship has been also demonstrated between the abundance of *H. pylori* in the stomach and intestinal microbiota. The abundance of *H. pylori* was associated with a lower amount of *Bifidobacteria* and a higher amount of *Candida albicans* in the intestine, as well as with the degree of dysbiosis in the intestine [34].

The discovery of *Helicobacter pylori* and other stomach colonizing microorganisms has highlighted the importance of microbiome in maintaining healthy body balance and disease development when the biodiversity and the quantity of microorganisms are disturbed.

How the microbiota changes in gastric cancer

Patients' stomach microbiota has been shown to change during progression to gastric cancer. Eun et al. [35] observed a change in the species abundance of gastric microbiota in gastric cancer compared with the microbiota of intestinal metaplasia patients and those with chronic inflammation. During the development of cancer, the diversity of the stomach microbiota decreases. A significant decrease in

the *Epsilonproteobacteria* class and the *Helicobacteriaceae* family and an increase in the *Bacilli* and *Streptococcaceae* classes were observed in patients with gastric cancer. Similar changes were also reported by Aviles-Jimenez et al. [36], who found a decrease in bacterial richness along with non-atrophic changes from intestinal metaplasia to gastric cancer. Hu et al. [37] observed a significant increase in *Betaproteobacteria* and *Gammaproteobacteria* and a decrease in *Alphaproteobacteria* in gastric cancer compared with superficial gastritis. Noteworthy is the significant increase in abundance of *Sphingobium yanoikuyae* in superficial gastritis, which has the ability to break down xenobiotic compounds, especially aromatic hydrocarbons that have toxic, genotoxic, mutagenic, and carcinogenic effects. Negative association with advanced gastric adenocarcinoma has been demonstrated [37]. Research confirms that *Helicobacter pylori* is predominant in the initial stages of the cascade of changes in the mucosa, and in later stages of the intestinal metaplasia, dysplasia, and adenocarcinoma the abundance of *Helicobacter* decreases, but new microorganisms then appear—*Proteobacteria* (including *Escherichia*, *Shigella*, and *Burkholderia*), *Firmicutes* (*Lactobacillus*, *Lachnospiraceae*, *Streptococcus* and *Veillonella*), and *Bacteroidetes* (*Prevotella*) [25, 26, 33]. It was confirmed by the research of Ferreira et al. [38], who examined the microbiota of 135 people—54 with gastric carcinoma and 81 with chronic gastritis (next-generation sequencing of the 16S rRNA was used). In patients with chronic gastritis, *Helicobacter* had the highest abundance, and *Streptococcus*, *Prevotella*, and *Neisseria* also appeared in large quantity. In contrast, gastric carcinoma showed a decrease in *Helicobacter* abundance, while several taxa were definitely more abundant—*Citrobacter*, *Clostridium*, *Lactobacillus*, *Achromobacter*, *Rhodococcus*, and *Phyllobacterium*.

In the research of Castano-Rodriguez et al. [39], the composition of the gastric microbiota was compared between people with functional dyspepsia and gastric cancer: 91 taxa were different, and the presence of *H. pylori* had an impact on the composition of the microbiota. A significant difference was found for 38 operational taxonomic units (OTU), 23 of them were enriched in gastric cancer, including *Lactococcus* OTU0002, *Fusobacterium* OTU0087, *Pasteurellaceae* unclassified OTU0022, *Staphylococcus* OTU0045, *Methylobacterium* OTU0018, *Veillonella* OTU0005, *Dialister* OTU0132, and *Leptotrichia* OTU0042.

Interesting results were obtained from the gastric microbiota study in Colombia, in which two populations—with low (Tumaco) and high cancer risk (Tuquerres) were considered. In the Tuquerres population, a significant amount of *Leptotrichia wadei* and *Veillonella* sp. was noticed, whereas

Tumaco was more abundant with bacteria recognized as a normal element of human microbiota, including *Staphylococcus* sp. [40]. Studies in the Korean population involving 556 people (268 patients with gastric cancer and 288 controls) showed that people who had high levels of *Helicobacter pylori* and *Cutibacterium acnes* were more likely to develop stomach cancer. In addition, the presence of *Prevotella capri* in patients increased the risk of cancer as compared with noncarriers. Research results point to *H. pylori*, *Prevotella capri*, and *Cutibacterium acnes* as strong risk factors and also suggest that *Lactococcus lactis* may be a protective factor in the development of stomach cancer, as its abundance was significantly higher in control than in cancer patients [21]. Interestingly, in the research of Hsieh et al. [41], attention was paid to the genera of *Clostridium*, *Fusobacterium*, and *Lactobacillus*, regardless of their role in the oncogenesis process, stressing, however, that their overabundance could be a diagnostic tool in gastric cancer.

Additionally, bacteria usually colonizing the oral cavity as commensals or opportunistic pathogens, including genera *Neisseria*, *Alloprevotella*, and *Aggregatibacter*, species *Streptococcus mitis*, *S. oralis*, *S. pneumoniae*, and the *Porphyromonas endodontalis* strain, were also found in gastric cancer [37]. It has been reported that oral bacteria were more abundant in gastric cancer compared with intestinal metaplasia and superficial gastritis. Attention was paid to several species of oral microbiota—*Streptococcus anginosus*, *Peptostreptococcus stomatis*, *Parvimonas micra*, *Slackia exigua*, and *Dialister pneumosintes*—they may play a key role in the development of the carcinogenesis process and could be used as noninvasive biomarkers to distinguish gastric cancer from superficial gastritis [42]. Other authors also note that the high level of periodontal pathogen colonization is associated with an increased risk of gastric precancerous lesions [43, 44]. A very interesting study was conducted by Sun et al. [45], which aimed to examine the characteristics of the oral microbiome in patients with gastric cancer and introduced screening tests for stomach cancer by using oral microbiome. It has been shown that changes in oral microbiome can be used as an indicator for gastric cancer in diagnostics. The following taxonomic units were considered in the assessment panel: *Veillonella*, *Prevotella*, *Leptotrichia*, *Rothia*, *Capnocytophaga*, *Aggregatibacter*, *Campylobacter*, *Megasphaera*, *Tannarella*, and *Granulicatella*. Moreover, the research by Wu et al. [46] indicated that even the microbiome of the tongue could be used in the early detection and prevention of stomach cancer. It was found that the greater abundance of *Firmicutes* and lower abundance of *Bacteroidetes* were associated with an increased risk of gastric cancer.

***Helicobacter pylori* and its association with gastric cancer**

A huge part of the research focuses on the participation of individual pathogens such as *Helicobacter pylori* and its ability to initiate disease such as gastritis or gastric cancer. Almost half of the world's population is made up of carriers of *H. pylori*. In recent decades, a decrease in prevalence has been observed, which may be related to the improvement of socioeconomic conditions [47]. However, in developing countries, *H. pylori* infection is still a big problem that affects about 80% of the people [31].

Initially, it was called *Campylobacter pylori*, while after the discovery of morphological, biochemical, and genetic differences between *Campylobacter pylori* and other bacteria of this genus, a new genus *Helicobacter* was created in 1898, and the human-isolated bacterium was called *Helicobacter pylori*. *H. pylori* belongs to the ϵ -proteobacteria and is Gram-negative, spiral shaped, microaerophilic flagellatum bacteria. Despite the relatively unfavorable conditions in the stomach, this is the only known *H. pylori* habitat. The bacterium has unique adaptations for survival and proliferation in the stomach environment, including urease activity, ability to penetrate through the gastric mucus layer (spiral shape, presence of flagella, mucolytic enzymes, the production of host mucin), and binding of bacterial surface adhesives to the corresponding receptors of the gastric epithelial cells [30, 31]. All clinical pathogenic strains are urease-positive, while urease-negative strains that are nonpathogenic can be found only in laboratory culture [29].

In 1994, the International Agency for Research on Cancer (IARC) recognized *Helicobacter pylori* as a class I carcinogen. *H. pylori*-induced carcinogenesis is primarily due to the expression and activity of VacA vacuolizing cytotoxin that is responsible for vacuolization of eukaryotic cells, and the activity of the CagA protein encoded by cag PAI pathogenicity island. The tumorigenic process is associated with the polymorphism of PAI genes. CagA interacts with host proteins via a dependent and independent phosphorylation pathway causing changes in motility, cell proliferation and polarity, resulting in a phenotype change of eukaryotic cells [14, 48]. The PAI also includes genes whose products build the so-called type IV secretion system (T4SS) responsible for the direct transfer of CagA toxin to the cytoplasm of gastric mucosal epithelial cells [49]. Additionally, VacA and CagA produced by *H. pylori* were detected in hepatic tissue with hepatocellular carcinoma, and it was additionally found that bacterial LPS promotes the growth and migration of liver cancer by increasing the level of IL-8 and TGF- β 1 [50].

Colonization of the gastric mucosa by *H. pylori* initially involves the antral part, and over time it spreads to the entire

mucosa initiating the inflammatory process. Each *H. pylori* infection ultimately leads to the development of chronic gastritis; however, most cases are without a complaint and do not require treatment. Only about 15% cases have symptoms [31].

Also, chronic inflammatory infection caused by *H. pylori* increases the risk of stomach cancer more than threefold [51]. Although, 1%–2% of people infected with *H. pylori* develop stomach cancer. The process of carcinogenesis begins with chronic inflammation that causes atrophic gastritis, intestinal metaplasia, dysplasia, and finally cancer. *H. pylori* usually colonizes the gastric antrum and causes antrum-predominant gastritis in most cases. The development of gastric mucosal lesions is known as Correa's gastric cancerogenesis cascade [26, 52].

Epstein-Barr virus (EBV) and its association with gastric cancer

Viruses such as EBV, CMV, and HHV6 were detected in gastric tumors, but among them EBV is the most important. EBV is one of the most common viruses in the world, and around 90% of the world's population has anti-EBV antibodies. Infection is usually asymptomatic [53]. The pathogen has the ability to determine latency in B lymphocytes. A decrease in host immunity may cause reactivation of the infection and make the virus enter the lytic cycle. EBV belongs to oncogenic viruses that promote proliferative processes. Burkitt's lymphoma and squamous cell carcinoma of the nasopharynx are among the most frequently mentioned EBV-related diseases, but a relationship with the development of gastric cancer and Hodgkin's lymphoma has also been shown [54, 55, 56].

EBV infection in the development of stomach cancer is associated with abnormal methylation of tumor suppressor genes, such as *RUNX1*, *RBM5*, and *PSME1* [15]. Virus proteins involved in the process of carcinogenesis, such as EBNA-1, EBNA-2, EBNA-3, LMP-1, and LMP-2 have been described. EBNA-1 is responsible, among others, for suppressing the *TP53* gene encoding the p53 protein, involved in the regulation of many cellular processes, such as DNA repair or the induction of apoptosis in response to DNA damage; EBNA-1 plays a major role in replication, activating transcription. EBNA-2, EBNA-3, LMP-1, and LMP-2 are necessary for lymphocyte immortalization and their retention in the lymphoblastoid phase. LMP-1 disturbs cellular metabolism, especially regulation of apoptosis and cell cycle; it leads to an increase in cytokine production, increased expression of adhesion molecules, and anti-apoptotic genes [54, 55]. The presence of EBV DNA is detected in 90% of gastric lymphoid epithelial carcinomas and in 5%–25% of gastric adenomas [14].

EBV and *H. pylori* cause chronic gastritis, and in the case of coinfection, the inflammatory process is significantly increased. *H. pylori* and EBV infections alter miRNA expression in host cells, which modulates the host's inflammatory immune response, promoting bacterial survival in the gastric mucosa and inhibiting apoptosis in EBV positive cells [57].

Proton pump inhibitors (PPI) and their influence on gastric microbiota

PPIs have been among the most-prescribed drugs in the world since they were launched in the 1980s. They work by blocking the H⁺/K⁺ ATPase of parietal cells. They are used to treat peptic ulcer disease (PUD), *H. pylori* infection, dyspepsia, and gastroesophageal reflux disease (GERD). Regardless of other factors, PPIs can cause gastric atrophy. They inhibit the secretion of hydrochloric acid in the stomach, thus affecting the environment in the stomach causing hypergastrinemia and enterochromaffin cell hyperplasia [51, 58]. It is worth emphasizing that gastric acidity plays a key role in filtering out bacteria and preventing the development of enteric infection [19, 31]. Many published papers pay attention to the increase in gastric pH caused by the use of PPI, which can lead to bacterial overgrowth. In the presence of chronic hypochlorhydria the defense mechanism against pathogenic bacteria is weakened, which, among other things, results in infections caused by bacteria such as *Clostridioides difficile* and *Campylobacter jejuni*. Acid suppression leads to the growth of luminal and mucosal bacteria other than *Helicobacter pylori*, which can exacerbate chronic inflammation [16, 17]. The simultaneous occurrence of *H. pylori* and other bacteria has a synergistic effect on the induction of a higher level of serum cytokines and an increased risk of atrophic gastritis. Additionally, some studies indicate that bacterial migration from the antrum to the gastric body and fundus occurs with long-term use of PPI [58].

PPIs are used in the eradication of *H. pylori*. Eradication allows the reduction of the risk of gastric cancer and noncardia gastric cancer [59]. However, the beneficial effect of *H. pylori* eradication is limited to patients without baseline preneoplastic lesions [60, 61]. Eradication can reverse chronic gastritis in most and atrophic gastritis in some patients, whereas in the case of intestinal metaplasia it can only slow down the development of gastric cancer [18]. It is worth noting the growing *H. pylori* resistance to drugs. The increase in resistance to metronidazole, clarithromycin, and levofloxacin should be the reason for susceptibility testing before treatment [62].

Long-term use of PPI (over three years) and *H. pylori* infection act synergistically to increase the risk of gastric cancer [51]. Brusselsaers et al. [63] also state that the risk of

developing cancer increases with the duration of use of PPI: the higher risk is in the first three years, but after five years the risk decreases. According to Peng et al. [64], the use of PPI increases the risk of gastric cancer about 2.5 times among patients with GERD (gastroesophageal reflux disease). Additionally, GERD patients are more likely to develop cancers of the upper gastrointestinal tract [65].

During PPI therapy, the most numerous group of microorganisms are the *Streptococcaceae*, followed by the *Prevotellaceae*, *Campylobacteraceae*, and *Leptotrichaceae* [66]. In addition, patients on long-term PPI treatment have a greater abundance of *Methylophilus* compared with those on short-term PPI treatment [67]. Higher pH causes the growth of oral bacteria: *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra*, *Slackia exigua*, and *Dialister pneumosintes*. In connection with the induction of various metabolic pathways, they may be involved in the progression of gastric cancer [42]. Also, the oral microbiota, which possesses nitrate reductase, produces N-nitroso compounds from food nitrates, which are known gastric carcinogens [51].

Research is needed to determine the maximum duration of PPI use when they are therapeutically active and do not increase the risk of cancerogenesis. Data on PPI are divergent, although the fact that in the US, despite the widespread long-term use of PPI, the occurrence of gastric cancer is relatively low is noteworthy [19]. There are also opinions like that of Zhang et al. [68] that PPIs can potentially be used as effective and relatively safe anticancer drugs that inhibit gastric cancer metastasis.

Conclusions

Biodiversity of microbiota of the human stomach may be more complex than we previously thought, and *H. pylori* colonization is that of the most important pathogen with clear impact on this diversity.

Despite the differences among various models and studies, there is strong evidence that the microbiota is necessary to maintain homeostasis and promote health, and that dysbiosis contributes to the host's susceptibility to infection and disease. The role of *H. pylori* in gastric microbiota is complex, and its connection with the inflammatory process and finally cancer has been proven. Another infectious agent, the Epstein-Barr virus, intensifies this process, which draws attention to the search for methods to identify the disease markers. Research in animal models could allow, in the future, the development of a strategy to manipulate the microbiota to prevent the development of stomach cancer. Also, with the help of molecular methods, which are increasingly used to explore the human microbiome, we can better understand the

structure and relationships between individual microorganisms and then use this knowledge for diagnosis and therapy.

Abbreviations

CagA: cytotoxin-associated gene A, **CFU:** colony forming units, **CMV:** Cytomegalovirus, **DNA:** deoxyribonucleic acid, **EBNA:** Epstein-Barr nuclear antigen, **EBV:** Epstein-Barr virus, **GERD:** gastro-esophageal reflux disease, **HERVs:** human endogenous retroviruses, **HHV6:** human herpesvirus 6, **IARC:** The International Agency for Research on Cancer, **IL-8:** interleukin 8, **LMP:** latent membrane protein, **LPS:** lipopolysaccharide, **miRNA:** microRNA, **rRNA:** Ribosomal ribonucleic acid, **OTU:** operational taxonomic units, **PPI:** proton pump inhibitors, **PUD:** peptic ulcer disease, **T4SS:** type IV secretion system, **TCGA:** The Cancer Genome Atlas, **TGF- β 1:** transforming growth factor beta 1, **VacA:** vacuolating toxin.

Authors' Contributions

M.D.: Drafting the article, literature review, writing the manuscript; D.P.-L.: Revising the article critically for important intellectual content, final proofreading and approval of the version for publication.

Conflict of Interest

The authors have no potential conflicts of interest to declare.

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