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A comparative study of the prevalence of *Helicobacter pylori* in the oral biofilms of a group of dental and non-dental undergraduates from Sri Lanka

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Dental health care workers (DHCW) are at a risk of occupational exposure to *Helicobacter pylori* from the aerosolized oral biofilms and saliva of patients. We designed this study to investigate the prevalence of *H. pylori* in the oral biofilms of a group of dental and non-dental undergraduates from Sri Lanka. After obtaining informed consent, oral biofilms were collected from 38 dental undergraduates (19 males and 19 females) undergoing clinical training and 33 non-dental undergraduates (14 males and 19 females). The participants were in the age range of 22–27 years and had healthy periodontium. Total DNA from the oral biofilms were extracted, and *H. pylori* DNA was detected using polymerase chain reaction (PCR) amplification of 16S rRNA gene of *H. pylori* using JW22-JW23 primers, and the results were confirmed using PCR amplification of *H. pylori-urease* specific HPU1-HPU2 primers. Out of 71 participants, 11 (28.95%) dental and 3 (9.09%) non-dental undergraduates had *H. pylori* in their oral biofilms indicating an overall prevalence rate of 19.72% (14/71). Thus, the prevalence of *H. pylori* in oral biofilms was significantly higher in dental undergraduates than in non-dental undergraduates ($p < 0.05$). An odds ratio of 4.07 indicated that dental undergraduates were four times more likely to harbor *H. pylori* in their oral biofilms than non-dental undergraduates. Foregoing data support the fact that there may be greater occupational risk of exposure to *H. pylori* for dental undergraduates during clinical training than that for non-dental undergraduates, warranting meticulous infection control practices during clinical dentistry.

Keywords: Dentistry, *Helicobacter pylori*, Infection control, Polymerase chain reaction, Occupational exposure

Introduction

Helicobacter pylori is a microaerophilic, gram−negative bacterium, often associated with gastric ulcers and chronic gastritis. Besides, *H. pylori* has been classified as a leading carcinogen especially with regard to gastric adenocarcinoma [1−3]. Even though nearly 50% of the global human population is affected by *H. pylori* only about 15% become symptomatic of *H. pylori* infection while majority remain asymptomatic [4]. The infection rate of *H. pylori* appears to be high in developing countries suggesting that the infection transmission is facilitated by poor sanitation, unawareness and crowded living conditions [5].

Even though the primary habitat of *H. pylori* is human stom−

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ach, it has also been isolated from the oral cavity, mainly in the oral biofilm and saliva [6,7]. Oral colonization by *H. pylori* is facilitated by gastro-esophageal reflux and poor oral hygiene of the patients. For instance, Song et al. [8] have reported that 97% of patients with gastritis harbored *H. pylori* in the oral biofilm. In another study, Gebara et al. [9] using polymerase chain reaction (PCR) found that 43% of the oral biofilm samples harvested from the patients with gastritis had *H. pylori*. As such, the oral cavity exists as the major extra-gastric reservoir of *H. pylori* that contributes to re-infections [7,10]. Furthermore, one of the major routes of transmission of *H. pylori* is through oral-oral route by direct or indirect contact [11,12]. However, prevalence of *H. pylori* in the oral biofilm of healthy individuals has not been explored adequately.

Several studies have claimed that various health care workers are at an occupational risk of acquiring *H. pylori* due to their risk of contamination with the oral and gastrointestinal contents of their patients [13,14]. Major routes of transmission of *H. pylori* within a population include oral-oral, gastro-oral, and faeco-oral routes [11,12]. Accordingly, gastroenterologists, nurses, and caregivers of mentally disabled are at a high risk of receiving *H. pylori* from their patients [13,15,16]. Similarly, dental healthcare workers (DHCW) may also be vulnerable to infection by *H. pylori* owing to their frequent exposure to aerosolized oral biofilm and saliva from their patients [17,18]. Further, dental undergraduates work closely with their patients and perform various aerosol generating clinical procedures such as oral surgeries, periodontal treatment, and dental restorations. However, comparative studies to assess the prevalence of *H. pylori* in the oral biofilm of dental and non-dental professionals are scarce. Therefore, the present study aimed to investigate the prevalence of *H. pylori* in the oral biofilms of a group of dental and non-dental undergraduates in Sri Lanka.

Materials and Methods

1. Study participants

This study consisted of two groups of undergraduates who were enrolled following convenient sampling and upon their informed consent. Group 1 consisted of 38 (19 males and 19 females) final year dental undergraduates who were in clinical training at the Faculty of Dental Sciences, University of Peradeniya, whereas Group 2 consisted of age and sex matched 33 (14 males and 19 females) final year non-dental undergraduates who had no patient contact during their training at the University of Peradeniya, Sri Lanka.

Exclusion criteria included smoking, alcohol use, betel chewing, diabetes mellitus, pregnancy and drug therapy such as anti-secretor, antacids, and antimicrobials. All the participants were undergraduates within 22–27 years of age having healthy periodontium with gingival bleeding on probing < 10% and loss of attachment < 3 mm [19]. Ethical approval for the study was obtained from the ethics review committee of the Faculty of Dental Sciences, University of Peradeniya (ERC/FDS/UOP/I/2018/11).

2. Collection of oral biofilm samples

The demographic data and the relevant medical and dental history of the participants were recorded using an interviewer administered questionnaire. Pooled oral biofilm samples were obtained with two sterile toothpicks passed around the tooth margins in two quadrants of the mouth following the methods described previously with slight modifications [20,21]. Each sample was collected into a sterile tube containing 500 μL of 1X TE buffer and stored at −20℃ until downstream processing.

3. DNA extraction

Total DNA was extracted from the oral biofilm samples according to the methodology described by Laine et al. [22]. In summary, biofilm samples were centrifuged at 300 × g for 10 minutes. The pellet was washed twice in 0.9% saline, resuspended in 100 μL of 50 mM NaOH, and boiled for 10 minutes. Samples were then neutralized with 14 μL of 1M Tris (pH 7.5) and centrifuged at 14,000 × g for 3 minutes. Resultant supernatants were stored at −20℃ until PCR amplification. Absorbance values at 260 nm and 280 nm of each sample were measured using spectrophotometer to determine DNA purity and concentration. The DNA of *H. pylori* ATCC 43629 strain was used as the positive control sample.

4. PCR amplification

1) Identification of *H. pylori* positive samples

To identify the *H. pylori* positive samples, PCR amplification of *H. pylori* DNA was performed according to the method described by Riggio and Lennon [6] using the primer pair JW22 and JW23 (Table 1) which targeted the 16S rRNA gene of *H. pylori*. Amplifications were performed using 15 μL total reaction volumes containing 7.5 μL 2X GoTaq Green® master mix

Reaction buffer (pH 8.5) (400 μM of each of dATP, dGTP, dTTP, dCTP, and 3 mM $MgCl_2$) (Promega, Madison, WI, USA), 0.5 μL of each primer, nuclease free water and template DNA. Cyclic process consisted of initial denaturation at 95℃ for 5 minutes, followed by 35 cycles consisting of 1 minute denaturation at 94℃, 1 minute of annealing at 60℃ and 1 minute extension at 72℃ followed by a final extension of 10 minutes at 72℃.

2) Confirmation of *H. pylori* positive samples

To confirm the presence of *H. pylori* DNA in the samples which gave positive results for PCR amplification with JW22 and JW23 primers, a second PCR was carried out using the primer pair HPU1 and HPU2 (Table 1) [21,23]. Amplifications were performed using a 15 μL total reaction volume containing 7.5 μL 2X GoTaq Green® master mix reaction buffer (pH 8.5) (400 μM of each of dATP, dGTP, dTTP, dCTP, and 3 mM $MgCl_2$), 0.5 μL of each primer, nuclease free water and template DNA. Cyclic process consisted of initial denaturation at 94℃ for 4 minutes, followed by 35 cycles consisting of 1 minute denaturation at 94℃, 1 minute of annealing at 45℃ and 1 minute extension at 72℃ followed by a final extension of 10 minutes at 72℃.

The PCR products were separated in 2% agarose gels in 1X TAE buffer (Tris-HCl [pH 8.0], 0.5% ethylene diamine tetraacetic acid and glacial acetic acid) stained with 3 μL of 2% ethidium bromide. A 100 bp ladder was used to compare the sizes of the PCR products.

5. Statistical analysis

Statistical analysis of the results was carried out using Minitab 14 statistical software (Minitab, State College, PA, USA) using chi-square test and the $p < 0.05$ was considered significant.

Table 1. Primers used to amplify 16S rRNA and *Urease* gene of *Helicobacter pylori*

Primer	Gene	Sequence	Annealing T_m
JW22[a]	16S rRNA	5' – CGT TAG CTG CAT TAC TGG AGA – 3'	60℃
JW23		5' – CAG CGC GTA GGC GGG ATA GTC – 3'	
HPU1[b]	*Urease*	5' – GCC AAT GGT AAA TTA GTT – 3'	45℃
HPU2		5' – CTC CTT AAT TGT TTT TAC – 3'	

[a]Previously used by Riggio and Lennon [6]; [b]Previously used by Eskandari et al. [21] and Medina et al. [23].

Results

Study included 38 dental undergraduates (19 males, 19 females) undergoing clinical training and 33 non-dental undergraduates (14 males, 19 females). All the study participants were in the age range of 22–27 year with satisfactory level of periodontal health.

Eleven out of 38 samples (28.95%) collected from dental undergraduates and 3 out of 33 samples (9.09%) collected from non-dental undergraduates were positive for 16S rRNA gene of *H. pylori*, exhibiting DNA bands of 295 bp (Fig. 1, Table 2). All the samples that gave positive bands for 16S rRNA gene of *H. pylori* upon PCR (JW22 and JW23 primers) produced positive bands of 411 bp upon PCR with HPU1 and HPU2 coding the *urease* gene (Fig. 1). Thus, out of the total of 71 participants, 14 individuals had *H. pylori* in their oral biofilms resulting an overall prevalence of 19.72% (14/71) (Table 2).

Statistical analysis revealed a significant difference between the prevalence of *H. pylori* in the oral biofilms of dental undergraduates with clinical exposure and non-dental undergraduates without clinical exposure (Pearson chi-square = 4.399, DF = 1, $p = 0.036$) (Table 2). An odds ratio (OR) of 4.07 indicated that

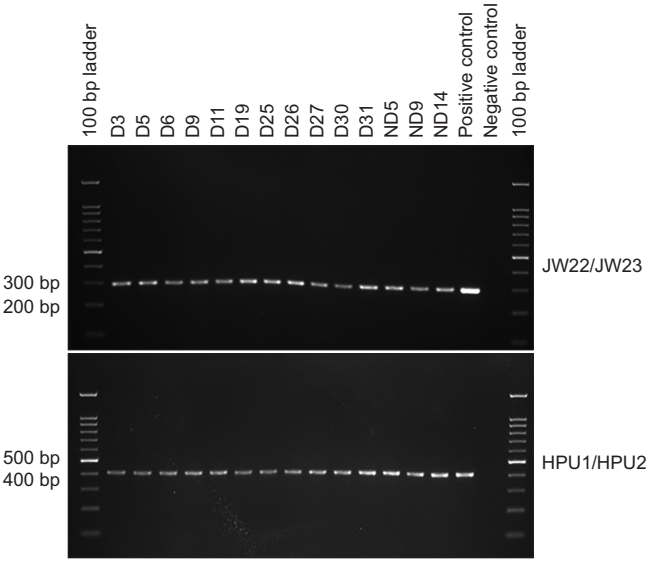

Fig. 1. Composite gel image exhibiting the polymerase chain reaction products obtained by the amplification of DNA extracted from oral biofilm samples of dental and non-dental undergraduates. Primer pair JW22 and JW23 targeted the 16S rRNA gene of *Helicobacter pylori* giving rise to an amplicon of approximately 295 bp whereas the primer pair HPU1 and HPU2 targeted the *Urease* gene giving rise to an amplicon of approximately 411 bp. Sample numbers are indicated at the top of the figure. The names of the primers used are shown at the right side whereas the corresponding band sizes are shown at the left side.

Table 2. *Helicobacter pylori* prevalence in oral biofilm from dental and non-dental undergraduates

H. pylori	Dental			Non-dental		
	Male	Female	Total	Male	Female	Total
Positive	5 (26.3)	6 (31.6)	11 (28.9)	1 (7.1)	2 (10.5)	3 (9.1)
Negative	14 (73.7)	13 (68.4)	27 (71.1)	13 (92.9)	17 (89.5)	30 (90.9)
Total	19 (100)	19 (100)	38 (100)	14 (100)	19 (100)	33 (100)

Values are presented as number (%).
$X^2 = 4.399$, $p < 0.05$.

dental undergraduates were more than four times as likely to harbor *H. pylori* in their oral biofilm as non-dental undergraduates with a 95% confidence interval (CI).

Discussion

Oral cavity remains the main extra-gastric reservoir of *H. pylori* [21]. Meanwhile, *H. pylori* has been attributed to the pathogenesis of some oral disease conditions such as aphthous ulcers, halitosis, periodontitis, and oral cancer [24]. However, prevalence of *H. pylori* in the healthy human oral cavity is inconclusive. Castro-Munoz et al. [25] have reported 13% prevalence of *H. pylori* in the oral cavity of Mexican children with an increased tendency of infection with age. Further, *H. pylori* has been demonstrated in the oral cavity of both gastric *H. pylori* positive and gastric *H. pylori* negative patients [20]. However, incidence of *H. pylori* in the oral cavity has been significantly associated with the presence of *H. pylori* in the stomach [21]. In a pioneering study to explore the prevalence of H pylori in the oral biofilm samples of chronic periodontitis patients, Riggio and Lennon [6] have revealed that nearly 38% of patients with chronic periodontitis had *H. pylori* in the oral biofilm.

Intriguingly, current study revealed that the overall prevalence of *H. pylori* in the oral biofilm of a healthy group of undergraduates was nearly 20%. Moreover, the prevalence of *H. pylori* in the oral biofilm of dental undergraduates and non-dental undergraduates was 29% and 9%, respectively and they were significantly different ($p < 0.05$). Furthermore, it was evident that the dental undergraduates were more than four times likely to harbor *H. pylori* in their oral biofilm than non-dental undergraduates (OR of 4.07; CI, 95%). In a comparable study, that examined the sero-positivity of *H. pylori* using ELISA among 60 Japanese dentists and a control group, Honda et al. [17] have demonstrated that the dentists are at nearly a four-time risk to have *H. pylori* infection. Accordingly, foregoing data suggests that the dental undergraduates are also prone to harbor *H. pylori* in their oral biofilm.

DHCW may be exposed to *H. pylori* through the aerosolized dental plaque and saliva of their patients [17,18]. In addition, contaminated dental instruments may transmit the pathogen within a dental healthcare setting [17]. Furthermore, Loster et al. [26] have reported that the presence of *H. pylori* in the oral cavities of DHCW may be related to their duration of clinical practice. In another prospective study that assessed the prevalence of *H. pylori* in DHCW serologically, Matsuda and Morizane [18] showed that DHCW are at nearly three times greater risk of being infected by *H. pylori* than non-clinical controls. Dental undergraduates during their clinical training perform clinical procedures that bring them to contact with oral biofilm, saliva and other oral secretions of their patients. As a result, dental undergraduates may acquire *H. pylori* leading to high prevalence of this organism in their oral biofilm compared to non-dental undergraduates. However, further studies are warranted to confirm this hypothesis.

Attempts to recover *H. pylori* from the oral cavity by culture methods have been hampered probably due to existence of *H. pylori* in a non-culturable coccoid form in the oral cavity [27,28]. The microaerophilic nature and the antagonistic effects of other oral microbes on *H. pylori* make it even more difficult to establish a suitable culturing method [27]. Additionally, small biofilm samples obtained from healthy individuals with good oral hygiene may limit the chances of isolation of *H. pylori* by culture methods. To overcome these limitations, a highly sensitive PCR based detection assay was utilized in the present study to detect *H. pylori* in oral biofilms, mainly targeting the 16S rRNA gene and *Urease* gene sequence of the bacterium.

H. pylori infection is one of the highly prevalent bacterial infections globally and it plays a major role upon the onset of several diseases such as chronic gastritis, gastric ulcers and duodenal ulcers [23,29]. The most effective way of protection from *H. pylori* infections during clinical practices is to minimize the contact of infectious materials such as aerosolized dental plaque and saliva of the patients. Hence, proper infection control practices such as hand hygiene, barrier protection like rub-

ber dam isolation and use of personnel protective equipment are recommended during clinical dental practice.

In conclusion, this study shows that nearly 20% of the current study sample harbor *H. pylori* in their oral biofilms. Prevalence of *H. pylori* in the oral biofilms of dental undergraduates in clinical training is significantly higher than that of non-dental undergraduates ($p < 0.05$). Dental undergraduates are more than four times likely to harbor *H. pylori* in their oral biofilm as non-dental undergraduates (OR = 4.07; CI, 95%). This indicates that dental undergraduates are susceptible to be infected by *H. pylori* inhabiting their patients' oral cavities during the clinical trainings. Hence, proper infection control practices are recommended for dental health care workers during clinical dentistry.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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