A. Does *H. pylori* Exist in Oral Cavity?

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The Urea Breath Test (UBT) C13, 14 is a good rapid diagnostic procedure used to identify stomach infections by H. pylori. It is based upon the ability of *H. pylori* to convert urea to ammonia and carbon dioxide. Urea breath tests are recommended in leading society guidelines as a preferred non-invasive choice for detecting *H. pylori* of stomach before and after treatment with diagnostic efficacy at 96.7% sensitivity and 96.2% specificity. However UBT is not a test for detection of *H. pylori* in the mouth. In medical practice, patients with negative results in UBT suggest that their stomach infection of *H. pylori* is cured. In fact, patients can present negative UBT results and yet exhibit *H. pylori* infection due to oral infection. The clinical study provides evidence that *H. pylori* oral infection is nonetheless present. In Asia, more than 20-30 % of the population suffered from oral *H. pylori* infection but had negative UBT results [1]. Our study also showed that saliva antigen screening test (HPS) which is oral fluid-based molecular diagnostics that could identify individuals who have no risk for *H. pylori* gastric but oral infection. It further identified persons with no symptoms but with antigenic evidence of possible oral *H. pylori* infection who are thus at risk for developing gastric disease. This information was not provided by UBT methods [2].

WHY UBT CAN'T DETECTING ORAL *H. PYLORI* ?

The principle of UBT test: patients swallow urea labelled with an uncommon isotope, either radioactive carbon-14 or non-radioactive carbon-13. In the subsequent 10-30 minutes, the detection of isotope-labeled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that *H. pylori* use to metabolize urea) is present in the stomach, and hence that *H. pylori* bacteria are present. In UBT test, since the urea labelled is not dissolved in the mouth, so there were no isotope-labelled carbon dioxide in exhaled breath that is why UBT can't provide information of *H. pylori* in the oral cavity. However, people even a medical professional may not have a clean mind that knows UBT can't detecting *H. pylori* in oral cavity. They consider UBT is a good standard test for detecting *H. pylori* no matter which organ harbor this bacteria. This is a great risk to missing or misleading the information of oral H. pylori.

DOES A SECOND COLONIZATION SITE OF *HELICOBACTER PYLORI* EXIST, IN ADDITION TO PRIMARILY RESIDING IN THE STOMACH?

Over the past twenty years, the existence of oral *H. pylori* infection has been controversial, without a definite conclusion. It was proposed that no living *H. pylori* exists in the oral cavity and that the positive results detected by PCR from oral samples indicate that the presence of *H. pylori* fragments, rather than living bacteria, or are due to reflux from the stomach, which is infected with H. pylori. *H. pylori* could not be cultivated from PCR-positive samples. The *H. pylori* originating from stomach reflux was thought to survive in the oral cavity for only a few hours because of the high oxygen concentration of the mouth. If the above-proposed idea is correct, then the fragmented or dead *H. pylori* should not have any negative effect on the drug eradication of *H. pylori* infections of the stomach [3].

However, the proposed idea contradicts with PCR studies, because there are a number of studies using PCR as the indicated research tool; they indicated PCR is a sensitive and reliable test for detecting oral *H. pylori* [4]. The proposed idea contradict with the fact of oral hypoxia environment, because the sub gingival plaque of the oral cavity has microaerophilic environments favorable for the growth of this bacterium, and *H. pylori* was detected in the supragingival plaque of individuals with *H. pylori* gastric diseases by a rapid urease test and real-time PCR analysis [5]. The proposed idea contradicts with eradication can't eliminate oral *H. pylori* infection, because there are a number of studies that show when patients received drug treatment for stomach H. pylori, the drug did not eliminate oral H. pylori. Also, a study shows that mouth-rinse treatment alone or combined with periodontal treatment can, to some extent, reduce the prevalence of oral *H. pylori* and improve the eradication rate of gastric *H. pylori* [4,6]. The proposed idea contradicts with *H. pylori* can be cultured in the oral cavity. In the PCR-positive saliva sample, can *H. pylori* be confirmed by culture? The answer is yes! One study showed that *H. pylori* from a saliva sample can be cultured in individuals with all positive test results of the oral cavity [4]. The proposed idea contradicts with the same original source of oral and stomach *H. pylori*. The remarkable

genotype diversity among stomach, saliva, and stool samples showed that more than one *H. pylori* genotype may exist in the same patient [7,8]. The proposed idea contradicts with the fact of lower rate of eradication on stomach *H. pylori* when oral *H. Pylori* positive. Miyabayashi et al, [9] found the eradication success rate was significantly lower in the oral H. pylori-positive cases (12/23, 52.1%) than in the negative cases (22/24, 91.6%) at 4 weeks after the therapy (p = 0028). Two years later, only 16 of the 23 (69.5%) oral H. pylori-positive cases were disease-free, as compared to 23 of the 24 (95.8%) oral H. pylori-negative cases (p =018). The eradication efficiency in the stomach was 85.8% (187/218), while in the oral cavity it was only 5.7% (9/158) (OR = 55.59, P < 0.00001). *H. pylori* were more difficult to eradicate in the oral cavity than in the stomach, and may be a source of re infection.

Krajden et al. in 1989 first reported on the culture of *H. pylori* gastritis in seventy-one patients with plaque; one plaque culture result was positive, and of all seventy-one saliva cultures, none of the patients presented a positive result [10]. Since then, many scientists have attempted to cultivate oral *H. pylori*, but these attempts were rarely successful. Indeed, culture-positive rates are very low among published studies from various countries. The key difficulties in cultivating oral *H. pylori* result from oral specimen collection, preservation, small colonies of *H. pylori* culture, and competition with other oral bacteria and *H. pylori* colonies. Because the concentration of *H. pylori* in stomach is three magnitudes higher than that of the oral cavity (105 CFU/mL versus102 CFU/mL [11,12]), it would be insufficient to use conventional stomach culturing techniques for detecting oral *H. pylori*. The method must be adapted to obtain a high positive rate of oral *H. pylori* culture. Some authors have drawn premature conclusions that "oral *H. pylori* cannot be cultured" and "the oral cavity is not a colonized site", which has become the main theoretical basis to oppose oral *H. pylori* colonization.

Because the majority of physicians and scientists in this field ignore the colonized cavities of H. pylori, approximately 20% of the population of Asia suffers from oral *H. pylori* infection. In China alone, more than 280 million people carry oral H. pylori, which results in 28 million recurrences of stomach *H. pylori* infection and the abuse of antibiotics by overused [13]. The massive antibiotic pollution that appears in food, water, and children's urine has become a serious concern worldwide [14]. Antibiotic abuse kills 80,000 Chinese people every year and leads to extra medical spending of 11.7 billion dollars across the country, which could become a global problem.

Recent studies have not only shown that *H. pylori* can be detected fairly consistently from the oral cavity but also demonstrated that the chances of recurrence of *H. pylori* infection is more likely among patients who harbor the organism in the oral cavity. Furthermore, initial results from clinical trials have shown that H. pylori-positive dyspeptic patients may benefit from periodontal therapy [15]. A study included 1050 subjects in total and oral *H. pylori* infection occurred in 60.29% of the subjects. The prevalence rates of oral *H pylori* in patients with periodontal diseases (63.42%) and caries (66.91%) were significantly increased than those without oral diseases (54.07%), respectively (P < 0.05), while the difference between subjects with recurrent aphthous

stomatitis and controls was not significant. In addition, the differences of positive rates of *H. pylori* with or without history of gastric ulcer were statistically significant (69.47% vs 58.26%, P<0.05). Presenting with periodontal diseases (OR 1.473; 95% CI 1.021 to 2.124), caries (OR 1.717; 1.127 to 2.618), and having history of gastric ulcer (OR 1.631; 1.164 to 2.285) increased the risk of *H. pylori* infection. Their conclusions: Oral *H. pylori* infection is common in adult Chinese, which is significantly associated with oral diseases including periodontal diseases and caries [16].

WHAT IS THE CONSEQUENCE, IF WE MISSING DIAGNOSIS OF ORAL *H. PYLORI*?

a. The progressive loss of efficacy of standard eradication therapies has made the treatment of *H. pylori* more challenging than ever.

Endoscopic-guided antibiotic susceptibility testing had previously been suggested to guide treatment after failure of second-line therapies. However, its role has expanded over the years, in accordance with the current Maastricht Guidelines. Several authors have dealt with this topic, developing both efficacy trials and cost-effectiveness trials against resistant *H. pylori* infections as well as infections in patients. However, results are not homogeneous enough to provide definite advice, because antibiotic resistance is not the only reason for treatment failure. Moreover, the culture-guided approach is surrounded by many practical issues, such as the availability of both endoscopy units and microbiology laboratories, and the need for a standard of quality that cannot be satisfied everywhere [17]. We proposed the key conception that *H. pylori* has second colonized site in oral cavity beside of stomach.

b. The traditional treatment of gastric infection be ineffective against oral infection.

Drug treatment on stomach *H. pylori* infection has no effective in *H. pylori* infection of oral cavity. *H. pylori* exist in between the teeth and gums called “bio- film membrane” (Bifilm), also known as plaque barrier. It is resistance when the drug into this area. Our clinical trial show conventional treatment for *H. pylori* eradication, but is not efficacy of oral *H. pylori* [4]. See Fig 1 The effectiveness of treatment on oral *H. pylori* infection.

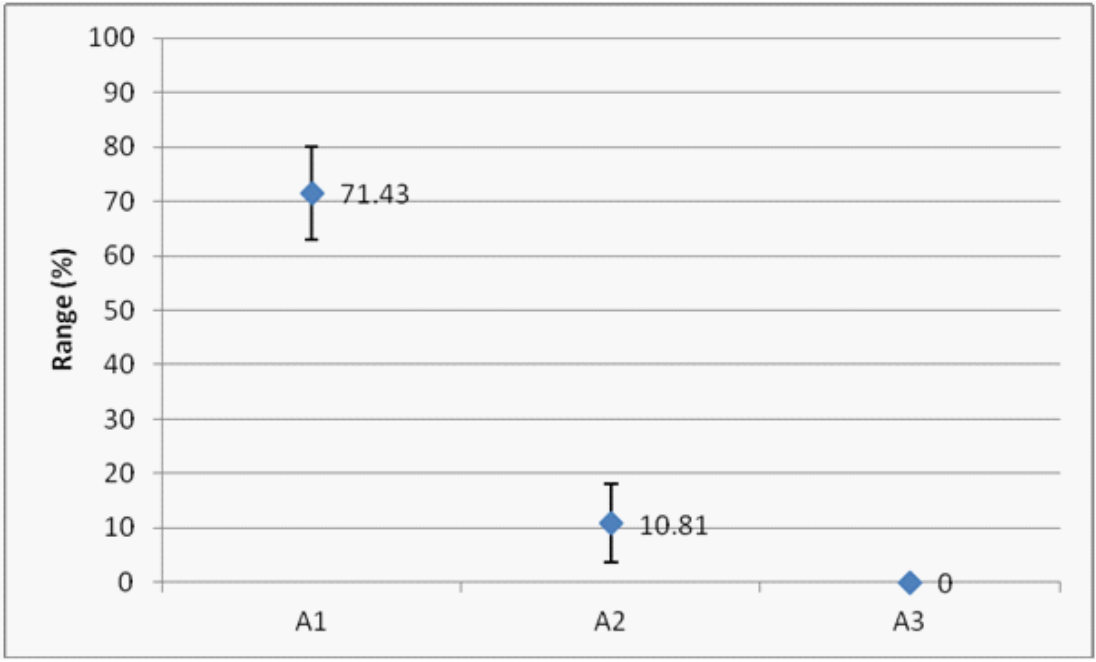

Figure 1: A1: patients received treatment with LGM* for oral infections, and DE** for stomach infections. The results indicated a 72.58% effectiveness rate on oral infections in the 95% of the CI ranges, as determined by changing HPS and PCR tests to negative. A2: Patients received DE, which was only 10.67% effective in the 95% of the CI ranges on oral infection. A3: Patients received Teeth Cleaning (TC). No patients showed changes in HPS, HPF or PCR. There was a significant difference of $p<0.05$ between A1 and A2, and between A1 and A3.

*LGM: the mouthwash solution contained lysine (0.4%) and glycerol monolaurate (0.2%). Oral rinsing with 20 ml of solution was done twice a day (in the morning and at night before bed) for one month.

*DE: standard dose Proton-Pump Inhibitors (PPIs) were used twice a day for 14 days. (PPIs include: lansoprazole 30 mg, omeprazole 20 mg, pantoprazole 40 mg, rabeprazole 20 mg, and esomperazole 40 mg). Also used were: 500 mg of tetracycline.

c. *H. pylori* sex transmit through oral sex.

The idea that *H. pylori* or another species of Helicobacter could cause urethritis has never before been proposed. There have been three conflicting studies conducted to determine if sexual contact plays any role in the transmission of *H. pylori* oral sex is one of the most common sexual practices in the world and it is possible that *H. pylori* could be transmitted via the act of fellatio to the urethra leading to infection. This organism may be the 'missing link' in explaining the large proportion of males with non-gonococcal urethritis where no other responsible organisms can be isolated. This is the first article to suggest a link etween *H. pylori* infection and urethritis [18].

Oral yeasts were isolated more frequently from normally-delivered neonates. The frequency of *H. pylori* genes in mothers' vaginal yeasts was significantly higher than in mothers' oral yeasts. A significant correlation was found between the occurrence of *H. pylori* genes in vaginal yeasts and that in neonates' oral yeasts, occurrence of *H. pylori* genes in mothers' vaginal yeasts or neonates' oral yeasts, and UBT+ results in mothers. Calbicans which colonizes the oral cavity of neonates through vaginal delivery or contact with environment or healthcare workers could be an important reservoir of *H. pylori*. Vaginal yeasts are more potent in accommodating *H. pylori* than oral yeasts. Accordingly, vaginal yeast is proposed as the primary reservoir of *H. pylori* which facilitates *H. pylori* transmission to neonates [19].

Oral sex (fellatio) is a very common sexual activity. *H. pylori* is mainly a gastric organism, but studies have reported that infected individuals may permanently or transiently carry *H. pylori* in their mouth and saliva [1]. The existing studies support the hypothesis that *H. pylori* could be a causative agent of non-gonococcal urethritis. It is possible that *H. pylori* may be transmitted via the act of fellatio in the urethra. Further research is required to explore the role of *H. pylori* in sexually transmitted urethritis [20].

ORAL FLUID-BASED MOLECULAR DIAGNOSTICS, SALIVA *H. PYLORI* ANTIGEN TEST

There are three important technologies developing to make a strong foundation of a colonized site of the oral cavity. PCR is a high sensitivity test for oral H. pylori, but it is not convenient in clinical settings. So, first a high sensitivity and specificity test in saliva such as HPS test should be established. Then we will have a much easier time of running clinical trial on a large number of patients to obtain a greater number of data, to find the positive correlation between oral and stomach *H. pylori* infection [2]. Second most important technology is developing a cell culture suitable for low concentration *H. pylori* in the oral cavity. After the method of a new cell culture is established, we would confirm if HPS technology can be confirmed by cell culture data [4]. As a final step, we need to develop a technology to eliminate *H. pylori* from the oral cavity instead of an antibiotic drug.

H. pylori antigen test for oral urease **(HPS):** It was specifically detected in saliva using a lateral flow immunochromatographic test device. The device for *H. pylori* antigen detection in saliva was identical to the device used for oral urease detection. The HPS test for saliva employed monoclonal antibody that was developed against oral urease. Test Procedure: No food or drink was allowed one hour prior to the test. A swab was put under the tongue for at least one minute. The swab was swirled vigorously for 15 seconds in a buffer solution, then we expunged as much liquid as possible from the swab by pressing and rotating the fiber portion against the wall of the tube. Two to three drops of saliva/buffer mixture were added into the sample well. As the test kit begins to work, one will see a purple color move across the result window in the center of the test disk. The presence of two color bands ('T' band and 'C' band) within the result window indicates a positive

result. The presence of only one purple color band indicates a negative result. Specificity: An in-house study was conducted with three separate lots of the HPS test to determine its specificity. The following common oral bacteria had been applied: *Actinomyces naeslundii, Actinomyces odontolyticus, Bifidobacterium dentium, Corynebacterium matruchotii, Gemella haemolysans, Granulicatella adiacens, Streptococcus gordonii, S. salivarius, S. sanguinis,* and *Veillonella parvula*. All of the above were analyzed and did not show interference or cross-reactivity with the test. Sensitivity: The test's sensitivity was 10 ng/ml HPS antigen [2].

The specificity of monoclonal antibodies used in antigen tests greatly influences the outcome of screening. At least 8 related Campylobacteraceae species have been detected in the oral microbiome [21,22]. BLAST searching of available flagellin and urease sequences suggested that some peptide homology exists among these species. Nonetheless, this study showed an apparently low incidence of oral antigen test cross-reaction with non- *H. pylori* bacteria.

CONCLUSION

1. A colonized site of *H. pylori* can exist in the oral cavity.
2. In medical practice, it is assumed that patients with negative UBT results are cured of their stomach *H. pylori* infection, but in fact, patients can present negative UBT results and exhibit oral *H. pylori*. This information was not provided by UBT methods.
3. If there is a live *H. pylori* colony in the oral cavity, then it would have a negative influence on the eradication of a stomach infection. In the classic *H. pylori* eradication programs, there are no clear measures of oral H. pylori; frequent relapses become more critical.
4. Oral Fluid-Based Molecular Diagnostics, Saliva *H. pylori* antigen test can detecting oral and stomach *H. pylori*.

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