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Colonization of *Helicobacter pylori* in the oral cavity – an endless controversy?

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ABSTRACT

Helicobacter pylori is associated with chronic gastritis, gastric or duodenal ulcers, and gastric cancer. Since the oral cavity is the entry port and the first component of the gastrointestinal system, the oral cavity has been discussed as a potential reservoir of *H. pylori*. Accordingly, a potential oral-oral transmission route of *H. pylori* raises the question concerning whether close contact such as kissing or sharing a meal can cause the transmission of *H. pylori*. Therefore, this topic has been investigated in many studies, applying different techniques for detection of *H. pylori* from oral samples, i.e. molecular techniques, immunological or biochemical methods and traditional culture techniques. While molecular, immunological or biochemical methods usually yield high detection rates, there is no definitive evidence that *H. pylori* has ever been isolated from the oral cavity. The specificity of those methods may be limited due to potential cross-reactivity, especially with *H. pylori*-like microorganisms such as *Campylobacter* spp. Furthermore, the influence of gastroesophageal reflux has not been investigated so far. This review aims to summarize and critically discuss previous studies investigating the potential colonization of *H. pylori* in the oral cavity and suggest novel research directions for targeting this critical research question.

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Introduction

Helicobacter pylori is a Gram-negative, rod-shaped, microaerophilic bacterium belonging to the order *Campylobacteriales* (see [Figure 1](#) for a field-emission scanning electron microscopy image of *H. pylori*) (O'Rourke 2001; Reshetnyak and Reshetnyak 2017). In most cases, this microorganism is positive for catalase, oxidase, and urease (Marshall and Goodwin 1987). Barry Marshall was the first to suggest a correlation between *H. pylori* and active chronic gastritis, duodenal ulcer, or gastric ulcer when he swallowed this bacterium bravely in a self-experiment (Marshall and Warren 1984), being awarded with the Nobel prize with Robin Warren in 2005. In subsequent studies, it was proven that *H. pylori* plays an essential role in the development of gastritis,

gastroduodenal ulcers, and gastric cancer (Dixon et al. 1996; Luman et al. 1996; Yamaoka 2010). *H. pylori* is classified as a Group 1 carcinogen for non cardia gastric carcinoma and low-grade B cell MALT gastric lymphoma by the International Agency for Research on Cancer (IARC) (IARC Monogr Eval Carcinog Risks Hum 2012). According to a sub-analysis from the Global Burden of Disease 2018 study, *H. pylori* was one of the primary causes of infection-attributable cancer cases worldwide in 2018 (Martel et al. 2020). Martel et al. highlighted that *H. pylori* may infect most adults once during their life course (Martel et al. 2013). Accordingly, a meta-analysis showed that about 4.4 billion individuals were infected with *H. pylori* worldwide in 2015 (Hooi et al. 2017).

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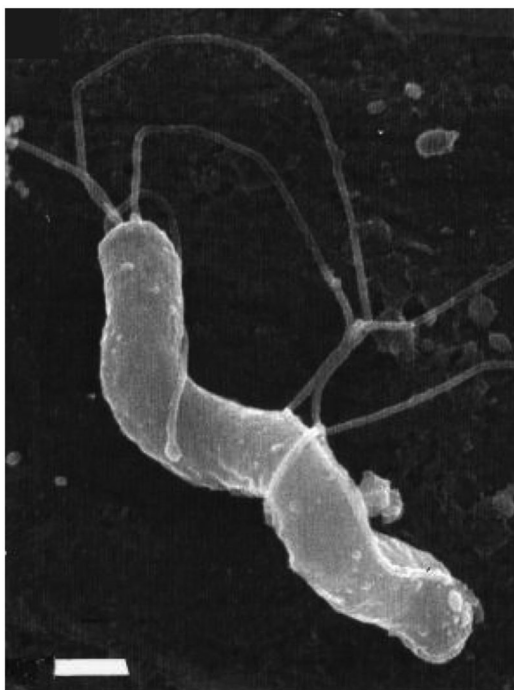


Figure 1. Morphology of *H. pylori*. Field-emission scanning electron microscopic (FESEM) image of a spiral-shaped *H. pylori* with five to seven sheathed polar flagella. The scale bar is 0.5 μm . This figure is reprinted from references (O'Rourke 2001; Reshetnyak and Reshetnyak 2017) with kind permission from the publishers.

As the oral cavity is the entry port and first component of the gastrointestinal system, researchers have also been interested in the presence of *H. pylori* in this niche (Singhal et al. 2011). There are a few studies in the literature claiming to have isolated this bacterium from dental plaque, but without any definitive proof in terms of whole genome sequencing of the given isolate or deposition in a culture collection (Agarwal and Jithendra 2012; Wang et al. 2014). Nevertheless, it was speculated that the oral cavity could also be a potential reservoir for this microorganism (Kignel et al. 2005; Agarwal and Jithendra 2012; Wang et al. 2014; Yee 2016; Urban et al. 2017). In 2003, Rickard et al. proposed that *H. pylori* was a typical colonizer of biofilms on the tooth surface in their diagrammatic representation of oral bacterial accretion on the tooth surface (Rickard et al. 2003). On the other hand, Bürgers et al. found no evidence for a link between oral and gastric *H. pylori* infections (Bürgers et al. 2008). In their study, the authors could not find any serum antibodies to *H. pylori* in patients who were found to have only oral *H. pylori* infections but without gastric involvement. The oral-oral transmission route is discussed as one of the most likely transmission pathways because *H. pylori* DNA has been found in gastric juices, vomitus, saliva,

and dental plaque (Gerhard et al. 1999; Brown 2000). For instance, kissing results in an average total bacterial transfer of about 80 million bacteria in 10 s and it may lead to the exchange of *H. pylori* (Al-Ahmad et al. 2012; Kort et al. 2014). From this finding, the question emerges concerning whether close contact such as kissing or sharing a meal, etc. can cause the transmission of *H. pylori* infections. The presence of *H. pylori* in the oral cavity may lead to further infection in the stomach. However, it is unclear whether *H. pylori* can colonize in the oral cavity at all. It is also unclear whether *H. pylori* is permanently resident or simply temporarily present in the oral cavity.

Most studies reporting on the detection of *H. pylori* in samples from the oral cavity (e.g. dental plaque or saliva) have been performed by using molecular techniques, such as polymerase chain reaction (PCR) methods (Westblom and Bhatt 1999; Kignel et al. 2005). For instance, Agarwal and Jithendra found *H. pylori* in subgingival plaque samples of eighteen (60%) patients with confirmed gastric *H. pylori* infection using PCR methods, but in 15% of control patients without gastric *H. pylori* infection (Agarwal and Jithendra 2012). Immunological methods such as the *Campylobacter*-like organism (CLO) gel test have also been used to detect oral *H. pylori*. Dane and Gurbuz tested dental plaque samples from 35 patients with gastric *H. pylori* infection using the CLO gel test and found that 29 (82.8%) of them were oral *H. pylori*-positive (Dane and Gurbuz 2016). By contrast, when using the conventional culture technique, detection results for *H. pylori* have been rather contradictory (Krajden et al. 1989; Shankaran and Desai 1995; Agarwal and Jithendra 2012). To date, no publication has definitively demonstrated cultivation of *H. pylori* from any sample taken from the oral cavity (Al-Ahmad et al. 2010, 2012). Therefore, this review aims to critically discuss the evidence of a resident or transient colonization of *H. pylori* in the oral cavity and provide novel research directions for further studies to eliminate the confusion regarding *H. pylori* in the oral cavity.

Detection of *H. pylori* with molecular methods

Molecular methods are widely used in the detection of *H. pylori* from oral samples (see Table 1 for a detailed overview of studies published since 2010). PCR, quantitative PCR (qPCR), nested PCR, and loop-mediated isothermal amplification (LAMP) are the most commonly used methods. Concerning the detection of *H. pylori*, the design of primers varies from study to study. Most of the primers designed in various studies focus on the 16S rRNA gene, 23S rRNA gene, *ureA* gene, *cagA*

Table 1. Studies published since 2010 detecting oral *H. pylori* with molecular methods.

Authors	Nation	Sample	Sample capacity	Method	Target genes	Total detection rate	Detection rate in gastric <i>H. pylori</i> -positive patients	Detection rate in gastric <i>H. pylori</i> -negative patients	Detection rate of other genes in <i>H. pylori</i> -positive samples
Seligová et al. (2020)	Slovakia	Saliva	81 patients admitted for upper gastrointestinal endoscopy 38 patients attending a dental clinic	nested PCR	16S rRNA	9.9%	13.2%	6.1%	—
Flores-Treviño et al. (2019)	Mexico	Dental plaque	87 patients requiring tooth extraction	qPCR	16S rRNA	60.5%	—	—	<i>vacA</i> : 100%; <i>cagA</i> : 21.7%
Hamada et al. (2019)	Japan	Saliva and extracted teeth	80 patients diagnosed with chronic non-atrophic gastritis 53 term newborns A 29-year-old Japanese female admitted to the gastroenterology clinic	PCR	<i>ureA</i>	18.4% saliva only; 9.2% extracted teeth only; 14.9% both sample types; 5.7% 60%	—	—	—
Zhao et al. (2019)	China	Tongue	131 subjects treated at Osaka University Dental Hospital	gene sequencing	16S rRNA	1.9%	—	—	—
Matamala-Valdés et al. (2018)	Chile	Oral swabs	110 healthy persons	PCR	16S rRNA <i>ureA</i>	100%	—	—	—
Kadota et al. (2019)	Japan	Dental plaque	108 adults with gastric <i>H. pylori</i> infection	Nested PCR	16S rRNA <i>ureA</i>	44.6%	—	—	—
Ansari et al. (2018)	Pakistan	Dental plaque	61 patients admitted to the Service of Gastroenterology	PCR	16S rRNA	40%	—	—	—
Nomura et al. (2018)	Japan	Inflamed dental pulp	70 admitted to endoscopy with a gastro-duodenoscope and 30 controls	Nested PCR	<i>ureA</i>	40%	—	—	—
Wongphutorn et al. (2018)	Thailand	Saliva	50 patients with chronic periodontitis and 50 periodontally healthy subjects	Real-time PCR; nested PCR	16S rRNA; <i>vacA</i>	Total: 64.5%	Total: 67.1%	—	<i>vacA</i> : 29.6%
Mendoza-Cantú et al. (2017)	Mexico	Dental plaque	154 obese adult individuals	qPCR	16S rRNA; <i>cagA</i> not mentioned	38%; 27% 46%#	—	—	—
Urban et al. (2017)	Poland	Saliva and dental plaque	49 patients admitted for a gastrointestinal endoscopy	PCR	16S rRNA	50.8%#	—	—	<i>vacA</i> : 100%; <i>cagA</i> : 29%; <i>babA2</i> : 17.6%
Medina et al. (2017)	Argentina	Saliva and dental plaque	54 patients with oral lichen planus (OLP); 40 healthy controls	PCR	<i>ureA</i>	Study group saliva: 80% dental plaque: 84.3% control group saliva: 46.7%	Study group saliva: 79.3% dental plaque: 84.5%	Study group saliva: 83.3%	—
Aksit Bircak et al. (2017)	Turkey	Saliva and dental plaque	72 patients with oral lichen planus (OLP); 40 healthy controls	real-time PCR	16S rRNA	Study group saliva: 80% dental plaque: 84.3% control group saliva: 46.7%	Study group saliva: 79.3% dental plaque: 84.5%	Study group saliva: 83.3%	<i>vacA</i> : 100%; <i>cagA</i> : 29%; <i>babA2</i> : 17.6%
Valadan Tahbaz et al. (2017)	Iran	Dental plaque	40 children and adolescents attending Osaka University Dental Hospital	PCR	16S rRNA	5% in both groups	—	—	<i>vacA</i> : 40%; <i>babA2</i> : 100%
Gastro-Muñoz et al. (2017)	Mexico	Oral swabs	56 dyspeptic adult patients	PCR	16S rRNA; <i>glimM</i>	13%; 5.5%	—	—	—
Patano et al. (2016)	Brazil	Saliva and tongue swabs	43 subjects living in Sofia	PCR	16S rRNA; <i>glimM</i> not mentioned	Saliva: 40.9% tongue swabs: 33.8%	—	—	—
Ismail et al. (2016)	UK	Dental plaque	46 children with upper gastrointestinal symptoms and gastric <i>H. pylori</i> infection	Nested PCR	295 bp fragment	40.8%	—	—	—
Kazanowska-Dygdala et al. (2016)	Poland	Dental plaque	30 patients with gastric <i>H. pylori</i> infection	Nested PCR	417 bp DNA	20% in leukoplakia 23% in OLP 0% in control	—	—	—
Amiri et al. (2015)	Iran	Dental plaque	40 children and adolescents attending Osaka University Dental Hospital	PCR and LAMP	<i>glimM</i>	PCR: 44%; LAMP: 66.7%	—	—	—
Ogaya et al. (2015)	Japan	Inflamed dental pulp and saliva	40 children and adolescents attending Osaka University Dental Hospital	PCR	<i>ureA</i>	Inflamed dental pulp 15% saliva 0%	—	—	—
Bharath et al. (2014)	India	Dental plaque	56 dyspeptic adult patients	Real-time PCR	<i>ureA</i>	0%	—	—	—
Boyanova et al. (2013)	Bulgaria	Dental plaque	43 subjects living in Sofia	PCR	<i>ureA</i>	33.9%	—	—	—
Cai et al. (2014)	China	Dental plaque and gargle	46 children with upper gastrointestinal symptoms and gastric <i>H. pylori</i> infection	PCR	<i>ureA</i> ; <i>vacA</i> 16S rRNA;	All: 2.3% 56.5%#	16S rRNA: 56.5%	—	<i>cagA</i> : 46.1%
Agarwal and Jithendra (2012)	India	Subgingival plaque	30 patients with gastric <i>H. pylori</i> infection	PCR	16S rRNA	60%	60%	—	—
Sepúlveda et al. (2012)	Chile	Dental plaque and tongue	18 patients admitted for endoscopy	Real-time PCR	<i>cagA</i> ; <i>vacA</i>	<i>cagA</i> : 16.7%; <i>vacA</i> : 5.6%#	—	—	3 <i>vacA</i> -/ <i>cagA</i> + samples and 1 <i>vacA</i> +/ <i>cagA</i> - sample; no <i>vacA</i> -/ <i>cagA</i> + sample
Hirsch et al. (2012)	Germany	Root canal; plaque	3 children with endodontically-infected deciduous teeth	PCR	16S rRNA	Root canal: 20%; plaque: 40%	—	—	—
Al-Ahmad et al. (2012)	Germany	Plaque; saliva, periapical exudate; tongue swabs	15 patients who tested positive for gastric <i>H. pylori</i> infection (163 oral samples)	PCR; nested PCR	417 and 109 bp fragment	Periapical exudate and tongue swabs: 6.6% plaque and saliva: 0%	6.6%	—	—

(continued)

Table 1. Continued.

Authors	Nation	Sample	Sample capacity	Method	Target genes	Total detection rate	Detection rate in gastric <i>H. pylori</i> -positive patients	Detection rate in gastric <i>H. pylori</i> -negative patients	Detection rate of other genes in <i>H. pylori</i> -positive samples
Wichelhaus et al. (2011)	Germany	Dental plaque	11 adolescent patients aged 11–15 years (93 samples)	Nested PCR	417 bp fragment	8.2%	–	–	–
Gao et al. (2011)	China	Dental plaque; gargle; tongue swabs	96 patients with chronic gastric diseases	PCR	<i>glmM</i>	Dental plaque: 82.3% gargle: 51.1%	59.6%	–	<i>cagA</i> : 14.6%
Silva et al. (2010)	Brazil	Supragingival and subgingival plaque	115 patients with complaints regarding the upper digestive tract	PCR	16S rRNA	Tongue swabs: 37.5% Supragingival plaque: 11.3% Subgingival plaque: 0%	14.9%	6.3%	–
Assumpção et al. (2010)	Brazil	Dental plaque	99 adult patients admitted to upper gastrointestinal endoscopy	PCR	<i>vacA</i>	72%	–	–	<i>cagA</i> : 81.7%

Sort order by decreasing publication year. –: not investigated in the respective study.
#No separate detection of each sample type given in the respective study.

gene, *vacA* gene, *babA2* gene, and *glmM* gene. The protein-coding genes encode components that are generally conserved between *H. pylori* strains and are important for the biology of the organism. For example, the *ureA* gene encodes for urease, which initiates urea hydrolysis to produce ammonia and neutralize gastric acid, creating a suitable pH environment for *H. pylori* survival and colonization (Tsuda et al. 1994; Dunn et al. 1997). Cytotoxin-associated gene A (*cagA*) can disrupt intracellular actin transport, stimulate inflammatory responses, and break down tight cellular junctions (Tohidpour 2016). CagA is encoded by the *cagA* gene and it is a highly immunogenic protein with an approximate weight of 140 kDa. Hatakeyama found the *cagA* gene in about two-thirds of *H. pylori* isolated from western countries (Hatakeyama 2004). Cytotoxin vacuolizing (VacA) protein – which causes vacuolization in epithelial cells, cell death, and the destruction of epithelial integrity – is encoded by the *vacA* gene and can be detected in all strains of *H. pylori* (Leunk et al. 1988; Phadnis et al. 1994). BabA (blood group antigen-binding adhesion) – a 78 kDa protein – is the first-identified and best-characterized adhesin of *H. pylori* and it is encoded by the *babA2* gene (Kalali et al. 2014). The *glmM* gene – also termed *ureC* – encodes phosphoglucosamine mutase, a member of the enzyme superfamily that converts glucosamine 6-phosphate (GlcN-6-P) to glucosamine 1-phosphate (GlcN-1-P) (Reuse et al. 1997).

The 16S rRNA gene has been most widely used in studies over the past decade. When considering studies using PCR amplifying 16S rRNA genes published since 2010, the reported detection rates of *H. pylori* in oral samples broadly range from 5% to 78.9% when considering all experimental subjects, irrespective of gastric complaints or proven gastric *H. pylori* infection (Song et al. 1999; Silva et al. 2010; Agarwal and Jithendra 2012; Hirsch et al. 2012; Cai et al. 2014; Aksit Bıçak et al. 2017; Castro-Muñoz et al. 2017; Mendoza-Cantú et al. 2017; Valadan Tahbaz et al. 2017; Ansari et al. 2018; Matamala-Valdés et al. 2018; Wongphutorn et al. 2018; Šeligová et al. 2020). The study performed by Silva et al. was the only one to report a 0% detection rate of *H. pylori* from subgingival plaque samples taken from 115 patients with complaints in the upper digestive tract (Silva et al. 2010). Zhao et al. reported a detection rate for *H. pylori* of 60% (48 out of 80) in tongue samples from patients diagnosed with chronic non-atrophic gastritis using 16S rRNA gene next generation sequencing (NGS) (Zhao et al. 2019).

In 1993, Li et al. described primers for a 417 bp DNA fragment encoding for the ribosomal protein S20 which is the primary binding protein that bridges the 5'

domain and the 3' minor domain of the 16S rRNA (Li et al. 1993). The authors reported absence of cross-reactivity when evaluating 166 non-*H. pylori* bacterial strains (including *Campylobacter cinaedi*, *Campylobacter coli*, *Campylobacter concisus*, *Campylobacter cryaerophila*, *Campylobacter foetus*, *Campylobacter jejuni*, *Campylobacter laridis*, *Campylobacter sputorum* subsp. *bubulus*, *Campylobacter sputorum* subsp. *sputorum*, *Campylobacter upsaliensis*) (Li et al. 1993). On the other hand, Šeligová et al. found these primers not to be specific as they could cross-react with *Barnesiella viscericola*, which can be found in the oral cavity (Šeligová et al. 2020). Therefore, further research needs to be done on this point.

Song et al. also used these primers targeting this 417 bp DNA fragment with nested PCR (Song et al. 1999). They included 40 randomly selected adult patients who visited the dental department. *H. pylori*'s detection rate in the oral cavity using this primer (100%) was found to be even higher than when using other 16S rRNA PCR (78.9%) (Song et al. 1999). In 2015, a study from Poland also used the nested PCR method amplified for the 417 bp fragment described above to study the distribution of *H. pylori* in the oral cavity of patients with leukoplakia and oral lichen planus (Kazanowska-Dygdała et al. 2016). Notably, *H. pylori* was only detected in samples from patients with leukoplakia or oral lichen planus but not from healthy patients. 20% of patients with leukoplakia and 23% of patients with lichen planus were found to be *H. pylori*-positive (Kazanowska-Dygdała et al. 2016). Employing the same method, Wichelhaus et al. detected oral *H. pylori* in 82% dental plaque samples from eleven adolescent patients who went for orthodontic therapy (Wichelhaus et al. 2011). As described above, the high detection rate in a healthy population raises questions about the specificity of the primers used for this 417 bp fragment (Wichelhaus et al. 2011). PCR (amplifying the 417 bp fragment) and nested PCR (amplifying a 109 bp fragment) were used in the study performed by Al-Ahmad et al. (2012). Only one out of fifteen patients with gastric *H. pylori* infection were detected with oral *H. pylori* using PCR with both primers. The authors excluded false-negative PCR results in the study since they used a PCR inhibition control comprising a plasmid, including the target gene (Al-Ahmad et al. 2012).

The *ureA* gene is also quite commonly used in oral *H. pylori* research. In most of the studies using the *ureA* gene as an amplification target, the detection rate of *H. pylori* in samples from the oral cavity ranges from 15% to 50.4% when considering all experimental subjects (Song et al. 1999; Boyanova et al. 2013; Bharath et al.

2014; Ogaya et al. 2015; Medina et al. 2017; Nomura et al. 2018; Hamada et al. 2019; Kadota et al. 2019). Kadota et al. described the case of a 29-year-old woman with the chief complaint of a stomach ache. The *ureA* gene of *H. pylori* was detected in oral specimens before they started triple antibiotic therapy (potassium-competitive acid blocker, amoxicillin, and clarithromycin) for *H. pylori* eradication. After this treatment, the gene could no longer be detected (Kadota et al. 2019). By contrast, Ogaya et al. found a 0% detection rate of oral *H. pylori* in salivary samples from 40 Japanese children and adolescents who went for root canal treatment without any gastric complaints. However, it is unsurprising that *H. pylori* was not detected in those young subjects, who exhibited a high probability of not being infected with *H. pylori* due to the lack of any gastric complaints (Ogaya et al. 2015). In 2019, Hamada et al. performed *ureA* gene PCR on saliva specimens and extracted teeth from 87 subjects to analyse *H. pylori*'s distribution among these specimens (Hamada et al. 2019). The *H. pylori*-positive rate in these samples was 18.4%. Eight samples exhibited *H. pylori* in saliva, and thirteen samples showed *H. pylori* in dental plaque taken from teeth that were extracted due to dental caries or periodontal disease (Hamada et al. 2019).

In the study mentioned above, Ogaya et al. detected *H. pylori* in 15% of samples obtained from infected dental pulps (Ogaya et al. 2015), while Nomura et al. found *H. pylori* in even 38.9% of specimens from infected dental pulps using the nested PCR system targeting the *ureA* gene (Nomura et al. 2018). The difference in detection rates compared with the study by Ogaya et al. (2015) may be attributed to the lower detection threshold of nested PCR as compared to conventional PCR. In another study, *ureA* PCR analysis was compared with the urease test for detecting *H. pylori* in 56 dental plaque samples from dyspeptic adult patients (Bharath et al. 2014). Here, a clear difference in detection efficiency was found (urease test showed a 71.4% *H. pylori*-positive rate, while *ureA* PCR showed a 33.9% positive rate) (Bharath et al. 2014). Besides *H. pylori*, other bacteria (such as *Staphylococcus epidermidis*, *Campylobacter ureolyticus*, *Streptococcus salivarius*, *Actinomyces* spp. and some strains of *Haemophilus parainfluenzae*) that are commonly found in the oral cavity exhibit strong urease activity (Dahlén et al. 2018). Since it is not clear whether the primers are specific to the *H. pylori ureA* gene, PCR analysis targeting this gene or testing for urease activity may lead to false-positive results due to the presence of other bacteria within the dental plaque exhibiting strong urease activity.

The *glmM* gene is not as commonly used for the detection of *H. pylori* as the *ureA* gene. The detection rate in studies using *glmM* gene PCR ranges from 5.5% to 66.7% when considering all experimental subjects (Gao et al. 2011; Amiri et al. 2015; Castro-Muñoz et al. 2017). Castro-Muñoz et al. investigated *H. pylori*'s distribution in the oral cavities of 162 healthy kindergarten children under five years of age (Castro-Muñoz et al. 2017). For the detection of *H. pylori*, they used PCR for 16S rRNA and *glmM* genes. As a result, 13% (21 out of 162) of the children were found *H. pylori*-positive with PCR for 16S rRNA, while only nine of those 16S rRNA-positive subjects were found to be *H. pylori*-positive with PCR for the *glmM* gene. The *glmM* detection rate in the 16S rRNA-positive samples was only 42.8% (Castro-Muñoz et al. 2017). Lu et al. described a similar *glmM* detection rate of 67.5% in 16S rRNA-positive samples (Lu et al. 1999). The difference in detection rate between 16S rRNA and *glmM* gene PCR may thus be due to differences in the specificity of the primers used (Castro-Muñoz et al. 2017). Without carefully analysing *H. pylori*-specificity of primers, a positive cross-reaction of the PCR with other *H. pylori*-related microorganisms such as *Campylobacter* spp. cannot be excluded (Al-Ahmad et al. 2010).

In 2015, Amiri et al. compared the efficiency of PCR and LAMP in oral *H. pylori* detection (Amiri et al. 2015). Forty-five samples of dental plaque from patients without any gastric complaints were investigated in this study. The target gene in this research was *glmM*. *H. pylori*'s detection rates in the dental plaque samples were 44% (20 out of 45) using PCR and 66.7% (30 out of 45) using LAMP, whereby in 33.3% (15 out of 45) both methods yielded positive results. The authors claimed that LAMP seems to be a more efficient method for oral *H. pylori* detection. However, compared with other studies using *glmM* gene PCR to investigate oral *H. pylori* in patients without gastric complaints, the detection rate of oral *H. pylori* in their work is relatively high. Therefore, the specificity of the primers used for detection of *glmM* may need further verification, as discussed above. Gao et al. also used PCR targeting the *glmM* gene to detect oral *H. pylori* in patients with gastric *H. pylori* infection (Gao et al. 2011). The *H. pylori* detection rate in oral samples (dental plaque, gargles, and tongue samples) was 56.9%, but only 14.6% of the *glmM*-positive samples were also detected as harbouring the *cagA* gene. The *H. pylori* infection rate of the oral cavity in the gastric *H. pylori*-infected population in this study was higher than in the non-gastric *H. pylori*-infected population in other studies (Gao et al. 2011).

The detection rate of the *vacA* gene in oral samples is relatively low compared with other genes, ranging from 2% to 5.5% when considering all experimental subjects (Sepúlveda et al. 2012; Boyanova et al. 2013; Valadan Tahbaz et al. 2017), while in a study from Thailand, the detection rate of *H. pylori* in saliva samples from healthy persons was 59% for the *vacA* gene, but still lower than the detection rate (65%) using 16S rRNA PCR (Wongphutorn et al. 2018). Notably, in the research conducted by Sepúlveda et al. (Sepúlveda et al. 2012), they did not detect the *vacA* and *cagA* genes simultaneously in any oral *H. pylori*-positive samples taken from patients with gastric complaints. Furthermore, it needs to be clarified whether cross-reactions with genes from other microorganisms may lead to false-positive results (Sepúlveda et al. 2012). Since the *vacA* gene cannot be found in all *H. pylori*-positive samples (Assumpção et al. 2010; Gao et al. 2011; Medina et al. 2017; Valadan Tahbaz et al. 2017), it may not be useful as a standard for oral *H. pylori* detection. Since the *vacA* gene is frequently but not always found in *H. pylori*, it can be concluded that the value of positive or negative results must be questioned when using *vacA* gene PCR for detection of *H. pylori* from oral samples.

In the studies using the *cagA* gene as a PCR target, detection rates of oral *H. pylori* range from 1.8% to 58% when considering all experimental subjects, and from 8.7% to 26.1% in gastric *H. pylori*-positive patients (Assumpção et al. 2010; Gao et al. 2011; Sepúlveda et al. 2012; Boyanova et al. 2013; Cai et al. 2014; Mendoza-Cantú et al. 2017; Valadan Tahbaz et al. 2017; Flores-Treviño et al. 2019). It is found that the *cagA* gene is not present in all *H. pylori* isolated from western countries. The *cagA*-positive *H. pylori* strains are more virulent than the *cagA*-negative strains (Hatakeyama 2004). Therefore, the *cagA* gene may not be worthwhile for oral *H. pylori* detection, but rather to characterize given strains in terms of whether they comprise this critical virulence factor.

The *babA2* gene has only been used in two studies for oral *H. pylori* detection in the past decade (Medina et al. 2017; Valadan Tahbaz et al. 2017). A study from Iran (Valadan Tahbaz et al. 2017) reported a 5% *H. pylori*-positive rate in supragingival and subgingival plaque samples from 50 periodontitis patients and 50 patients without periodontal disease. They detected *H. pylori* with PCR using a 16S rRNA primer set and found that the *babA2* gene was detected in all five 16S rRNA-positive samples (Valadan Tahbaz et al. 2017). On the other hand, Medina et al. reported that the *ureA* gene could be detected in 50.8% (31/61) of patients who

attended Gastroenterology service (Medina et al. 2017). However, only three of those *ureA*-positive oral samples were seen with the *babA2* gene (Medina et al. 2017). These findings support the notion that caution is required when *babA2* PCR is conducted for detecting *H. pylori* because strong heterogeneity in the detection rates of the *babA2* gene has been shown depending on the PCR primer sets used (Šterbenc et al. 2020).

Compared with other detection methods, *H. pylori*'s oral detection rate is relatively high when using molecular techniques. PCR is a highly sensitive technique, but the specificity of the used primers is of paramount importance. Since cross-reactions have been observed between *H. pylori* and different members of *Campylobacter* spp. that can be frequently detected in the oral cavity by using checkerboard DNA-DNA hybridization (Ximénez-Fyvie et al. 1999; Al-Ahmad et al. 2010), cross-reaction between *Campylobacter* spp. and *H. pylori* in molecular methods may cause false-positive results and explain the high detection rates. For instance, Šeligová et al. recently pointed out that many primers designed or used in previous studies had flaws. The major drawback was nonspecificity at the 3' ends, as shown *in silico* by a fast search in GenBank that excluded the *Helicobacter* taxid (Šeligová et al. 2020). Another important aspect is gastroesophageal reflux, which can bring *H. pylori* from the stomach to the oral cavity. In western and eastern societies, the prevalence of gastroesophageal reflux disease is about 19% to 44% (Ho et al. 2006; Yönm et al. 2013). The high prevalence of gastroesophageal reflux among citizens can also influence the detection of oral *H. pylori* because efflux may temporarily bring *H. pylori* or "fragments" of *H. pylori* from the stomach, which may lead to positive PCR detection. Moreover, it could be speculated that DNA of *H. pylori* may reach the oral cavity by a hiccup and hence be detected by the PCR as a sensitive molecular method.

Detection with immunological and biochemical methods

Besides molecular methods like PCR, immunological and biochemical methods have also been described for investigating the presence of *H. pylori* in the oral cavity (Matamala-Valdés et al. 2018; Wongphutorn et al. 2018; Zhao et al. 2019). The following paragraphs summarize studies using such immunological and biochemical methods, and Table 2 shows details of studies using immunological methods published in 2010 or later.

Immunological methods used for the detection of *H. pylori* vary from study to study. The saliva *H. pylori*

antigen (HPS) test and the *H. pylori* flagellin test (HPF) are lateral flow, immuno-chromatographic tests to detect the *H. pylori* urease antigen (HPS) or the flagellin antigen (HPF), respectively (Yee et al. 2013). The CLO test (also known as the rapid urease test) is a quick diagnostic test for *H. pylori*. It is designed to test *H. pylori*'s ability to secrete the urease enzyme, which catalyses the conversion of urea to ammonia and carbon dioxide (Dane and Gurbuz 2016). Indirect immunofluorescence assays (IFA) using fluorescence-labeled monoclonal antibodies (IgG anti-*H. pylori* antibodies) against *H. pylori* have also been used (Wongphutorn et al. 2018).

A total of 277 patients (159 with stomach pain and 118 with no stomach complaints) were included in the study by Wang et al., 201 (110 with stomach pain and 91 with no stomach complaints) of whom were tested with HPS and HPF. 70.1% (141/201) of these patients were found to be oral *H. pylori*-positive according to positive results in both HPS and HPF. The detection rates of oral *H. pylori* in the symptomatic and asymptomatic group were 87.3% and 49.5%, respectively (Wang et al. 2014). No cross-reactivity with *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Bifidobacterium dentium*, *Corynebacterium matruchotii*, *Gemella haemolyans*, *Granulicatella adiacens*, *Streptococcus gordonii*, *Streptococcus salivarius*, *Streptococcus sanguinis*, and *Veillonella parvula* was found using HPS and HPF. However, cross-reactivity with *H. pylori*-related organisms such as *Campylobacter* spp. that have strong potential to give false-positive results were not considered (Wang et al. 2014).

An anti-*H. pylori* antibody (a polyclonal rabbit antibody to *H. pylori*) was used to stain samples from 50 oral lichen planus patients for microscopic evaluation and detection of *H. pylori* (Hulimavu et al. 2014). No *H. pylori* was detected in both 50 tissue samples from oral lichen planus patients and ten samples from normal buccal mucosal biopsies. Since none of these patients had gastric complaints, this finding is unsurprising (Hulimavu et al. 2014).

Ding et al. (2015) used HPS and HPF test to detect *H. pylori* in the oral cavity of subjects who went for an oral health examination (Ding et al. 2015). A total of 1050 patients were tested, 633 (60.3%) of whom were found to be *H. pylori*-positive. 69.5% of patients with a history of gastric ulcer carried oral *H. pylori*, compared with 58.3% of patients without a history of gastric ulcer. A statistically significant difference was found between these two groups. Detection rates of *H. pylori* in patients with caries (66.9%) and periodontal diseases (63.4%) were higher than those without oral diseases

Table 2. Studies published since 2010 detecting oral *H. pylori* with immunological and biochemical methods.

Author	Nation	Sample	Sample capacity	Method	Detection rate
Zhao et al. (2019)	China	Tongue scraping and swabs	80 patients diagnosed with chronic non-atrophic gastritis	Anti-cagA IgG antibody	43.7%
Matamala-Valdés et al. (2018)	Chile	Oral swabs	53 term newborns	Anti- <i>H. pylori</i> IgG antibody	1.9%
Wongphutorn et al. (2018)	Thailand	Saliva	110 healthy persons	Indirect IFA using a mouse anti- <i>H. pylori</i> IgG antibody	51%
Yu et al. (2017)	China	Dental plaque	4321 adults	Saliva <i>H. pylori</i> antigen (HPS) test	59.6% patients; 82.8% controls;
Dane and Gurbuz (2016)	Turkey	Dental plaque	35 patients with gastric <i>H. pylori</i> infection and 35 patients without gastric <i>H. pylori</i> infection (control)	CLO gel test	22.9%
Ding et al. (2015)	China	Saliva	1050 patients admitted for oral health examination	HPS	60.3%
Hulimavu et al. (2014)	India	OLP and buccal mucosa biopsies	tissue blocks from 50 OLP cases and buccal mucosa biopsies from 10 healthy cases	Anti- <i>H. pylori</i> antibody (rabbit polyclonal)	0%
Wang et al. (2014)	China	Saliva	201 patients (110 with stomach pain and 91 with no stomach complaints)	HPS & <i>H. pylori</i> flagellin test (HPF)	HPS & HPF positive: 87.3% symptomatic; 49.5% asymptomatic;
Boyanova et al. (2013)	Bulgaria	Dental plaque	43 subjects living in Sofia	Immunofluorescence with monoclonal antibodies (MCA) (121F3, 122E9, 123B11, and 161F8)	2.3%
Namiot et al. (2010)	Poland	Supragingival plaque	155 patients aged 19–78 years	Immunological test (horseradish peroxidase-labeled monoclonal antibodies against <i>H. pylori</i>)	65.6%

Sort order by decreasing publication year. -: not investigated in the respective study.

(54.1%). The authors concluded that oral *H. pylori* infection is closely related to periodontal diseases and caries. However, the lack of careful validation of the specificity of the tests is a shortcoming of this study (Ding et al. 2015). In 2016, Dane et al. tested dental plaque samples from 35 patients who had endoscopically-diagnosed cases of *H. pylori*-related gastritis and 35 healthy patients with the CLO gel test (Dane and Gurbuz 2016). Twenty-nine of 35 (82.8%) gastritis patients were found oral *H. pylori*-positive, while eight of 35 (22.9%) healthy patients were found to be positive. A high prevalence of *H. pylori* was found in dental plaque. The authors concluded that the oral cavity was a vital reservoir for *H. pylori*. Unfortunately, cross-reactivity to *Campylobacter* spp. – which are frequently isolated from dental plaque – was not evaluated in this study (Dane and Gurbuz 2016).

Yu et al. (2017) performed a study to detect oral *H. pylori* infections among 4321 adults in samples from dental plaque by using HPS (Yu et al. 2017). They detected *H. pylori* in 59.6% of the young age sub-group (<45 years), while the rate of positive detection of *H. pylori* was 25.5% in the elder sub-group (75–89 years). One strength of this study is that researchers tested cross-reactivity of HPS with thirteen bacterial strains (*Streptococcus gordonii*, *Streptococcus mutans*, *S. salivarius*, *S. sanguinis*, *Veillonella parvula*, *Porphyromonas gingivalis*, *Gemella haemolysans*, *Granulicatella adiacens*, *Campylobacter rectus*, *Corynebacterium matruchotii*, *Bifidobacterium dentium*, *Actinomyces naeslundii*, and *A. odontolyticus*) and obtained a negative result (Yu et al. 2017). While *Campylobacter rectus* is known to be urease-negative (Noël et al. 2018), a recent study showed that the *Campylobacter lari* group – which is typically isolated from humans – was found to be positive for urease (Boukerb et al. 2019). Based on this, HPS and CLO tests that test for urease antigens may have cross-reactivity with this group of bacteria, potentially leading to false-positive detection results of *H. pylori*.

Wongphutorn et al. investigated *H. pylori*'s prevalence in 110 saliva samples from asymptomatic persons in north-eastern Thailand (Wongphutorn et al. 2018). Samples were tested with IFA using a mouse anti-*H. pylori* IgG antibody. From 110 saliva samples, 57 (51.8%) were positive according to IFA. However, cross-reactivity of IFA using this antibody (especially with *H. pylori*-related microorganisms) remains to be clarified in further studies (Wongphutorn et al. 2018).

A study from Chile focussed on oral swabs from 53 term newborns (Matamala-Valdés et al. 2018). They detected *H. pylori* with immunofluorescence using rabbit polyclonal IgG anti-*H. pylori* antibodies marked with

FITC. Subsequently, the authors took images with fluorescence microscopy, finding only one of 53 samples to be *H. pylori*-positive. This result was consistent with the PCR amplified for *cagA* and *vacA* genes in this study. However, unfortunately cross-reactivity was not tested in this study (Matamala-Valdés et al. 2018).

In 2019, Zhao et al. performed research on samples from the gastric mucosa and tongue scrapings collected from 80 patients with chronic gastritis (Zhao et al. 2019). The *H. pylori* status was confirmed by 16S rRNA gene sequencing. The *cagA* status was confirmed by investigating anti-*cagA* immunoglobulin G (IgG) in serum. The authors found *H. pylori*-negative samples (32 of 80), *cagA*-negative *H. pylori* infections (13 of 80), and *cagA*-positive *H. pylori* infections (35 of 80). Notably, 27% of *H. pylori* infections were found to be *cagA*-negative (Zhao et al. 2019). However, as stated above, *cagA* is not present in all *H. pylori* isolated from western countries and rather serves as an indicator of the virulence of a given *H. pylori* strain (Hatakeyama 2004).

A total of 277 patients were included in the research conducted by Wang et al., with 50.9% (141 out of 277) being found oral *H. pylori*-positive according to positive results in both HPS and HPF (Wang et al. 2014). However, it is known that flagellin can also be found in the *Campylobacter jejuni* group (Salah Ud-Din and Roujeinikova 2018). Based on this, HPF – which tests for the flagellin antigen – may have cross-reactivity with this group of bacteria.

Moreover, immunofluorescence with monoclonal antibodies was used to detect oral *H. pylori* (Boyanova et al. 2013). Four monoclonal antibodies (121F3, 122E9, 123B11, and 161F8) were used in this study. *H. pylori* could only be detected in the dental plaque from one out of 43 patients. 121F3 was negative in this strain, while 122E9, 123B11, and 161F8 were positive. However, no further information on these antibodies is given, despite that there was no cross-reactivity with *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, and *Yersinia enterocolitica* (Boyanova et al. 2013).

Namiot et al. collected samples of supragingival plaque from 155 patients aged 9–78 years (Namiot et al. 2010). They detected *H. pylori* with an immunological method using a kit to detect *H. pylori* antigens in stool samples. One hundred and one patients (65.6%) were found to be oral *H. pylori*-infected (Namiot et al. 2010). The only drawback is that no data on the specificity of this kit is given.

With immunological methods, attention should be paid to cross-reactions between *H. pylori* and other

bacteria. Although some studies have investigated the cross-reactions between *H. pylori* and other bacteria (Wang et al. 2014), almost none of them focus on the cross-reactions between *H. pylori* and *H. pylori*-related organisms such as *Campylobacter* spp. In the Chinese study by Yu et al., researchers tested cross-reactivity of HPS with *Campylobacter rectus* and obtained negative results (Yu et al. 2017). Nonetheless, it is known that flagellin can also be found in the *Campylobacter jejuni* group (Salah Ud-Din and Roujeinikova 2018). Therefore, the cross-reactivity of HPF remains to be clarified. It is worth noting that some studies discovered serological detection with low specificity as common surface antigens of *Campylobacter jejuni* and *Campylobacter coli* may lead to serological cross-reactivity (Webberley et al. 1992; Bodhidatta et al. 1993; Glupczynski et al. 1993). Therefore, this cross-reactivity may cause false-positive results of immunological *H. pylori* detection in oral samples. As stated above, it has recently been shown that the *Campylobacter lari* group isolated from humans was positive for urease (Boukerb et al. 2019). Based on these findings, HPS and CLO – both of which test for the urease antigen – may cross-react with this group of bacteria. Likewise, CLO even suggests by its name that it is suitable for the detection of *Campylobacter*-related organisms but not specifically for *H. pylori*. If researchers want to use CLO in oral *H. pylori* detection, they need to make a rigorous assessment of CLO's cross-reactivity. On the other hand, as highlighted above regarding DNA-based molecular methods, the impact of the high prevalence of gastroesophageal reflux among subjects on the detection of oral *H. pylori* cannot be ignored because efflux may temporarily bring *H. pylori* from the stomach, which may lead to positive immunological detection.

Detection with culture technique

When using the culture technique, *H. pylori*'s detection rates from oral samples are usually relatively low compared with those using the previously-mentioned detection methods. The culture technique is time- and laboratory-intensive and requires experience among the staff. Furthermore, for proofing the culture of *H. pylori* from an oral sample, it is crucial to provide data from whole genome sequencing of the given strain (Whittam and Bumbaugh 2002). Furthermore, deposition of a suchlike isolate in an international culture collection would be highly worthwhile in order to provide authenticated biological material for confirmation of the results and for further studies (Smith 2003). Unfortunately, no *H. pylori* strain isolated from the oral

cavity has ever been deposited in any national or international culture collection. Accordingly, the Human Oral Microbiome Database (HOMD; www.homd.org) comprises data on the genomes of twelve *H. pylori* strains, none of which, however, have been isolated from the oral cavity. In the following, selected studies are described that used the culture technique for detection of *H. pylori* from oral samples, while Table 3 summarizes the details of studies published since 2005 investigating the presence of *H. pylori* in the oral cavity by culture technique.

In 1989, Krajden et al. collected samples of dental plaque, saliva, and gastric biopsies from 71 patients undergoing endoscopy (Krajden et al. 1989). It was the first study attempting to culture *H. pylori* from oral samples. All such oral and gastric samples were cultured on 10% laked horse blood with Skirrow formula. The researchers successfully recovered *H. pylori* from 29 (40.8%) of 71 gastric biopsy samples. At the same time, no *H. pylori* were recovered from the salivary samples, while *H. pylori* could only be cultured from one of 71 dental plaque samples. Consequently, the authors did not consider saliva and dental plaque to be relevant reservoirs of this organism (Krajden et al. 1989). To the best knowledge of the authors, this isolate has unfortunately neither been investigated by other groups nor deposited in any culture collection.

Likewise, the detection rate of oral *H. pylori* in research conducted by Goosen et al. was only 3.4%. In this research, dental plaque and saliva samples were collected from 58 randomly-selected clinically healthy volunteers and cultured on brain heart infusion agar plates with 5% sheep blood. *H. pylori* isolates were identified with the urease test and catalase activity test, which does not rule out false-positive detection, as discussed above (Goosen et al. 2002).

Allaker et al. did not find any positive culture results from dental plaque samples taken from *H. pylori*-positive children according to gastric biopsy results, while PCR amplified for a 411 bp DNA fragment of the *ureA* gene exhibited a 25% *H. pylori* detection rate (Allaker et al. 2002). Their results were quite similar to those from two other studies: Teoman et al. found *H. pylori* in 28.3% of dental plaque samples using *ureA* gene PCR, although they failed to isolate *H. pylori* from any of those dental plaque specimens with the culture method (Teoman et al. 2007), while Umeda et al. made attempts to isolate *H. pylori* with *H. pylori* selective medium (specific formula unknown) from oral samples of Japanese subjects and confirmed the result by morphological observation, catalase test, oxidase test, and nested PCR. While *H. pylori* was identified in one sample

using PCR, it could not be isolated by culture technique at all (Umeda et al. 2003).

In 2004, researchers tried to culture *H. pylori* from saliva and dental plaque samples from 100 female subjects using solid selective, enriched medium with 5% horse blood (Cześnikiewicz-Guzik et al. 2004; Czesnikiewicz-Guzik et al. 2005). The authors claimed to have successfully cultured *H. pylori* from 54.1% of the saliva samples and 48.3% of the supragingival plaque samples. The detection rate of oral *H. pylori* in gastric *H. pylori*-positive patients was 55.9%, and the detection rate of oral *H. pylori* in gastric *H. pylori*-negative patients was 48%. The result was confirmed by checking for bacterial urease, catalase, and oxidase activity (Cześnikiewicz-Guzik et al. 2004; Czesnikiewicz-Guzik et al. 2005). However, for instance, *Brucella* spp. also show a positive reaction on oxidase, catalase, and urease tests (Koestanti et al. 2018). Therefore, testing for urease, catalase, and oxidase may simply not be sufficiently selective to confirm the detection of *H. pylori*.

Wang et al. performed *H. pylori* culture from saliva samples (Wang et al. 2014; Yee 2016). They also tried to confirm the result with the oxidase test, catalase test, *H. pylori* urease test and HPF, and microscopy following Gram-staining. They claimed to have successfully cultured this bacterium from 86% of HPS and HPF positive samples (Wang et al. 2014). In another study, Boyanova et al. reported successful isolation of *H. pylori* on blood agar with Columbia agar base and 1% Isovitalex from one dental plaque sample out of 43 samples from subjects randomly selected among citizens (Boyanova et al. 2013).

Agarwal and Jithendra also claimed to have successfully cultured *H. pylori* on Columbia blood agar with 5% defibrinated sheep blood with antibiotic supplements from nine samples of subgingival plaque collected from patients with confirmed gastric *H. pylori* infection (Agarwal and Jithendra 2012).

In the study of Hirsch et al. (2012), ten samples taken from root canals and corresponding supragingival plaque were collected from three children with endodontically-infected deciduous teeth (Hirsch et al. 2012). 16S rRNA PCR and culture methods (GC agar plates with 10% horse serum containing vancomycin, trimethoprim, nystatin, and colistin) were used to investigate the prevalence of *H. pylori* in those samples. With the PCR method, two samples of the root canal and four plaque samples were found to be *H. pylori*-positive. Interestingly, *H. pylori* was successfully cultured from both root canal samples with PCR-positive results, while *H. pylori* could not be cultured from any of the plaque samples. The authors concluded that root canals of

Table 3. Studies published since 2000 detecting oral *H. pylori* by culture techniques.

Author	Nation	Sample	Sample capacity	Culture medium	Total detection rate	Detection rate in gastric <i>H. pylori</i> -positive patients	Detection rate in gastric <i>H. pylori</i> -negative patients
Wang et al. (2014)	China	Saliva	277 patients (159 with stomach pain and 118 with no stomach complaints) 43 subjects living in Sofia	Trypticase soy agar (TSA) + 5% blood plate	Symptomatic: 86.7% asymptomatic: 85.6%	-	-
Boyanova et al. (2013)	Bulgaria	Dental plaque		blood agar with Columbia agar base and 1% Isovitalex	2.3%	-	-
Agarwal and Jithendra (2012)	India	Subgingival plaque	30 patients from the gastroenterology department	Columbia blood agar + 5% defibrinated sheep blood with antibiotic supplements	30%	-	-
Hirsch et al. (2012)	Germany	Root canal; plaque	3 children with endodontically-infected deciduous teeth (10 samples)	Gonococci (GC) agar plates with 10% horse serum (containing vancomycin, trimethoprim, nystatin, and colistin)	Root canal: 20%; plaque: 0%	-	-
Al-Ahmad et al. (2012)	Germany	Plaque, saliva, subgingival exudate; tongue	15 patients tested positive for <i>H. pylori</i> in stool antigen test (163 oral samples)	<i>H. pylori</i> selective supplement (DENT) agar, yeast-cysteine blood agar and Columbia agar	0%	-	-
Teoman et al. (2007)	Turkey	Dental plaque	67 dyspeptic patients admitted for upper gastrointestinal endoscopy 100 female subjects	Brain heart infusion (BHI) agar containing 7% horse blood and DENT agar	0%	-	-
Cześnikiewicz-Guzik et al. (2004, 2005)	Poland	Saliva and supragingival plaque		solid selective, enriched medium (<i>H. pylori</i> agar, Becton Dickinson) with 5% horse blood	Saliva: 54.1%; plaque: 48.3%	saliva: 54.9%; plaque: 56.9%	saliva: 53.2%; plaque: 42.9%
Umeda et al. (2003)	Japan	Dental plaque; saliva; tongue plaque	18 Japanese subjects suffering from gastritis and peptic ulcers admitted to the hospital of Tokyo Medical and Dental University	<i>H. pylori</i> selective medium	5.6%#	-	-
Allaker et al. (2002)	UK	Dental plaque	100 children admitted for upper gastrointestinal tract endoscopy	BHI agar containing <i>H. pylori</i> selective supplement (Oxoid SR147)	0%	-	-
Goosen et al. (2002)	South Africa	Dental plaque and saliva	58 randomly-selected clinically healthy volunteers	brain heart infusion agar plates with 5% sheep blood	Dental plaque: 1.7% saliva: 1.7%	-	-

Sort order by decreasing publication year. -: not investigated in the respective study. #No separate detection of each sample type given in the respective study.

endodontically-infected teeth – but not dental plaque – could be a reservoir for *H. pylori* (Hirsch et al. 2012). PCR (amplifying for the 417 bp fragment discussed above) and nested PCR (amplifying for a 109 bp fragment) were used in the research performed by Al-Ahmad et al. (Al-Ahmad et al. 2012), as mentioned above. While one out of fifteen patients with gastric *H. pylori* infection was detected with oral *H. pylori* using PCR, *H. pylori* could not be cultured from that sample with three different growth media (DENT agar, yeast-cysteine blood agar plates, and Columbia blood agar) (Al-Ahmad et al. 2012). The authors concluded that *H. pylori* are not residents but only transient in the oral cavity.

The methods used in previous studies for confirming *H. pylori* culture or isolation have been controversial. In almost all of the studies, the oxidase test, catalase test, *H. pylori* antigen test, or microscopy observations were used to “confirm” *H. pylori* isolation by culture technique. The selectivity of these methods for *H. pylori* detection in previous studies may be insufficient. For instance, *Brucella* spp. also show a positive reaction on oxidase, catalase, or urease tests (Koestanti et al. 2018). Therefore, potential cross-reactions may exist, and thus these methods are not sufficient to prove the isolation of *H. pylori*. By contrast, whole-genome sequencing of the isolates and subsequent deposition of the organisms in a culture collection is needed to confirm the *H. pylori* colonization of the oral cavity. Application of whole-genome sequencing on the isolated bacterial strain is crucial to prove that a given isolate is *H. pylori* and not another *H. pylori*-like microorganism. Furthermore, deposition in culture collections is crucial to provide authenticated biological material and allow other researchers to study this organism (Smith 2003). Until there is data from whole-genome sequencing of at least one oral *H. pylori* isolate and deposition of this isolate in any national or international culture collection, positive proof that the oral cavity is a reservoir for this bacterium is almost impossible.

Future research directions

Despite the large number of studies dealing with the topic of oral colonization of *H. pylori* as summarized above, it remains unclear whether *H. pylori* colonizes the oral cavity residently, transiently or even at all. Along with differences in the detection methods used in previous studies, the huge diversity in the sample types (e.g. plaque, saliva, biopsies, tongue scrapings etc.) and in the study populations (e.g. in terms of disease status, age, country, sample size) may influence

the reported detection rates. As such, it makes it very difficult to accurately compare reported detection rates even when the technique is the same or similar.

One major drawback of the existing studies using molecular or immunological methods is the lack of investigating the specificity or cross-reactivity of the given methods. For instance, it has been reported that cross-reactions with *H. pylori* have been observed between different members of *Campylobacter* spp. when using molecular techniques (Ximénez-Fyvie et al. 1999; Al-Ahmad et al. 2012). Therefore, it is evident that cross-reactions between *Campylobacter* spp. and *H. pylori* may cause false-positive results when using molecular methods. Common antigens shared by *Campylobacter* spp. and *H. pylori* – which may lead to false-positive results in immunological methods – have also been found in previous research (Tanabe et al. 2003). Therefore, cross-reaction tests with *H. pylori*-like microorganisms (such as *Campylobacter* spp.) are needed to ensure specificity in molecular and immunological methods.

Furthermore, other points also need to be considered; for instance, a coccoid form of *H. pylori* has been reported in several studies (Roe et al. 1999; Rudnicka et al. 2014). It was found that the transformation of *H. pylori* from its spiral to its coccoid form is induced under stress conditions (such as antibiotics or environmental change; see Figure 2) (Roe et al. 1999) and that the coccoid form is a protection mechanism against such stress conditions (Azevedo et al. 2007). To prepare the coccoid state of *H. pylori*, *H. pylori* isolates were cultured in tubes containing 3 mL double distilled water with 1.5×10^8 CFU/mL bacteria under microaerophilic conditions and harvested after 1 or 2 months of incubation. The coccoid form of *H. pylori* was proven by using optical microscopy and LAMP. The prepared coccoid *H. pylori* cultures were then plated on Brucella blood agar under microaerophilic conditions at 37 °C for seven days. 90% of coccoid *H. pylori* could not be “resurrected” on Brucella blood agar, and only 10% of coccoid *H. pylori* could be re-cultured on the same agar (Chamanrokh et al. 2015). Likewise, Cellini et al. prepared the coccoid form *H. pylori* and tried to culture it on Brucella blood agar under the same conditions as described above, but they failed to “revive” *H. pylori* (Cellini et al. 1994). This may explain why *H. pylori* can be detected in the oral cavity by molecular methods while it hardly can be cultured. Nonetheless, it cannot be excluded that such coccoid forms of *H. pylori* would turn viable again when returning to the stomach. Accordingly, Cellini et al. inoculated coccoid *H. pylori* intragastrically in the stomachs of mice and observed

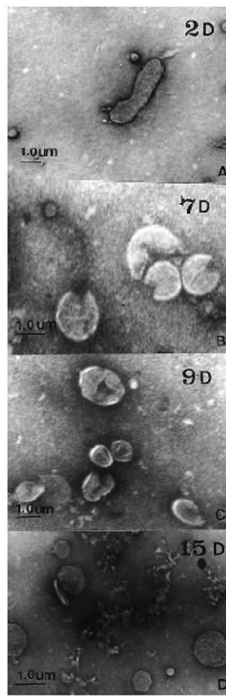


Figure 2. Morphological transformation of *H. pylori* from spiral to coccoid form under stress conditions. Transmission electron microscopic (TEM) images exhibiting the morphological transformation of *H. pylori* from its spiral to its coccoid form under stress conditions. *H. pylori* was cultured on Brucella blood agar with 5% horse serum and antimicrobial agents (10 mg/L vancomycin, 5 mg/L colistin, 5 mg/L trimethoprim and 5 mg/L amphotericin B) for a total of 15 days. On day 2, day 7, day 9, and day 15, its morphology was pictured by TEM: A: Day 2 (2D): bacillary form of *H. pylori*; B: Day 7 (7D): U-shaped form; C: Day 9 (9D): doughnut shaped form; D: Day 15 (15D): full coccoid form. The scale bar shows 1 μm . This figure is reprinted from reference (Roe et al. 1999) with kind permission from the publisher.

alterations of the stomach tissue under transmission electron microscopy (TEM). They found a bacillary form of *H. pylori* in mice stomachs two weeks after inoculation, which shows successful inoculation and replication of the coccoid *H. pylori*. Furthermore, they observed histopathologic changes in the murine gastric mucosa after inoculation of coccoid *H. pylori*, albeit which were less pronounced than after inoculation of bacillary *H. pylori*. Interestingly, inoculation of both forms of *H. pylori* led to a systemic antibody response towards *H. pylori* in all colonized mice (Cellini et al. 1994). Based on this fact, *H. pylori* may be viable but not culturable in its coccoid form in the oral cavity, although it may be “revived” in the human stomach when swallowed. Therefore, the coccoid form of *H. pylori* can be considered as a “viable but not culturable” (VBNC) state of *H. pylori* (Rudnicka et al. 2014). In the VBNC state, *H. pylori* usually shows decreased metabolic activity and little or no ability to replicate (Roe et al. 1999). Most human

pathogens (including *H. pylori*) exhibit a VBNC form, and bacteria in this state may play an essential role in recurrent and drug-resistant infections (Ozçakir 2007). It has been found that low levels of AI-2 (Autoinducer-2) in supragingival plaque may allow dental *H. pylori* to colonize the oral cavity as non-culturable forms (Krzyżek and Gościński 2018). But there is still not much research on the ability of *H. pylori* to colonize in oral biofilms or respective *in vitro* models. In this light, it will be interesting to study whether *H. pylori* changes to its coccoid form after it encounters human saliva or typical oral bacteria and whether it can be “resurrected” again or not.

While researchers have found *H. pylori* in drinking water, seawater, vegetables, and animal food (Quaglia and Dambrosio 2018) or even on refrigerated ready-to-eat food (Poms and Tatini 2001), it remains unknown whether the oral cavity can be “contaminated” with *H. pylori* by consuming *H. pylori*-infected food. On the other hand, only a few studies have successfully cultured this bacterium from water or food, which means that positive results in many reports may simply reflect contamination with either dead *H. pylori* organisms or even naked DNA.

Likewise, positive detection of *H. pylori* may also be due to gastroesophageal reflux, which could bring *H. pylori* or “fragments” from the stomach to the oral cavity. Therefore, it may be interesting to specifically investigate a cohort of *H. pylori*-positive patients suffering from reflux for oral detection of *H. pylori* by molecular, immunological and culture-based methods. As the estimated half-life of cell-free DNA has been described to range from several minutes up to two hours (Kustanovich et al. 2019), taking samples at least two hours after reflux might be a way to eliminate the influence of gastroesophageal reflux in such a cohort of patients.

Combining all of these aspects mentioned in the paragraphs above, Figure 3 depicts the potential “cycle” of *H. pylori* in the human body. Due to the lack of deposition of oral *H. pylori* strains in national or international culture collections, collaboration among researchers is particularly meaningful. Building a network of the researchers who have reported isolating *H. pylori* from the oral cavity would be very worthwhile to confirm these isolates as *H. pylori*, e.g. by whole genome sequencing approaches. Overall, future studies investigating the potential colonization of *H. pylori* in the oral cavity should consider some important aspects, which are outlined in Figure 4. This may also help either to prove or to rule out a potential oral-to-oral transmission route of *H. pylori*.

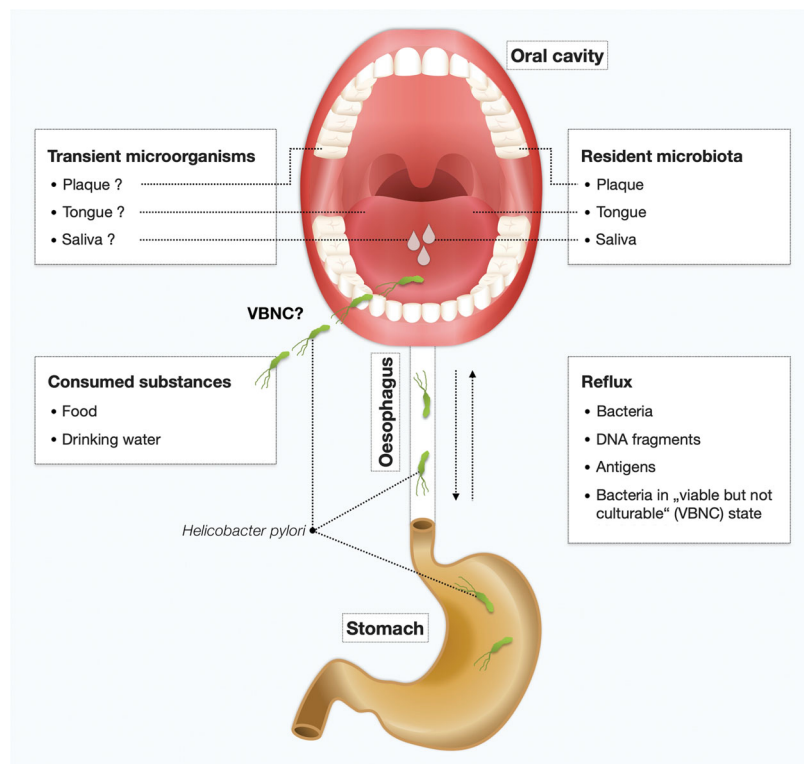


Figure 3. The cycle of *H. pylori* in the human body. The question on whether *H. pylori* is resident or transient in the oral cavity (saliva, dental plaque, or tongue microbiota) remains to be clarified. *H. pylori* can be introduced in the oral cavity by contaminated substances including food and drinking water. There may also be a relationship between gastric and oral *H. pylori* infection. *H. pylori* can be swallowed and may subsequently cause infections in the stomach. Conversely, reflux can bring back viable organisms, DNA fragments or antigens of *H. pylori* to the oral cavity from the stomach of *H. pylori*-infected individuals. This may lead to positive results in detection with molecular methods, immunological methods or culture methods. Furthermore, *H. pylori* may be present in a viable, but not-culturable (VBNC) state (*i.e.* coccoid form).

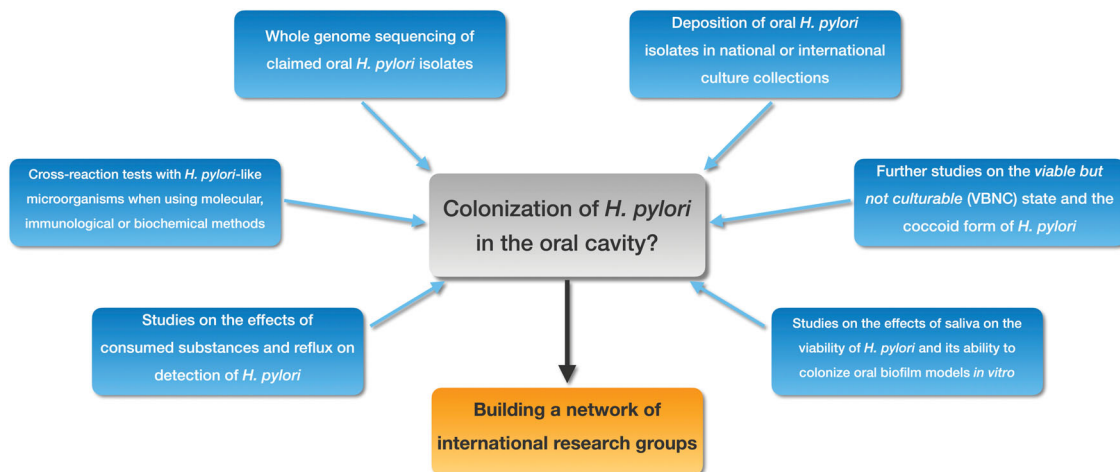


Figure 4. Future research directions. Important aspects for future studies investigating the potential colonization of *H. pylori* in the oral cavity:

- Whole genome sequencing of oral *H. pylori* isolates.
- Deposition of oral *H. pylori* in national or international culture collections.
- Research on the VBNC state and the coccoid form of *H. pylori*.
- Studies on the effects of saliva on viability of *H. pylori* and on the ability of *H. pylori* to colonize biofilms formed from oral bacteria *in vitro*.
- Effects of consumed substances and reflux on the detection of oral *H. pylori*.
- Cross-reaction tests with *H. pylori*-like microorganisms when using molecular or immunological methods.

To achieve these goals, we advocate establishing a network of researchers who have reported isolating *H. pylori* from the oral cavity.

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