Oral *Helicobacter pylori*: Interactions with host and microbial flora of the oral cavity

Helicobacter pylori w jamie ustnej – interakcje z mikrobiotą gospodarza

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Abstract

The role of the oral cavity as a reservoir for *Helicobacter pylori* is still a controversial issue. There is a great number of articles indicating the presence of this pathogen in the oral cavity, but discrepancies among techniques for *H. pylori* detection and the variations in the patients tested often make it difficult to formulate a final verdict. Outer membrane proteins (OMPs) are one of the most important factors determining colonization of *H. pylori* in the oral cavity. Among them, the key role is attributed to BabA, SabA and NapA, all of which promote adherence and retention within this area. The oral cavity is characterized by the co-existence of numerous microorganisms which may potentially affect the physiology and morphology of *H. pylori*. The presence of coccoid-stimulating factors and relatively low levels of AI-2 in the early- to mid-stages of supragingival plaque allow dental *H. pylori* to colonize this niche as nonculturable spherical forms. On the other hand, subgingival plaque characterized by high numbers of periopathogens, capable of synthesizing high concentrations of AI-2, may favor the presence of mixed populations of spiral and coccoid *H. pylori* forms. This review article provides an up-to-date knowledge about the ability of oral *H. pylori* to interact both with the host and the local microflora of the oral cavity.

Key words: biofilm, *Helicobacter pylori*, oral cavity, dental plaque, microflora

Słowa kluczowe: biofilm, *Helicobacter pylori*, jama ustna, płytka nazębna, mikroflora

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Every living organism, to maintain the stability of its internal environment, must constantly adapt at the molecular, cellular and physiological levels. The gastrointestinal tract is the largest surface of the body exposed to the external environment. Therefore, the presence of mucous membranes and a well-functioning immune system is essential to protect the host organism.¹

The first barrier in the digestive system, including the oral cavity, is the mucus layer. The mucus matrix forms a strongly hydrophilic colloid, mainly composed of water (about 95%), but also lipids (phospholipids, cholesterol), nucleic acids, polysaccharides and proteins (antibodies, defensins, growth factors).² Antibodies found in saliva belong mainly to 2 classes, i.e., IgA (90–98%) and IgG (1–10%). Their presence contributes to the inactivation and opsonization of microorganisms, which are then detected by the antigen presenting cells (APCs) and phagocytosed. In the oral cavity, antibody production comes through the stimulation of B cells, located within the mucosa-associated lymphoid tissue (MALT). The main region of their recruitment is nasopharynx-associated lymphoid tissue (NALT), which consists of the adenoids and palatine tonsils of Waldeyer's ring. Salivary defense proteins play an important role in the protection of the oral environment. These include, but are not limited to: lysozyme, bactericidal increasing proteins (BPIs), palate lung and nasal epithelial clone proteins (PLUNCs), salivary amylase, cystatins, proline-rich proteins, statherin and peroxidases. The function of these proteins is to adsorb to the microbial surface and form ion channels, which leads to the microorganism's destruction. In addition, some of these compounds also possess immunomodulating activity and the ability of chelating metal ions from the local environment (Fe^{3+} , Ni^{2+} , Cu^{2+}).³

Another important component of mucus is the high molecular weight oligomeric glycoproteins, namely mucins. More than 20 mucins covering the epithelial surface of the respiratory, digestive and reproductive tracts have been detected in the human body.⁴ MUC1, MUC4, MUC19, MUC5B and MUC7 are produced in the oral cavity, while the dominant and most important groups are the last two.^{4,5} MUC5B is a compound with a molecular weight in excess of 1000 kDa and consists of disulfide-bonded subunits.³ MUC5B is a member of the gel-forming mucin family, secreted by the submandibular, sublingual, palatine and labial mucous cells of the salivary glands.⁴ MUC7 is a secretory mucin present in the form of monomers (180–200 kDa) or dimers. It is located mainly in the submandibular and sublingual salivary glands.^{3,4} Mucins and defense proteins play a crucial role in the formation of acquired pellicle, i.e., a thin layer (0.5–1 μm) of saliva components with the ability to bind to calcium hydroxide. These components have receptors for microbes and, thus, in the surface immune exclusion process, con-

tribute to the immobilization of microorganisms and protection against host tissue invasion.^{3,5} Despite the protective function, this process also has a disadvantageous aspect associated with the formation of biofilm by microorganisms colonizing the oral cavity.^{3,4}

Populations of microbes often form highly organized complexes called biofilms. These structures are made up of microbial cells embedded within the extracellular matrix, which accounts for nearly 90% of biofilm and consists of exopolysaccharides, proteins, lipids and extracellular DNA (eDNA). Biofilm provides a protective environment against adverse conditions including temperature, pH and oxidation-reducing potential fluctuations, presence of antimicrobial agents (antibiotics, enzymes) and the action of innate and adaptive immune systems. Moreover, besides the protective function, biofilm also determines co-adhesion and aggregation of the cells, formation of a strongly hydrated environment (protection against drying) and sorption of organic and inorganic compounds from the surrounding environment.⁶ The formation of biofilm is also related to another important process, supervising the physiology of microorganisms, called quorum sensing (QS). In the course of this mechanism, microbes communicate using small molecular signaling molecules, which allow them to sense their density and integrate actions that resemble multicellular organisms.⁷ With respect to the intestinal flora, the oral cavity is one of the most colonized areas in the human body. The number of bacterial species localized in this region is estimated at 600–800.⁸ In addition to the enormous diversity of microorganisms, the oral ecosystem is characterized by succession, microbial composition variations and changes in conditions within the different morphological regions of the oral cavity.⁹ For this reason, oral biofilm is a very dynamic environment modified by availability and type of nutrient substances, pH, microbial toxic metabolite concentration, host immune response and shear forces.¹⁰ The surface of the teeth is covered by the saliva components forming the acquired pellicle.⁹ This area is colonized by microorganisms capable of linking to these components, referred to as early colonizers, i.e., *Streptococcus* spp., *Actinomyces* spp., *Gemella* spp., *Veillonella* spp., *Selenomonas* spp., and *Capnocytophaga* spp.^{8–11} *Fusobacterium nucleatum* is known to act as the “bridge bacterium”. This is due to the long, filamentous shape and numerous multivalent adhesins exposed on the surface of this bacterium. These features make it possible to co-adhere with *Streptococcus* spp. and recruit Gram-negative rods (late colonizers), including: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans*.^{8,12} In many scientific studies, it is also suggested that the oral cavity may be a reservoir site for Gram-negative, microaerophilic *Helicobacter pylori*.^{5,13–17}

Colonization of the oral cavity by *H. pylori*

H. pylori infections occur most often in childhood, and clinical symptoms may develop over many years. This microorganism inhabits the gastric mucosa, where it is able to survive despite the therapy used. Persistent infections induce chronic gastritis, which is associated with the risk of developing gastric or duodenal ulcers and gastric cancers.¹⁸ Transmission from person to person seems to be the main mechanism of the bacteria spreading. It is suggested that environmental reservoirs, such as contaminated water and food or infected animals, also exist. The factors influencing the frequency of *H. pylori* infection include age, sex, genetic predisposition, immunological status, and hygiene level.¹⁶

The outer membrane proteins (OMPs) presence is a crucial factor determining host colonization by *H. pylori*. It is estimated that 4% of the genome of this bacterium encodes information for the production of these structures.¹⁹ The ability of direct adhesion to host epithelial cells is a process responsible for persistent infections, because it contributes to the protection against the removal of microbes from the mucous layer and enables delivery of virulence factors to eukaryotic cells.^{19,20}

One of the most important *H. pylori* adhesins is BabA (blood group antigen binding adhesin A), which allows interaction with fucosylated Lewis b (Le^b) and H type 1 antigens. For this reason, the protein has a strong affinity to MUC5AC, mucin secreted in the stomach environment. Additionally, BabA also determines adhesion with saliva components, i.e., MUC5B, proline-rich proteins and salivary agglutinin (gp-340).²⁰

The second essential adhesin produced by *H. pylori* is SabA (sialic acid-binding adhesin). This protein promotes the adherence of bacteria to the inflamed stomach tissue, which is characterized by increased mucin sialylation (the process of linking sialic acid residues to Lewis antigens). This property also affects the ability of *H. pylori* to associate with salivary glycoproteins, such as MUC5B, MUC7, carbonic anhydrase VI, zinc- α -2-glycoprotein or heavy chains of sIgA1. The expression of SabA is regulated by the pH of the environment, and the amount of *sabA* mRNA decreases under acidic conditions.²⁰ This feature makes it possible that in more neutral pH conditions (e.g. in the oral cavity) SabA production can play a key role in *H. pylori* oral mucosa colonization.

The third vital adhesin expressed by almost all clinical *H. pylori* strains is NapA (neutrophil-activating protein). This protein has the ability to stimulate myeloperoxidase secretion and the production of reactive oxygen species (ROS) by neutrophils. Therefore, this factor most likely contributes to the release of nutrients from destroyed tissues.²¹ *H. pylori* NapA has been shown to be capable of binding to the sulfur oligosaccharides of saliva, i.e., sulfo-Le^a, sulfo-galactose and sulfo-*N*-acetyl-glucosamine.²²

The significance of these proteins was confirmed by an oral *H. pylori* virulence factors analysis, because the presence of all these adhesins was detected in this microbe. Furthermore, the ability of oral *H. pylori* strains to secrete factors associated with tissue damage, inflammation initiation and nutrient uptake, i.e., VacA (vacuolating cytotoxin A), CagA (cytotoxin-associated antigen A), and serine protease HtrA, was observed.²³ The ability of *H. pylori* to cause pathological changes in the oral cavity is uncertain. In the stomach environment, this bacterium has many factors facilitating persistent colonization of the gastric mucosa. These properties include the ability to invade eukaryotic cells, disrupt cell-cell contact, promote inflammation, inhibit immune cell activity (including T cells) and induce excessive host cell proliferation.²⁴ *H. pylori* colonizing the oral cavity is also suggested to be involved in the development of various ailments in this region, such as halitosis, glossitis, recurrent aphthous stomatitis and dental caries.²⁵ Studies on the human acute monocytic leukemia cell line (THP-1) have shown that these microorganisms may be responsible for causing pathological changes in the oral cavity. The main factor responsible for this effect was CagA, because strains producing this protein contributed to significant increases in inflammatory cytokine secretion (IL-6, IL-8, IFN- γ) as compared to non-producing strains.²⁶ The capacity of *H. pylori* to interact with the host and modulate the local environment was also demonstrated by the ability of this bacterium to induce elevated MUC5B and MUC7 levels by three- and twofold, respectively. The increase in the amount of these components, which are receptors for oral *H. pylori*, can promote colonization and retention within the oral cavity.²⁷ Despite this, there is a need to increase the number of studies showing a direct relationship between *H. pylori* infection/colonization and oral pathophysiology.

Epidemiology of oral *H. pylori* infections

There are many diagnostic methods for the detection of *H. pylori*, but none of them are 100% specific and sensitive. Invasive methods require removal of the gastric mucosa. Histopathological examination allows for accurate assessment of the degree and type of the gastric mucosa inflammation. Hematoxylin and eosin staining are used for the visualization of inflammatory cells, while Giemsa or Genta staining serve for microorganism detection. The sensitivity of the method is 89–91% and the specificity is 91–100%. Culture methods have a very high specificity (about 100%), but much lower sensitivity (78–94%). In addition, the culture of this bacterium is necessary to determine the sensitivity of *H. pylori* to antibiotics. Rapid urease testing is the simplest, cheapest and fastest test determining urease activity in biopsy specimens. This method has a sensitivity of 82–94% and

a specificity of 96–96%. PCR is a highly useful method because, apart from the detection of bacteria (the highest accuracy in 16S rRNA gene detection), it also provides information about the production of virulence markers, such as *cagA* or *vacA*. Improved PCR methods with colorimetric detection of *H. pylori* DNA using isothermal helicase-dependent amplification result in sensitivity and specificity of up to 95%. Non-invasive methods are divided into active (detecting active *H. pylori* infection) or passive (showing evidence of contact with bacteria). Among the active assays are: the fecal antigen test which is used to determine the presence of *H. pylori* antigens in feces (sensitivity and specificity 92% and 94%, respectively), and the urease test. The latter is based on the detection of *H. pylori* urease activity during active infection. The level of labeled CO₂ derived from the decomposition of the labeled urea ¹³C is measured. Sensitivity and specificity are 91% and 94%, respectively. Serological tests belong to passive assays and are based on the detection of IgG anti-*H. pylori* antibody levels in the plasma. The biggest disadvantage of these tests is the lack of the ability to delineate between active infection and temporal exposure to *H. pylori* antigens. This method is characterized by a high sensitivity (90–97%), but a low specificity (50–96%).^{28,29}

The role of the oral cavity as a reservoir for *H. pylori* is still a controversial issue. There are a great number of articles indicating the presence of this pathogen in the oral cavity, but discrepancies among techniques for *H. pylori* detection and the variations in patients tested often make it difficult to formulate a final verdict. One of the newest and more accurate meta-analyses conducted by Sayed et al. suggests that dental plaque can be an alternative, extragastric site of *H. pylori* residence.¹⁴ Briefly, the frequency of *H. pylori* detection depends on the diagnostic technique used. The prevalence of bacteria detection using urease tests in most studies ranged between 50% and 100%. PCR studies have even greater divergence in results, i.e., 0–100% (of which only 7/35 studies showed a frequency greater than 50%). Culture methods have a low sensitivity, with prevalence lower than 50% (half of studies with prevalence lower than 10%). Inhomogeneous results were obtained using immunoassays (2 studies with frequencies above 65% and 2 with very low prevalence, 0% and 11%).¹⁴

Namiot et al., in oral *H. pylori* detection, used immunoassays intended routinely for fecal antigen detection.^{30–32} The authors suggest that these tests can be successfully used to detect *H. pylori* in the oral cavity after appropriate sample preparation (preincubation in a microaerophilic atmosphere). This procedure increases the incidence of *H. pylori* more than twice.³¹ Using this method, a high prevalence of these microorganisms in dental plaque was reported, i.e., 58.7%³² and 65.6%.³⁰ It was suggested that plaque reduction and oral health had no effect on the amount of antigens of these bacteria.³²

Interesting results were obtained using molecular techniques. The presence of *H. pylori* in the oral cavity of patients with gastric *H. pylori* was reported in 46% of the subjects. Moreover, a relationship between these bacteria and increased approximal plaque index (API) and bleeding on probing (BOP) values has been noted. Within strains colonizing the oral cavity, only 16% expressed *cagA*, suggesting that this virulence factor is not critical in causing pathological changes to the oral cavity mucosa.³³ In another study using PCR, the DNA of oral *H. pylori* was isolated more frequently in patients with oral ailments (leukoplakia and oral lichen planus; 20% and 23%, respectively) than in those without these diseases (0%). The potential of these microorganisms to promote inflammatory conditions in the oral environment was proposed on this basis.³⁴

There is also a relationship between the presence of pathological gingival pockets and *H. pylori* infections. Umeda et al. found that in patients with gastric *H. pylori* infection, the detection frequency of these bacteria in the oral cavity was 4-fold higher (41.2%) when patients had gingiva with a pocket depth of ≥4 mm than those with healthy periodontium (9.1%).³⁵ Similar observations were made in a large-scale epidemiological study of 4504 patients by Dye et al.³⁶ It has been shown that pocket depth greater than 5 mm correlates positively with *H. pylori* seroprevalence. Despite the ability of *H. pylori* to colonize dental plaque, this area is not the only site of colonization. Other regions include the tonsils and dorsal mucosa of the tongue.^{15,37}

Many studies based on genetic or serological tests in *H. pylori* detection have shown the presence of this bacterium in the oral cavity or stomach, while the number of studies demonstrating the effective isolation of this microbe from the oral environment is still very low. Hirsch et al.²³ successfully managed to isolate live *H. pylori* from samples taken from dental canals. In the morphological observation, using field-emission scanning electron microscopy, the presence of a mixed population of spiral rods and aggregates of coccoids was observed. Cultivation of the samples taken from supragingival plaque was unsuccessful, suggesting that in this niche, *H. pylori* occurred only in the coccoid form. The presence of particular *H. pylori* morphological forms in various areas of the oral cavity may indicate that the physicochemical parameters and local microbiological flora may affect the transformation process of *H. pylori*.

Interactions of oral *H. pylori* with microbial flora

The diversity of environmental factors in the oral cavity contributes to the development of specific microbiota in different regions.¹¹ Tooth-associated biofilm can be divided into the supragingival (on the exposed surface of the

enamel) and subgingival (below the gums and inside the periodontal pocket) regions.⁹

Supragingival plaque is characterized by a low level of species diversity and the dominance of *Streptococcus* spp., *Actinomyces* spp. and *Lactobacillus* spp.³⁸ The presence of these microorganisms is related to the intense saccharolytic metabolism associated with the fermentation of carbohydrates to organic acids, and the subsequent acidification of the local environment. In addition to the acidification, the process of supragingival plaque maturation is also linked with the formation of microaerophilic conditions. This is the result of the oxygen consumption by the NADH oxidase in the Embden-Meyerhof-Parnas pathway. This process is accompanied by the appearance of *F. nucleatum* and late colonizers, which are sensitive to high oxygen levels.¹¹

In vitro studies have shown that *F. nucleatum* supports the growth of *P. gingivalis* in the presence of elevated oxygen concentrations (10% and 20%) and lack of CO₂. Under such conditions, the oxygen-sensitive capnophilic *P. gingivalis* was unable to survive in monoculture. During the co-culture with *F. nucleatum*, a strong growth of this bacterium was observed.³⁹ The ability to generate a capnophilic environment by *F. nucleatum* is a feature that could potentially promote the survival of another bacterium, for which the main factor limiting growth is access to CO₂, i.e., *H. pylori*.⁴⁰ It has been observed that *H. pylori* has a strong capacity to co-aggregate with *Fusobacterium* spp. naturally isolated from dental plaques (*F. nucleatum* and *F. periodontium*).⁴¹ The tendency to co-aggregate with *F. nucleatum* was also confirmed in another experiment.⁴² In addition, it has been determined that *P. gingivalis* may also be involved in such interaction and that this is a fimbriae-dependent process.⁴² On this basis, it can be concluded that the presence of *H. pylori* is strongly associated with the physiological functioning of these two bacterial species (*F. nucleatum* and *P. gingivalis*) within the dental plaque, and vice versa.

Streptococcus spp. is the dominant genus in supragingival plaque. The ability of these bacteria to ferment carbohydrates into acidic products (acetic, formic and lactic acid) reduces local pH values near 5, during high availability of fermentation substrates.⁹ These are the conditions in which *P. gingivalis* and *F. nucleatum* are unable to survive, because the minimum pH tolerated by these microorganisms is 6.5 and 5.5, respectively.¹¹ Hence the close association of microbes capable of buffering acidic pH is highly desirable. The presence of bacteria able to utilize lactic acid, the main fermentation product of *Streptococcus* spp., may protect the supragingival plaque from excessive acidification.⁸ *H. pylori* has been shown to have genes responsible for the uptake and metabolic conversion of D- and L-lactose.⁴³ The pH-buffering process in supragingival plaque can also be mediated in an ammonia-dependent manner. *H. pylori* produces urease, an enzyme which converts urea into CO₂ and ammonia.

The urease produced by *H. pylori* has a very high activity. It has been shown that the rate of urea hydrolysis by the enzyme of this bacterium is $36 \pm 28 \mu\text{M}/\text{min}/\text{mg}$ of bacterial proteins. This result is twice as high as that of *Proteus mirabilis* and 10 times higher than in other urinary tract pathogens.⁴⁴ In vitro studies have demonstrated that the ability to produce ammonia is a key contributor to the preservation of the species diversity in oral biofilm. The presence of physiological concentrations of urea in the culture medium (1–10 mM urea in saliva) determined the bacterial population heterogeneity. However, in a culture with strains defective in urease production or the absence of urea in the culture medium, there was a drastic decrease in the viability of periopathogens (*P. gingivalis*, *F. nucleatum*, *Prevotella intermedia*), *Neisseria subflava* and *Streptococcus oralis*, and the dominance of biofilm by cariogenic bacteria, i.e., *Streptococcus* spp. (other than *S. oralis*) and *Lactobacillus* spp.⁴⁵

Supragingival plaque consists mainly of early colonizers, *Streptococcus* spp. and *Actinomyces* spp., which may potentially modulate the physiology of oral *H. pylori*. It has been observed that such microorganisms have the ability to inhibit *H. pylori* growth in vitro.⁴² Diffusible compounds secreted by *Streptococcus mutans*⁴⁶ and *Streptococcus mitis*⁴⁷ contribute to a dramatic decrease in *H. pylori* viability. However, microscopic observation has shown that this effect was caused by the conversion of *H. pylori* to nonculturable coccoid forms. This mechanism is consistent with the lack of possibility to isolate this bacterium from supragingival plaque, in which *Streptococcus* spp. is the dominant microbial genus.^{9,23} Streptococci are a source of SDSF (*Streptococcus* diffusible signal factors), which may be involved in the morphological transformation of *H. pylori* into coccoid forms.⁴⁸ In an experiment, Khosravi et al. determined the effect of *H. pylori* and *S. mitis* co-culture on the gene expression of both bacteria.⁴⁷ It was observed that streptococci expressed genes encoding phosphoglycerate kinase (PGK) only when co-existing with *H. pylori*. PGK is a glycolytic enzyme responsible for the conversion of 3-phosphoglycerol aldehyde to 1,3-bisphosphoglycerate. PGK is a surface protein produced by streptococci in increased amounts during the biofilm phase and is responsible for cell-cell interactions.⁴⁹ During co-culture in *H. pylori*, significant reduction in oxidative stress proteins, including glutathione metabolism enzymes, thioredoxin, flavodoxin and thiol peroxidases, have been observed. On the other hand, the amount of proteins involved in RNA degradation and nucleotide excision repair were increased.⁴⁷ The reduction of genes encoding *H. pylori* antioxidants may potentially be due to increased physical contact between the bacterial cells and PGK-dependent aggregation/biofilm promotion in *H. pylori*.⁴⁹ Despite the use of filters (pore size 0.22 μm) for the spatial separation of both microorganisms, it cannot be excluded that interactions between PGK, present on the surface of membrane vesicles secreted by *Strepto-*

coccus ($<0.2\ \mu\text{m}$), and *H. pylori* cells still may exist.⁵⁰ The increase in the amount of *H. pylori* proteins responsible for the DNA rearrangement can be beneficial under stressful conditions by rapidly selecting well-adapted strains, for example to antibiotics presence.^{51,52} Because of this, the co-aggregation of both bacteria in dental plaque can promote the survival of *H. pylori* in the oral cavity.

One of the key signaling substances produced within dental plaque is autoinducer-2 (AI-2).⁸ This compound is a chemorepellent agent that promotes the dispersion of *H. pylori* aggregates/biofilms and initiates negative chemotaxis against the source of these signals.⁵³ Hence, the colonization of this niche by *H. pylori* should be impeded or even impossible. Commensal plaque bacteria secrete relatively low concentrations of AI-2 and respond to much lower levels of these substances in the environment (less than 100 pM) than periopathogens, considered to be strong AI-2 producers (nanomolar concentrations).⁹ The amount of AI-2 produced endogenously by *H. pylori* corresponds to the concentrations of signaling compounds secreted by periopathogenic microbes ($\sim 37\ \text{nM}$).⁵³ Therefore, the presence of coccoid-stimulating factors and relatively low levels of AI-2, in the early- to mid-stages of supragingival plaque allow dental *H. pylori* to colonize this niche as nonculturable spherical forms. On the other hand, subgingival plaque characterized by a high density of periopathogens, and thus also higher concentrations of AI-2, may favor the presence of mixed populations of spiral and coccoid *H. pylori* forms.

Subgingival biofilm is composed of many species of microbes, including the dominance of *Actinomyces* spp., *Tannerella forsythia*, *Fusobacterium nucleatum*, *Spirochetes* and *Synergistetes*.³⁸ In addition, the presence of *A. actinomycetemcomitans*, *Streptococcus parasanguinis*, *Filifactor alocis*, *Eubacterium* spp., *Prevotella* spp., *Porphyromonas* spp., and *Campylobacter* spp. is also detected.^{11,54} Metabolism of the bacteria located within this niche is characterized by the breakdown of nitrogen compounds (amino acids, peptides, proteins) derived from gingival crevicular fluid (GCF). This niche possesses a neutral pH, abundance of secondary metabolites, short chain fatty acids (SCFAs), and ammonia.^{9,11,12}

In a study, Henne et al. observed that in patients with periodontal disease, *Campylobacter rectus* could be detected at elevated levels in diseased subgingival sites compared to the healthy control group.⁵⁵ The reverse relationship was observed in the case of *Campylobacter concisus*. Such microbial shift was accompanied by more frequent isolation of “red complex” bacteria, including *P. gingivalis* 15.9% vs 0%, *F. nucleatum* 33.9%, vs 3.1%, and *T. forsythia* 28.2% vs 0.1%, respectively. A similar relationship was also observed in another Gram-negative bacterium from the Epsilonproteobacteria class, i.e., *H. pylori*. Hu et al. investigated the effect of *H. pylori* on inflammation severity and the presence of oral pathogens.²⁶ In samples taken from gingival lesions, the incidence of *P. gingivalis*,

P. intermedia, *F. nucleatum* and *T. denticola* was significantly higher when *H. pylori* co-existed. This was related to the exacerbation of periodontal disease. Interestingly, the inverse relationship between the presence of *H. pylori* and the pathogenic bacterium *A. actinomycetemcomitans* was also observed. The reason of this phenomenon is not known, but it cannot be ruled out that *H. pylori* can limit the amount of L-lactic acid in the local environment, which is a growth factor for *A. actinomycetemcomitans*.⁸ The mechanism of competition for lactic acid availability is uncertain and should be confirmed in experimental studies.

Influence of oral *H. pylori* on gastric infections and eradication efficacy

Colonization of the oral cavity by *H. pylori* can promote reinfections, even when antibiotic therapy has taken place. The ability to form biofilm and coccoid forms by oral *H. pylori*, and the presence of a relatively low biocide concentration in saliva often make it impossible to achieve therapeutic success. In a meta-analysis by Zou et al., it has been shown that in patients with gastric *H. pylori*, the incidence of oral *H. pylori* was significantly higher (45%, 490/1088) than in those without gastric infection (23.9%, 196/821).¹³ It was also determined that the eradication rate of systemic therapy was significantly higher in cases of gastric *H. pylori* (85.8%, 187/218) and extremely low for oral *H. pylori* treatment (5.7%, 9/158). Gao et al. determined the effect of triple therapy (systemic therapy) and triple therapy combined with oral treatment, on the eradication rate of gastric *H. pylori*.¹⁵ Combination therapy proved to be more effective, as one year after the end of treatment the incidence of these bacteria was twice as low (32.4%) as compared to the exclusive use of systemic therapy (62.8%). A similar relationship, showing an increased degree of *H. pylori* eradication after combination therapy, has been demonstrated in the meta-analyses of Ren et al.⁵⁶ and Bouziane et al.⁵⁷ It was found that the relative chance of gastric *H. pylori* recurrence in patients who underwent oral and gastric therapy was reduced by 63%.⁵⁷

Conclusions

The role of the oral cavity as a reservoir for *H. pylori* is still a controversial issue. There are a great number of articles indicating the presence of this pathogen in the oral cavity, but discrepancies among techniques for *H. pylori* detection and the variations in patients tested often make it difficult to formulate a final verdict. Urease and PCR assays have a high level of positive results in oral *H. pylori* detection, while culture methods have an extremely low sensitivity. Despite large discrepancies in the results

obtained, it is suggested that the oral cavity may be an extragastric *H. pylori* reservoir, which contributes to re-infections after successful eradication therapies. This location is characterized by the co-existence of numerous microorganisms that naturally colonize the oral cavity. That may potentially affect the physiology and morphology of *H. pylori*. It appears that in the supragingival plaque, *H. pylori* occurs in the form of nonculturable coccoids, which often prevent the detection of such bacteria using standard culture techniques. Subgingival plaque is associated with high amounts of periopathogens, and thus also higher concentrations of AI-2, which determines the presence of *H. pylori* as mixed spiral and coccoid subpopulations. Based on the considerations presented in this review, it is suggested that there is a need to increase the number of studies that identify the relationship between *H. pylori* and other microorganisms, including oral flora. Identification of these mechanisms will make it easier to understand the physiology of oral *H. pylori* and will help to create new, alternative methods to increase the effectiveness of standard systemic therapy.

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