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Original Article

Association between untreated caries and cariogenic bacteria in adolescents in Taiwan

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KEYWORDS
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Abstract *Background/purpose:* There is a paucity of research focused on salivary bacteria analyzed through real-time polymerase chain reaction (qPCR) among adolescents. The current study determined the quantity of *Streptococcus mutans* (SM) and *Lactobacillus* (LB) in saliva obtained from Taiwanese adolescents and investigated the association between the oral bacteria and untreated dental caries.

Materials and methods: This cross-sectional study recruited Taiwanese students aged 10–18. Saliva was collected using a Salivette kit and then analyzed through qPCR. The relative quantification values of SM and LB were coded based on mean fold ratios, with values >2 coded as high and other values coded as low. Untreated dental caries was assessed through standard oral examinations. Univariate and multivariate logistic regression models were used to estimate the association between the levels of bacteria in the saliva of the study participants and the presence of untreated caries.

Results: The study involved 421 adolescents. 56 (13.3%) had both SM and LB values of >2 and were coded as having high levels of bacteria, whereas the other 365 (86.7%) students were coded as having low levels. The multivariate logistic regression analysis revealed that adolescents who had high combined salivary SM and LB levels had an odds ratio of having untreated dental caries of 2.05 (95% CI = 1.09, 3.86, $P = 0.027$) compared with those who had low salivary SM and LB levels.

Conclusion: The results of the present study indicate that salivary SM and LB levels are significantly associated with adolescents having untreated caries.

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Introduction

Oral diseases pose a considerable global public health challenge. According to the Global Burden of Disease 2017 Study, 532 million children have untreated dental caries in their deciduous teeth, and 2.3 billion adolescents and adults have caries in their permanent teeth.[1] However, most oral health policies focus only on children younger than 12 years or adults older than 65 years, meaning no clear public policies for maintaining oral health have been developed for individuals aged 13—64 years. To ensure a lifetime of good oral health, it is crucial to establish strong dental hygiene habits in adolescence that continue into adulthood.[2] Moreover, adolescents who do not brush their teeth frequently or do not pay adequate attention to their oral health may accumulate substantial layers of dental biofilm throughout their lives, increasing their risk of developing chronic oral diseases.[3]

Although several risk factors such as dietary habits, oral hygiene practices, genetics, awareness of oral health, and sociocultural environment for developing dental caries are frequently addressed in public policies on oral health,[4,5] the primary cause of dental caries is the demineralization of hard tooth tissues due to acids produced by acidogenic and aciduric/acidophilic oral microorganisms.[6] Among these microorganisms, *Streptococcus mutans* (SM) is primarily responsible for the development of human dental caries, whereas *lactobacillus* (LB) plays a vital role in the progression of caries.[6,7] Several studies have revealed a significant association between salivary levels of SM and LB and the subsequent onset of caries.[8,9] However, few studies have focused on salivary SB and LB levels in adolescents. In one such study from 2015, Sgan-Cohen et al. recruited 286 12-year-old schoolchildren from Palestine, analyzed their salivary SM and LB levels, and found that children with higher SM levels were at a higher risk of experiencing dental caries.[10] In another study from 2019, Lalloo et al. collected salivary SM and LB data from 239 children (average age = 8.79 years) and found that children with higher SM and LB counts had significantly more caries than those with lower counts (prevalence rate ratio = 1.51 for SM, 1.52 for LA, $P < 0.05$ for both).[11] However, these studies utilized commercial kits to analyze SM and LB amounts, meaning they may have underreported bacterial levels. Moreover, these studies exhibit substantial methodological variability, and their results may not be reproducible.[12]

A more reliable method for oral bacteria detection and quantification is real-time polymerase chain reaction (qPCR),[13,14] which enables sensitive and specific quantification of oral bacterial colonies. Nurelhuda et al. used qPCR to analyze the oral bacteria in saliva samples from 140 12-year-old children from Sudan. They found that children with higher salivary SM and *Streptococcus sobrinus* levels were at a higher risk (odds ratio [OR] = 3.0) of having untreated caries than children with lower levels of these bacteria were.[15] Additionally, in a qPCR study from 2013, Sánchez-Acedo et al. recruited 614 schoolchildren from Spain between the ages of 12 and 15 years; they determined that adolescents with high salivary levels of SM were significantly more likely to experience caries than those without.[16] Although these two studies focused on adolescents, they did not collect data on LB, which may be limiting the practical implications of the findings on adolescents' oral health. Consequently, the present study quantified both SM and LB levels in saliva samples obtained from Taiwanese adolescents between the ages of 10 and 18 years and then investigated the associations between oral bacteria and the presence of untreated caries.

Materials and methods

Study population

Participants in this cross-sectional study were randomly selected from a sample of 10,436 children recruited for a nationwide survey conducted in Taiwan from 2019 to 2020.[17] A sample size calculation indicated that a sample of at least 400 individuals was required to detect a mean fold difference in salivary bacteria of 0.3 with a power of 0.8 and a significance level of 0.05. Therefore, 421 students were recruited, with 107 from elementary schools (aged 10—12 years), 197 from junior high schools (aged 13—15 years), and 123 from senior high schools (aged 16—18 years). With regard to the latter laboratory procedure (DNA extraction within 24 h), we chose schools in Taipei City, New Taipei City, and Keelung City. The participants were healthy, did not use tobacco, and had not been on antibiotics during the 3 months prior to saliva collection. The study was approved by the Research Ethics Committee of Taipei City Hospital (approval number: TCHIRB-11003022), and it conformed to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Structured questionnaire and untreated caries examination method

Structured questionnaires were administered to all included schools. In the elementary schools, parents completed both the informed consent form and questionnaire at home and returned them to the school, whereas in the junior and senior high schools, the students completed the questionnaires themselves. Each questionnaire was divided into three parts to obtain information regarding the parents' education levels, the adolescents' oral-health-related knowledge, attitudes, and practices (KAP), and

the adolescents' dietary habits, including the frequency of consuming sugary drinks and milk.

Parents with an education level of junior high school or lower were coded as having a low education level, those who had only completed high school were coded as having a moderate education level, and those who had completed college or more were coded as having a high education level. The students' oral-health-related knowledge, assessed on a 10-point scale, was coded by score range as low (0–4), moderate (5–7), or high (8–10). The students' oral-health-related attitudes, measured on a 7-point scale, were coded as either low (0–4) or high (5–7). The frequency of consuming sugary drinks and milk was coded as low if the participants consumed such beverages no more than once per week (including "1–3 times in a month" and "never") and as high if consumption exceeded once per week (including "1–3 times in a week," "4–6 times in a week," "1–2 times in a day," or "3 or more times in a day").

Eight calibrated dentists conducted oral examinations in the schools by using community periodontal index probes, disposable mouth mirrors, and an intraoral light-emitting diode. Data on caries were collected through visual-tactile examinations without radiographs. Diagnoses of untreated dental caries were made at the cavitation level by considering the World Health Organization criteria and considering both primary and permanent dentition.[18]

The examining dentists were trained according to the criteria before the examination. Duplicate examinations were conducted for 30 students (7.1%) to assess the reliability of the diagnoses, and the data were used to calculate inter-examiner and intra-examiner agreements. The inter-examiner reliability for the eight examiners ranged between 0.85 and 0.98 (Cohen's kappa coefficient), whereas the intra-examiner reliability ranged between 0.79 and 0.95.

Collection of salivary microorganisms

Salivette® (SARSTEDT, Nümbrecht, Germany) was used for saliva collection following the manufacturer's instructions with some modifications. Students were instructed to avoid toothbrushes before sampling. A clean cotton roll inside the plastic tube of Salivette® was carefully taken out with disinfected gloved fingers. Each student was asked to place the cotton roll into their mouth and chew on it for exactly 1 min. Afterward, the student was asked to put the cotton roll containing saliva back into the plastic tube, which was then sealed tightly and labeled. Tubes were transported to the laboratory as soon as possible at 4 °C. In the laboratory, plastic tubes were centrifuged at 13,500×*g* for 10 min to remove saliva from the cotton roll. Two ml phosphate buffer saline (PBS) was then added to the roll and centrifuged at 13,500×*g* for 10 min. PBS containing cells or microorganisms attached to the roll was then transferred to another centrifuged tube for centrifugation at 13,500×*g* for 10 min. The supernatant was removed, while the pellets were stored at 4 °C and subjected to DNA extraction within 24 h.

DNA extraction & qPCR

DNA in the pellets was extracted with TOOLS Buccal Swab and Saliva DNA Kit (BIOTOOLS Co., Ltd, New Taipei City, Taiwan) following the manufacturer's instructions. Pellets were resuspended with the GBHA solution (500 μL) containing proteinase K and then heated at 65 °C for 30 min. An aliquot of the solution (350 μL) was then mixed with the GBBS buffer (500 μL) and then incubated at room temperature for 5 min. The mixture was subjected to a spin column and centrifuged at 13,500×*g* for 1 min. The spin column was then washed with the iced-cold RBD buffer, followed by the PBWE buffer, and then 75% alcohol. DNA attached to the spin column was resolved by 50 μL TB buffer. Concentrations of DNA were measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The extracted DNA was analyzed with qPCR to obtain relative levels of total bacterial counts, SM, and LB in each sample. In a total reaction volume of 20 μL, the mixture consisted of a specific primer pair for bacteria (16S rRNA primer Fw: TCCTACGGGAGGCAGCAGT/Rv; GGACTACCAGGGTATCTAATCCTGTT;[19] SM primer: Fw: CCTACGGGAGGCAGCAGTAG/Rv: CAACAGAGCTTTACGATCCGAAA;[20] LB primer: Fw: TGG AAA CAG RTG CTA ATA CCG/Rv: GTC CAT TGT GGA AGA TTC CC;[21] a final concentration of 1 nM), 10 μL FastStart Universal SYBR® Green Master (ROX) (Cat. No. 04913914001, Roche, Mannheim, Germany), 1 μL sample DNA and 8 μL sterile distilled water. Reactions were performed in duplicate for each sample, using the StepOnePlusDM Real-Time PCR System (Corning, New York, United States). Cycle parameters were set as 1 cycle of denaturing at 94 °C for 3 min, 30 cycles of 30 s at 94 °C, 30 s at 60 °C and 60 s at 72 °C, and end temperature at 72 °C for 5 min. The relative level of SM or LB in each sample was calculated by the $2^{-\Delta\Delta Ct}$ method.[22,23] ΔCt of the target of a sample, i.e. ΔCt of SM or LB, was calculated by subtracting the cycle threshold (Ct) value of the target of the sample, i.e. SM or LB, from the Ct value of 16S rRNA. ΔCt of the calibrator was calculated by subtracting the average Ct value of SM or LC from the average Ct value of 16S rRNA. Then, each sample's mean fold ratio for the level of SM or LB relative to the average level of all samples was calculated as $2^{-(\Delta CT \text{ of SM or LC} - \Delta CT \text{ of SM or LC calibrator})}$.

All procedures, including saliva collection and qPCR, were performed by one author (H.-Y. Mai).

Statistical analysis

Relative levels of SM and LB were assessed by using the mean fold ratio, with both values > 2 coded as high and others coded as low. Differences in demographic characteristics, oral health-related KAP, and dietary habits were analyzed between the adolescents with high and low salivary SM and LB levels by using Mantel–Haenszel chi-square tests. Univariate and multivariate logistic regression models were used to estimate the association between participants' salivary bacteria levels and the presence of untreated caries. Adjustments were made in the regression analyses for potential confounding factors, such as the participants' age, sex, parental education level, oral-health-related KAP, and dietary habits. All statistical tests were performed using SPSS (IBM Corp., Chicago, IL, USA), and the level of significance was set at $P < 0.05$ (two-tailed).

Results

Table 1 presents the distribution of the participants' relative salivary SM and LB levels. With respect to SM, 196 adolescents (46.6%) had a relative value < 1, 100 (23.7%) had a value between 1 and 2, and 125 (29.7%) had a value > 2. With respect to LB, 176 students (41.8%) had a relative value < 1, 140 (33.3%) had a value between 1 and 2, and 105 (24.9%) had a value > 2. Of the 421 adolescents, 56 (13.3%) had both SM and LB values > 2 and were coded as having high combined SM and LB levels, whereas the other 365 (86.7%) were coded as having low combined SM and LB levels.

Table 2 presents the demographic information, oral hygiene KAP, and dietary habits of the study population, categorized on the basis of the levels of SM and LB in their saliva. A total of 60.7% of the students coded as having high combined levels of SM and LB in their saliva had untreated caries, which was a significantly higher proportion than the 43.2% of students coded as having low amounts of salivary bacteria ($P = 0.015$). The adolescents with high combined SM and LB levels in their saliva were more likely to be junior high school or senior high school students and more likely to be girls, although no statistically significant differences were observed. With respect to parents' education level and students' oral-health-related KAP, the high- and low-level groups had similar distributions. With respect to dietary habits, 23.2% of the adolescents with high combined SM and LB levels in their saliva rarely drank milk, which was a significantly higher proportion than the 9.1% of children with low amounts of salivary bacteria who rarely drank milk ($P = 0.005$).

Table 3 presents the results of univariate and multivariate logistic regression analyses of the association between salivary levels of SM and LB and untreated caries in the participants. In the univariate analysis, adolescents who had high combined levels of SM and LB in their saliva had an odds ratio 2.02 times higher than that of students with low levels of salivary bacteria (95% confidence interval [CI] = 1.14, 3.60, $P = 0.016$). After adjustment for potential confounding factors, including age, sex, parents' educational level, adolescents' oral-health-related KAP, and dietary habits, multivariate logistic regression analyses revealed that adolescents who had high combined levels of SM and LB in their saliva had an OR of 2.05 (95% CI = 1.09, 3.86, $P = 0.027$) of having untreated dental caries compared with those who had low SM and LB levels in their saliva. In summary, the model indicates that levels of SM and LB in saliva may play an independent role associated with untreated caries.

Table 1 Distribution of relative quantification of bacteria in the study participants.

	S. mutans		*Lactobacillus*	
Bacteria value	N	%	N	%
<1	196	46.6	176	41.8
1–2	100	23.7	140	33.3
>2	125	29.7	105	24.9

*Bacteria value: The mean fold ratio for the level of *S. mutans* and *Lactobacillus* relative to the average level, calculated as $2^{-(\Delta CT \text{ of } SM \text{ or LC} - \Delta CT \text{ of } SM \text{ or LC calibrator})}$.

Discussion

The present cross-sectional study combined laboratory qPCR and field data, revealing that high combined levels of SM and LB in saliva are associated with a higher risk of having untreated caries in adolescents. The result showed nearly half of the study population (45.6% [192/421]) had untreated dental caries. Although this overall prevalence was comparable with data reported in other studies,[24] there were studies reporting a lower prevalence of untreated caries in individual age groups (12.7% in those aged 12–15 years and 20.4% in those aged 16–19 years).[25] The results of the present study reveal that untreated dental caries remain a severe problem in Taiwan, suggesting a potential area of focus for future public policy and preventive care. The results may be helpful for dentists, policymakers, and educators to promote oral health.

The present study demonstrated that adolescents who have high combined SM and LB levels in their saliva have a significantly higher risk (OR = 2.05, 95% CI = 1.09, 3.86) of having untreated caries compared with that of those who have low SM and LB levels in their saliva (Table 3). This result is consistent with data collected by other cross-sectional studies, including two studies conducted by Sgan-Cohen et al. and Lalloo et al., in which commercial kits were used for microbial analyses.[10,11] Additionally, Relvas et al. recruited 190 13-year-old adolescents from Portugal in 2014, analyzing their saliva samples by Mitis Salivarius Bacitracin agar and Man Rogosa Sharpe agar kits. Their results showed that adolescents with SM and LB $> 10^3$ colony-forming units had a significantly higher OR (8.66 for SM, 2.11 for LB) of having more than five decayed, missing, or filled dental surfaces.[26] SM is frequently identified in the lesion of human dental caries, especially in the formative stages. Regarding as the main pathogen, the bacterium has the ability to produce acid, resist acidic compounds, and synthesize extracellular polymers.[27] LB, another major acid-producing bacteria, can colonize human teeth and is associated with the progression of lesions that induce subsequent dental caries.[28] Although some studies have indicated an uncertain association between LB and caries, the results of the present study suggest, in addition to SM, that high levels of salivary LB may be more harmful than previously believed. An association between SM, LB, and dental caries has also been observed in preschool children and older individuals,[29,30] indicating that "SM + LB" may be a useful indicator of the risk of developing dental caries throughout a person's lifespan.

Several methods can be advocated toward this age group. Fluoride varnish, for example, has been demonstrated to reduce the incidence of dental caries in children and adolescents by 37%–43% in a systematic review, indicating its strong antibacterial effect.[31] In the last decade, probiotics are also considered as an important alternative therapy, which has been demonstrated to replace oral pathogens with healthy bacteria.[32] In a study investigating the effect of probiotics on caries prevention, Zare Javid et al. recruited 66 18–30-year-old students and randomly

Table 2 Demographic information, oral health-related KAP, and dietary habits in the study participants categorized based on levels of salivary bacteria.

	Low combined amounts* (N = 365)		High combined amounts* (N = 56)		
Variables	N	%	N	%	*P*
Untreated caries	158	43.2	34	60.7	0.015
Age group					0.19
10–12	93	25.5	8	14.3	
13–15	168	46.0	29	51.8	
16–18	104	28.5	19	33.9	
Sex					0.37
Female	159	43.6	28	50.0	
Male	206	56.4	28	50.0	
Father's educational level[a]					0.69
Low	72	19.9	9	16.4	
Middle	165	45.6	24	43.6	
High	125	34.5	22	40.0	
Mother's educational level[a]					0.65
Low	60	16.5	12	21.4	
Middle	187	51.5	28	50.0	
High	116	32.0	16	28.6	
Oral health-related knowledge					0.72
Low	104	28.5	17	30.4	
Middle	110	30.1	19	33.9	
High	151	41.4	20	35.7	
Oral health-related attitude					0.98
Low	222	60.8	34	60.7	
High	143	39.2	22	39.3	
Tooth brushing twice a day					0.51
No	100	27.4	13	23.2	
Yes	265	72.6	43	76.8	
Use of fluoridated toothpaste[a]					
No	160	44.8	30	54.5	0.18
Yes	197	55.2	25	45.5	
Frequency of sugary drinks[a]					0.44
Seldom	76	20.9	10	17.9	
Often	279	76.9	46	82.1	
Everyday	8	2.2	0	0.0	
Frequency of milk[a]					0.005
Seldom	33	9.1	13	23.2	
Often	256	70.5	31	55.4	
Everyday	74	20.4	12	21.4	

*Relative levels of *S. mutans* and *Lactobacillus* were assessed by using the mean fold ratio, with both values > 2 coded as "high combined amounts" and others coded as "low combined amounts."

[a] With missing data.

assigned them to an intervention group (probiotic yogurt) and a control group (conventional yogurt). Salivary SM and LB were analyzed by culturing on Mitis Salivarius agar and in Rogosa agar media, respectively. After 2 weeks of yogurt consumption, the intervention group experienced a significant reduction in SM and LB levels relative to the control group.[33] Probiotics may decrease SM and LB levels by preventing the formation of dental plaque either directly by adhering to the tooth surface or indirectly by neutralizing free electrons.[34]

Yogurt or probiotic-containing dairy products are not a routine component of diet. However, the present study revealed that 23.2% of the adolescents with high combined SM and LB levels rarely drank milk, a proportion significantly higher than the 9.1% in all other adolescents (Table 2). This result was consistent with the findings of two studies indicating that milk sugar lactose is less cariogenic than sugar commonly found in other beverages, when it leads to a smaller pH drop in plaque than glucose, fructose, and sucrose do.[35] In addition, milk could also increase the concentration of calcium and phosphate in the dental plaque, when compared to the control subjects who chewed paraffin wax.[36] The effect is likely to be related to caseain, a protein enriched in milk. Casein is known for its ability to conjugated with amorphous calcium phosphate on the demineralized enamel surface, promoting calcium

Table 3 Results of univariate and multivariate logistic regression analyses conducted to investigate the association between levels of salivary bacteria and untreated caries in the study participants.

	Univariate	Multivariate
Variables	OR (95% CI)	OR (95% CI)
Salivary bacteria amounts		
High vs. low	2.02 (1.14, 3.60)*	2.05 (1.09, 3.86)*
Age		
13—15 vs. 10—12	1.21 (0.74, 1.99)	0.93 (0.52, 1.66)
16—18 vs. 10—12	3.02 (1.75, 5.22)***	1.95 (1.01, 3.77)*
Sex		
Male vs. female	1.05 (0.71, 1.55)	1.02 (0.66, 1.56)
Father's educational level		
Middle vs. low	1.10 (0.65, 1.85)	1.33 (0.73, 2.42)
High vs. low	0.49 (0.28, 0.85)*	1.04 (0.51, 2.10)
Mother's educational level		
Middle vs. low	0.79 (0.46, 1.36)	0.86 (0.46, 1.58)
High vs. low	0.32 (0.17, 0.58)**	0.48 (0.23, 1.01)
Oral hygiene knowledge		
Middle vs. low	0.68 (0.41, 1.12)	0.93 (0.52, 1.65)
High vs. low	0.60 (0.37, 0.96)*	0.88 (0.51, 1.52)
Oral hygiene attitude		
High vs. low	0.63 (0.43, 0.94)*	0.92 (0.58, 1.46)
Tooth brushing twice a day		
Yes vs. no	0.81 (0.52, 1.24)	0.93 (0.55, 1.57)
Use of fluoridated toothpaste		
Yes vs. no	0.60 (0.41, 0.89)*	0.70 (0.44, 1.12)
Frequency of sugary drinks		
Often vs. seldom	1.84 (1.12, 3.02)*	1.64 (0.95, 2.85)
Everyday vs. seldom	3.28 (0.73, 14.68)	3.43 (0.68, 17.23)
Frequency of milk		
Often vs. seldom	0.80 (0.43, 1.50)	0.98 (0.49, 1.97)
Everyday vs. seldom	0.57 (0.28, 1.18)	0.97 (0.43, 2.19)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.
****Relative levels of *S. mutans* and *Lactobacillus* were assessed by using the mean fold ratio, with both values > 2 coded as "high" and others coded as "low."

precipitation and surface remineralization.[37] Whether milk or other dairy products can successfully prevent the formation of caries in Taiwanese children might require more long-term follow-up studies to evaluate further.

This study selected saliva as the sampling medium rather than dental plaque because plaque cannot reliably be used to predict levels of oral bacteria due to the considerable variation in bacterial counts on tooth surfaces.[38] Furthermore, salivary SM and LB levels reflect an individual's total oral load of these bacteria,[39] consisting of bacteria present not only on the teeth but also on the tongue and the mucosal surfaces. Therefore, the use of saliva samples in the current study provides a general picture of the microbial load of SM and LB. Additionally, Salivette® is a convenient saliva-collection device, containing a cotton swab that allows each participant to easily insert it into the mouth on their own. It has even been used successfully to collect saliva for SARS-CoV-2 detection, which is shown to be comparable to the result obtained from nasopharyngeal swabbing.[40]

qPCR is a sensitive, rapid, valid, and reliable method for quantification of bacteria. Thus, it is well-suited to epidemiological research such as the present study. Li et al. determined that qPCR was highly sensitive and highly specific for analyses of saliva samples. They collected saliva from 344 preschool children between the ages of 3 and 5 years and the children's mothers in 2017. They then compared the level of salivary SM measured by qPCR or a commercial test kit. Their results indicated that, although results provided by two techniques were correlated, approximately 10% more salivary samples were tested positive for SM when qPCR was used, indicating that this method more sensitively and specifically detects salivary bacteria levels than commercial kits do. Such increased sensitivity and specificity are especially helpful to the present study. Additionally, where our objective was to determine the associations between the level of SM and LB colonization and the incidence of dental caries, qPCR, but not commercial test kit, can provide data related to more than one cariogenic bacterial species.[12] However, although specific primers were utilized to identify the targeted microorganisms, primer bias may still exist. Furthermore, the universal primer employed in this study targeted the 16S rRNA gene, potentially resulting in an underestimation of the mean fold ratio.[15] Thus, the observed SM and LB levels may have been underestimated and may not reflect the actual bacterial counts. Future longitudinal studies should be conducted to quantify SM and LB levels at different growth stages to provide more evidence for peak bacterial concentrations, and the single-point salivary analysis might also be considered, predominantly conducted during the morning hours to ensure a stable intraoral environment.

The present study has additional limitations. First, the study examined only SM and LB concentrations, which may not completely represent the relationship between all microorganisms and untreated caries among adolescents. However, we adjusted for potential confounding factors, with information obtained through the questionnaire, such as toothbrushing habits and frequency of sugary drink consumption, and we are confident that our findings remain valid and hold practical implications for oral care improvement. Second, the cross-sectional design of the present study does not permit inference of causality, and a single saliva sample provides a record of microbial counts at one particular point in time. However, dental caries is a chronic disease that develops over a long period during which bacterial counts may fluctuate in response to the changing oral environment.[38] Finally, Taiwan does not treat its water supply with fluoride and has a high proportion of adolescents with dental caries. Thus, our results may not be generalizable to countries where water is treated with fluoride or adolescents have more effective oral care.

In conclusion, the present study demonstrated that adolescents with high combined SM and LB levels in their saliva (as detected through qPCR) were 2.05 times more likely (95% CI = 1.09, 3.86) to have untreated dental caries than those with low SM and LB levels in their saliva were. This finding indicates that "SM + LB" can be microbiological indicators that can be utilized in designing a caries screening program to enhance oral health in Taiwanese adolescents.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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