

## Dental Caries—A Hiding Niche for *Helicobacter Pylori* in Children

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**Background:** *Helicobacter pylori* (*H. pylori*) is one of the human pathogens proven to be present in the oral cavity due to microaerophilic nature of the dental biofilm. The present study aimed to investigate the presence of *H. pylori* in cavitated carious lesions of children by polymerase chain reaction (PCR). **Study design:** Forty-eight children aged between 4 to 7-years attending outpatient Pediatric clinic were enrolled in the study. Caries status and caries severity were assessed using the dmft and ICDAS caries index. Dentine samples were collected for DNA isolation for the detection of *H. pylori* by PCR. **Results:** *H. pylori* was detected among 30% of children with severe caries lesions detected by PCR. Overall, the mean  $\pm$  SD of the dmft score for *H. pylori* positive children was higher compared to the negative control. Amongst the *H. pylori*-positive group, the decayed (mean-dt) number of teeth were significantly higher ( $p < 0.05$ ) than the other group. Moreover, association between severity of caries lesions (codes 5 and codes 6) and presence of *H. pylori* were significant ( $p < 0.05$ ) when compared negative group. **Conclusion:** The results demonstrate presence of *H. pylori* in the cavitated, non-gastric niche of children with severe caries, which possibly could serve as a reservoir for microbial dissemination to other sites of the body.

**Keywords:** *Helicobacter pylori*, cavitated caries lesion, polymerase chain reaction (PCR), oral microbiome.

### INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is one of the common human pathogens affecting up to 50% of world population<sup>1, 2</sup>. Infection of this Gram negative, microaerophilic and spiral-shaped bacterium is believed to be a significant etiologic factor for gastritis, gastric and duodenal ulcers, and gastric cancer.<sup>3</sup> However, only about 15% of infected individuals develop disease in their lifetime depending on the virulence factor of the organism and other host and environmental factors.<sup>4</sup>

Reports of the presence of *H. pylori* in the oral cavity are conflicting.<sup>5, 6</sup> Some researchers concluded that the presence of *H. pylori* in the oral cavity is transient and carries no clinical significance.<sup>7-9</sup> Contrary, numerous studies reported presence of *H. pylori* in various oral- microbial habitats including plaque, saliva, tongue, and tonsillar area in the oro-pharynx.<sup>10-12</sup> These investigations observed a significant relationship between extra-gastric occurrence of *H. pylori* and the gastric ailments due to the stated microbes.<sup>13-15</sup> Additionally, some reports suggested that oral habitats serve as a reservoir for re-infection from *H. pylori*.<sup>16, 17</sup> According to Suk *et al*<sup>18</sup>, oral biofilm may provide an optimal pH, temperature, and microaerophilic environment for *H. pylori* to grow.

Despite availability of different treatment protocols, such as, eradication therapy with systemic antibiotics, proton pump inhibitors, and colloidal bismuth subcitrate, the high re-infection rate of *H. pylori* suggests that the organism may reside in other areas of the body that are not affected by systemic antibiotics and this could possibly serve as source of recurrent infection.<sup>1, 19</sup>

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To date, there are no data available in the literature regarding the cavitated caries lesions, as a habitat, for *H. pylori*. In the present study we aimed to investigate, if caries lesions would act as reservoir of *H. pylori* using Polymerase Chain Reaction (PCR).

## MATERIALS AND METHOD

Forty-eight children with severe caries -lesions, aged from 4 to 7-years attending outpatient Pediatric Dentistry clinics at University of Sharjah- Dental hospital, UAE, participated in the study. All participants were medically fit at the time of dental-clinical examination, cooperative, and underwent full dental examination. Un-cooperative child, or children on antibiotics or with tooth anomalies, were excluded from the study. Informed consents were obtained from the parents or caregivers of each participant in this study under the protocol approved by the REC, University of Sharjah (REC-18-02-18).

Caries status of every child participants was recorded according to the World Health Organization criterion (1997), using dmft index.<sup>20</sup> The severity of class 1 and class 2 cavities was ascertained according to the ICDAS caries severity criteria codes.<sup>21</sup> A pediatric dentist conducted all clinical examinations for all child participants. Children with four or more, asymptomatic decayed primary molars with caries severity of code 5 or code 6, were included in this study. Plaque scores of the participants were recorded using the Quigley and Hein Plaque Index, modified by Turesky and colleague.<sup>22</sup> Socio-demographic and oral health-related data were collected using a questionnaire.

Identified occlusal cavitated caries lesion and proximal cavitated caries lesion with caries severity of ICDAS code 5 or code 6, were cleaned and dried with water and air using a triple syringe. The pH of the cavities were noted using a pH indicator strip (Spezilindikator, Merck, Germany) before cleaning according to the previously described protocol by Carlen et al.<sup>(23)</sup> The dentine samples were collected using sterile spoon-excavator (1.5mm-LM Dent). The samples were collected in phosphate-buffered saline (PBS) and stored at -20°C until use.

DNA extraction and purification from the collected samples were done using QIAamp DNA Mini Kit (Qiagen, Germany) as per the manufacture's protocol. Quantity and Quality of the extracted-DNA were assessed by Qubit using Qubit® dsDNA HS High Sensitivity- Assay Kit (Invitrogen, USA). PCR specific primers targeting 16S-rRNA sequence, forward 5'CTGGAGARACTAAGYCCTCC3' and reverse 3'GGAATACT-CATTGCGAAGGCGA5' used to detection *H. pylori* with the following conditions 94°C for 5 min, 40 cycles of 94°C for 1min, 58°C for 1min and 72°C for 1 min, followed by 72°C for 7 mins. The PCR reaction was performed using Veriti Dx thermal cycler, Applied Biosystems, USA. Purified genomic DNA from *H. pylori*-positive sample was used as a positive control. PCR-products were assessed by 2.0% (w/v) agarose gel electrophoresis.

To gain robust results, with 15 *H. pylori* -positive children, we compared twice the number of *H. pylori* -negative children. This increases the power of the present study to detect effects. The overall results were described using descriptive statistics. To analyze the relationship between socioeconomic status, oral hygiene, dental visits, and the presence of *H. pylori* and in caries dentin samples Pearson's chi-squared test was used. The student *t*-test was employed

to test the significance of the association between the caries status, oral conditions, and presence/absence of *H. pylori*. The  $p \leq 0.05$  was deliberated as statistically significant. Statistical analyses were performed using SPSS-version 22 (SPSS Inc., Chicago, Ill., USA).

## RESULTS

A total of 48 children aged between 4 to 7-years with severe dental caries were enrolled in the present study. The prevalence of *H. pylori* was found to be 31.37% (n=15) in the deep-dentine carious lesions of these children. The number of decayed teeth with a cavitated lesions involving less than half of the tooth code 5 and severe cavitated lesions involving more than half of the tooth code 6 were highly positive for *H. pylori* (Figure 1). The PCR-analysis of DNA obtained from clinical isolates shows a significantly high prevalence of *H. pylori* in the proximal deep-dentine caries lesions (Figure 2). In the proximal deep dentin cavitated lesions (caries code 6), we found an abundance of *H. pylori* in the acidic pH environment. Agarose gel electrophoresis of PCR assay of *Helicobacter pylori* in the tested DNA samples of children is represented in (Figure 3).

Socio-demographic and oral health related behavior data are summarized in (Table 1). The mean- ages of children in both groups were similar. The influence of variables such as family income, family size, and mothers' educational level did not contribute significantly to the deep dentine *H. pylori* positive and negative groups. Over 85% of the children brush their teeth without parental assistance, once per day, while, less than 15% of the children underwent brushing performed by the parents or their caregivers in either of the studied groups. Children in the *H. pylori*-negative group attended for their dental care more than once, which was significantly more frequent than those tested positive for *H. pylori*.

The mean  $\pm$  SD dmft score for *H. pylori* positive children was higher compared to the *H. pylori* negative control, though the dissimilarities were not significant between the two groups of children with severe caries lesions. Amongst the *H. pylori* positive group, the decayed (mean-dt) number of teeth were significantly higher than the other group. We observed a statistically significant association ( $p < 0.05$ ) between positivity for *H. pylori* and severity of caries lesions (codes 5 and codes 6) when compared to *H. pylori*, negative groups. The effect of salivary pH and level of plaque scores variables did not significantly contribute to the extensive caries *H. pylori* positive and negative groups (Table 2).

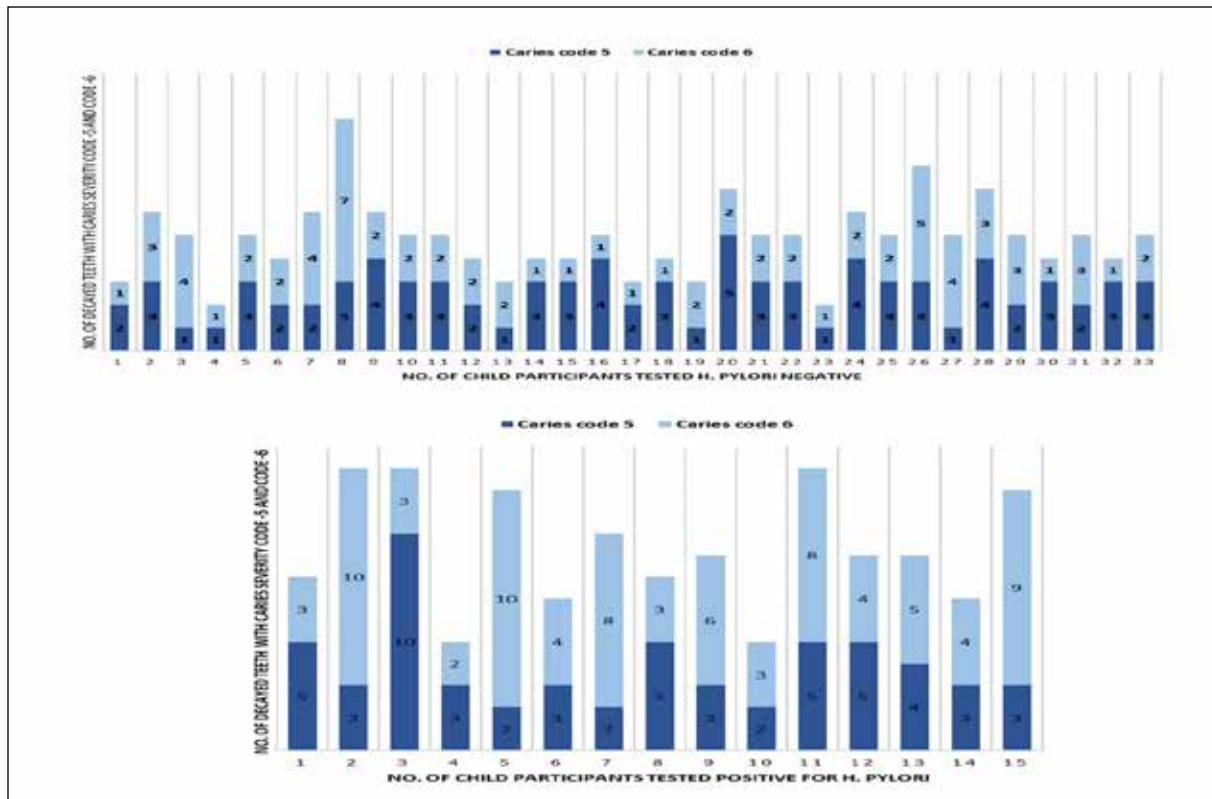


Figure 1: Decayed teeth with caries-severity codes 5/6, classified according to ICDAS and presence or absence of *H. pylori* among (n=48) child participants

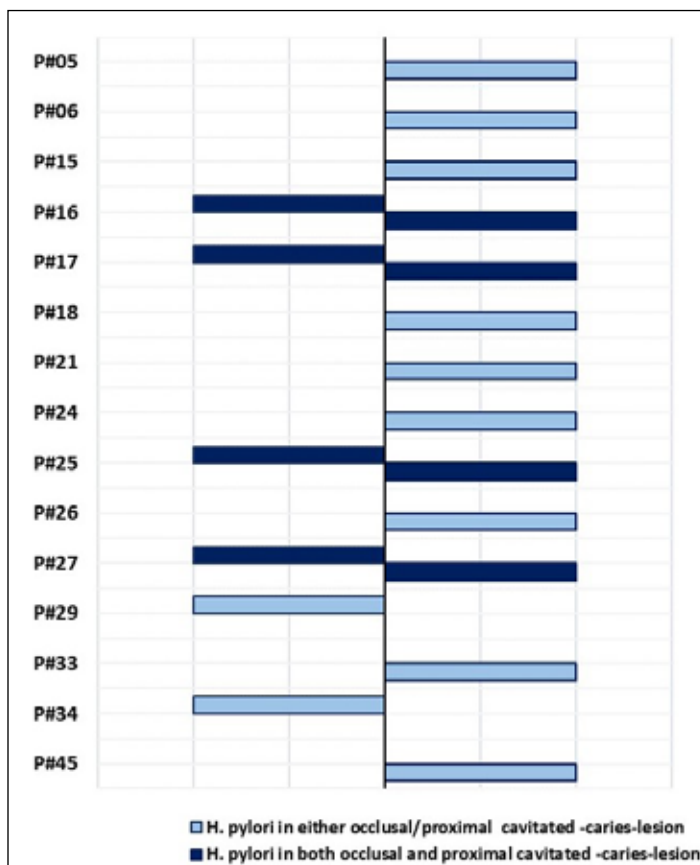


Figure 2: Presence of *H. pylori* in occlusal and proximal cavitated-caries-lesions ( $p < 0.001^{**}$ )

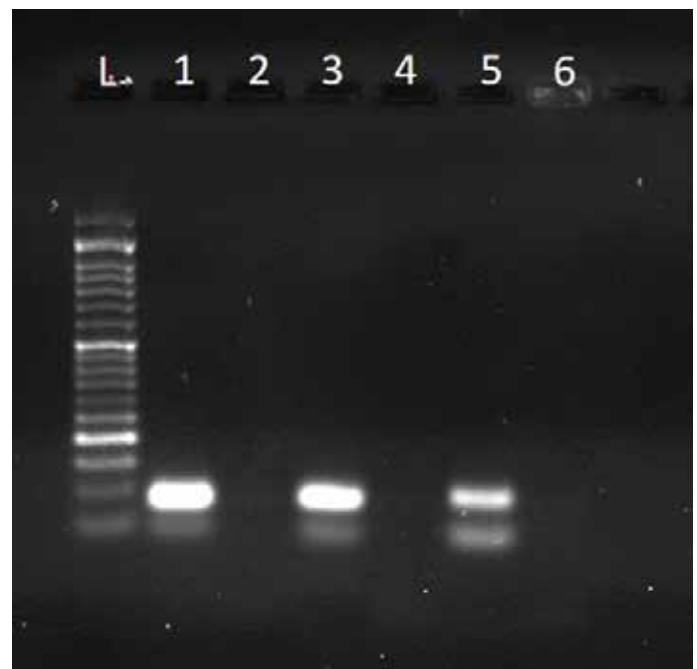


Figure 3: Agarose gel electrophoresis of PCR assay of *Helicobacter pylori*.

Lane L- 100 bp ladder, Lane 1- Positive control (~150bp), Lane 2- Negative control, Lane 3,5- Samples positive for *H. pylori* showing amplification of ~150bp and Lane 4,6 Samples negative for *H. pylori* infection.

**Table 1. Socio-demographic characteristics and oral-health behaviors of the sample population**

Characteristics	H. pylori (+) (n=15)	H. pylori (-) (n=33)	p-value
<b>Gender</b>			
male	8 (53.3%)	14 (42.4%)	0.53 †
female	7 (46.6%)	19 (57.6%)	
<b>Age</b> (mean age-years)	5.13±0.92	5.24±0.79	0.46 †
<b>Family income</b>			
Low income bracket (5,000-9,999) AED	8 (53.3%)	17 (51.5%)	0.33 †
Middle/high income bracket (>10,000) AED	7 (46.6%)	16 (48.5%)	
<b>Education level of mother</b>			
Less than high/high school	9 (60%)	18 (54.5%)	0.28 †
College/University education	6 (40%)	15 (45.5%)	
<b>Family size</b>			
Up to 4 people	2 (13.3%)	5 (15.2%)	0.83 †
Five or more people	13 (86.6%)	28 (84.8%)	
<b>Maternal Dental visit/s</b>			
Never	5 (33.3%)	12 (36.4%)	0.33 †
Once or >1 time a year	10 (66.6%)	21 (63.6%)	
<b>Child Dental visit/s</b>			
Never	11 (73.3%)	13 (39.4%)	0.04* †
Once or >1 time a year	4 (26.6%)	20 (60.6%)	
<b>Frequency of brushing</b>			
1 time per day	12 (80%)	26 (78.8%)	0.41 †
> 1 time per day	3 (20%)	7 (21.2%)	
<b>Who brushes child's teeth?</b>			0.62 †
Child	13 (86.6%)	29 (87.9%)	
Parent /Caregiver	2 (13.3%)	4 (12.1%)	

† Student's t-tests; † \*Chi-square test. Values are presented as mean ± SD or n (%).

**Table 2. Caries status and oral findings of the sample population**

Variables	H. pylori (+) (n=15) Mean ±SD	H. pylori (-) (n=33) Mean ± SD	p-value*
Mean dmft (SD)	14.2±4.04	11.76±3.47	0.42
Mean dt (SD)	11.33±3.02	6.97±2.05	0.001*
<b>Caries severity (ICDAS)</b>			
Caries code 5	3.87±2.03	2.61±1.05	0.04*
Caries code 6	5.47±2.89	2.24±1.35	0.001*
Plaque index	0.69±0.52	0.67±0.34	0.81
Salivary pH	7.32±0.52	7.27±0.27	0.65

\*p≤0.05, obtained using t-tests

## DISCUSSION

*H. pylori* infection can occur during childhood through oral ingestion, and when it ensues, it thrives through the child's lifetime unless eradicated by medical intervention.<sup>24</sup> Several studies demonstrated that the presence of this microbe in saliva and dental biofilm, making the oral cavity a potential reservoir for reinfection and a cause of failure of eradication treatment.<sup>25</sup> To the best of our information, to date, no data have yet been published regarding the presence of *H. pylori* in severe caries-lesions in such a young age-group.

In agreement with other studies, our study demonstrates that *H. pylori* associated with high caries rates.<sup>26-28</sup> Whether the detected *H. pylori* in the current study is due to long-term colonization or transient reinfection from reflux is a point of further investigation. However, close association between severity of caries and the presence of *H. pylori* observed in our study suggests that these microbes are early plaque colonizers which could have tipped the balance of the plaque ecosystem in favor of *Streptococcus mutans*.<sup>27</sup> Thus, possibly contributing to the severity of caries lesions, as reported by Zhu *et al.*<sup>28</sup> This could be ascribed to the fact that identified *H. pylori* aciduric potential helps flourish and continue their progeny, regardless of the antagonistic low pH conditions in deep cavities.

In the present study, *H. pylori* was more characterized in proximal than in occlusal cavitated lesions. This finding can be explicated on the basis that occlusal cavities are more subjected to ecological changes in pH, dilution with water/saliva and mechanical cleansing by mastication and brushing. Contrary, proximal cavities may have provided shelter for *H. pylori* to prosper, as they are not influenced either by strong masticatory forces or salivary flow and are difficult to clean spaces.<sup>29,30</sup> Vanderas and colleagues in a 4-year's longitudinal study observed that, as the cariogenic factors intensified, the progression gets faster in proximal lesions compared to carious occlusal cavities.<sup>31</sup>

Intriguingly, both occlusal and proximal severe caries lesions (caries-code 6) demonstrated a higher prevalence of *H. pylori* in this study. This distinctive distribution could be an expression of the microaerophilic characteristics of *H. pylori*.<sup>32</sup> Theoretically, oxygen exposure possibly reduced as the depth of the lesion increases, which provides an ambient environment for these microbes to survive.

The current study showed a strong statistical correlation between the caries lesion's low pH environment and the occurrence of *H. pylori*. This could be explained by the fact that the microaerophilic microbes can sustain low pH, and their possible synergistic interaction with other acidogenic and aciduric microbes in the biofilm microenvironment made them flourish in those hidden niches.

In agreement with Escobar *et al.*,<sup>33</sup> frequency of dental checkup was significantly related to the prevalence of *H. pylori* in our study. Though, the data associated with the frequency of tooth brushing did not show any influence on the presence or absence of *H. pylori*, whether done by the child alone or with assistance from a caregiver. These findings highlight the importance of routine dental checkup and early diagnosis of carious lesions in children. The cavitated lesion may represent

a risk factor for these microbes in the oral cavity.<sup>1</sup> Deep- cavities carrying *H. pylori* is not just a habitat,<sup>(34)</sup> these niches can serve as a reservoir for infections elsewhere in the body.

Investigating the occurrence of *H. pylori* in carious lesions, as well as using the PCR technique, known for its sensitivity might be considered as points of strength in the current study. This is an exploratory study and further investigation of the microbe's antigenic types will help understand their probable role in gastric pathology.

## CONCLUSION

Deep cavitated caries lesions may serve as a reservoir for *H. pylori*. These hidden niches provide thriving ground for *H. pylori*, which can be a source of extra-oral transmission and infection to the digestive tract.

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