The Relationship Between Salivary *Lactobacillus* Sp. And DMF-T Caries Index In Female Boarding School Students

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Received 27 June 2026; Revision 1st 20 August 2026, Accepted 24 August 2026; Published online 24 August 2026

Keywords:

Boarding school students; Dental caries; DMF-T index; *Lactobacillus* sp.; Saliva

ABSTRACT

Background: *Dental caries remains one of the most prevalent chronic diseases in Indonesia and is associated with changes in the oral microbiota. Lactobacillus sp. is bacteria that contribute to caries progression and is found as microbiological indicators of caries activity. However, data regarding the relationship between salivary Lactobacillus sp. counts and caries severity in boarding school students remain limited. This study aims to determine the relationship between salivary Lactobacillus sp. colony counts and caries severity using DMF-T index among female students in boarding school.*
Method: *An analytical observational study with a cross-sectional design was conducted among 28 female students. Caries severity was assessed using DMF-T index and categorized into mild (0-2), moderate (3-4), and severe (>5). Unstimulated saliva samples were collected and cultured on MRS agar. Samples were incubated anaerobically, and colony counts were counted. The association between colony count and DMFT category was analyzed using Spearman's rank correlation test, while differences among DMF-T categories were evaluated using the Kruskal–Wallis and Mann–Whitney U tests.*
Result: *A significant positive correlation was observed between Lactobacillus sp. colony count and DMFT index (r=0.443; p=0.018). The Kruskal–Wallis test showed significant differences in Lactobacillus sp. counts among DMFT categories (p = 0.018). Significant differences in bacterial counts were observed between severe and both mild (p = 0.013) and moderate (p = 0.017) caries index, but not between mild and moderate index (p = 0.718).*
Conclusion: *Salivary Lactobacillus sp. colony counts were significantly associated with dental caries severity among female boarding school students.*

DOI: http://dx.doi.org/10.30659/medali.8.2.94-101
MEDALI Jurnal: Media Dental Intelektual accredited as **Sinta 4 Journal** (https://sinta.kemdikbud.go.id/journals/profile/13665)
2337-6937/ 2460-4151 ©2025 National Research and Innovation Agency

How to Cite: *Rahmatari et al*. The Relationship Between Salivary Lactobacillus Sp. And DMF-T Caries Index In Female Boarding School Students. MEDALI Jurnal: Media Dental Intelektual, v.8, n.2, p.94-101, February 2026.

INTRODUCTION

Dental caries is a disease that is formed through multifactorial factors between acid-producing bacteria (acidogenic), dietary intakes (high fermentable carbohydrates) and host factors including teeth and saliva that happen for long periods of time. Recent evidence further emphasizes that dental caries is associated with dysbiosis of the oral microbiome rather than the activity of a single pathogenic species alone[1]. It remains one of the most prevalent chronic diseases worldwide, affecting individuals across all age groups. In Indonesia, Riset Kesehatan Dasar (Riskesdas 2018) in Survei Kesehatan Indonesia (SKI 2023) report estimated national caries prevalence of 88.8%, with average DMF-T index of 7.1 and the highest rates observed in individuals aged 5-9 years and 35 years above[2]. This data highlights the urgent need for preventive strategies and treatments, particularly among vulnerable demographic groups.

One of the oral pathogens that caused caries is *Lactobacillus* sp[3] . *Lactobacillus* sp. is gram positive bacteria that has received considerable attention because of their acidogenic and aciduric characteristics[4]. This bacteria is capable of fermenting variety of dietary carbohydrates and surviving in low-pH environments, so it can contribute to lesion progression[5]. While *Streptococcus mutans* is often attributed as an initiator of dental caries, *Lactobacillus* sp. is frequently associated with the progression and severity of established carious lesions. Increased salivary *Lactobacillus* sp. counts have been reported in individuals with active dental caries and may serve as a microbiological indicator of caries activity[6,7].

The standard epidemiological tool for measuring dental caries experience is Decayed, Missing and Filled Teeth (DMF-T) index, which has been widely used by World Health Organization (WHO). The DMF-T index provides a cumulative score representing the total number of permanent teeth that are decayed (D), lost due to caries (M), or restored because of caries (F), with individual scores ranging from 0 to 28 [8]. A higher DMF-T score reflects a greater dental caries and is commonly used to evaluate oral health status among adolescents. Adolescence is considered a critical period for caries development because dietary habits, oral hygiene behaviors, and hormonal changes may influence both the oral environment and microbial composition[8].

Students attending boarding schools are a population with oral health issues. Living in a community environment may have an impact on dietary patterns, dental care availability and oral hygiene habits. Shared eating habits, frequent consumption of fermentable carbohydrates, and limited opportunities for professional dental services may increase the risk of developing the oral environment. Adolescent females may be particularly vulnerable because hormonal changes during puberty can alter saliva composition and oral microbial ecology, potentially affecting caries susceptibility[9]. Furthermore, recent research suggests that age related lifestyle factors and pubertal changes should be considered when evaluating the relationship between oral microbiota and dental caries among adolescents.

Although many studies have investigated the association between oral microorganisms and caries, data regarding the relationship between salivary *Lactobacillus* sp. colony counts and caries experience among female

boarding school students remain limited, particularly in the Indonesian area. Understanding this relationship may provide valuable information for the development of microbiology-based caries prevention strategies and early risk assessment programs in adolescent populations. Therefore, this study is aimed to analyze the relationship between salivary *Lactobacillus* sp. colony count and DMF-T caries levels among female boarding school students.

RESEARCH METHOD

This study used a cross-sectional observational study to examine the relationship between salivary *Lactobacillus* sp. colony count and DMF-T caries levels among female students at boarding school in Gresik, East Java, Indonesia. Data collection was conducted in February 2026. Ethical approval for this study was obtained from Universitas Airlangga Faculty of Dental Medicine Health Research with ethical clearance number: 0006/HRECC.FODM/I/2026. Prior to data collection, informed consent was obtained from all participants after explanation of study objectives, procedures, potential risks and benefits.

The population in this study was female students residing at boarding school in Gresik. A total of 28 students were enrolled as participants which were selected using simple random sampling technique. The inclusion criteria were female students who were permanent residents of boarding school and were willing to participate after providing informed consent. Exclusion criteria were those with systemic diseases, who had consumed medicines and were not willing to participate in this study.

Dental caries status was assessed through direct intraoral clinical examination conducted by dentists using dental mirror and dental explorer probe (sonde) under adequate light, in accordance with World Health Organization (WHO) criteria for caries diagnosis[10]. Each participant's DMF-T score was calculated as the sum of decayed (D), missing due to caries (M) and filled permanent teeth (F)[6]. The DMFT score for each participant was recorded and subsequently categorized into three DMF-T level: mild with DMF-T score 0-2, moderate with DMF-T score 3-4 and severe with DMF-T score more than 5.

Unstimulated saliva samples were collected from each participant in the morning between 06.00-07.00 WIB[5]. Participants were instructed not to eat, drink, brush their teeth, or use mouthwash for two hours before saliva collection. Approximately 2 mL of saliva was collected using a spitting method into a sterile, labelled container and immediately placed in a cool box for transport to the laboratory within 2 hours[10,11]. Saliva samples were homogenized and serially diluted using sterile physiological saline solution. The saliva was taken in 100 µl and was inoculated by streaking onto de Man, Rogosa and Sharpe (MRS) agar medium (Merck 1.10660.500), which is selective for the isolation and cultivation of *Lactobacillus* sp (Figure 1). The inoculated plates were incubated at 37°C for 48 hours using anaerobic jar (Merck, 1.13681.0001) under anaerobic environment (AnaeroPack, MGC, Japan)[10,12]. Following incubation, colonies exhibiting morphological characteristics consistent with *Lactobacillus* sp. were counted manually using a colony counter. Colony morphology was assessed based on color, shape, margin, and distribution on the agar surface[13]. The number of bacterial colonies was expressed as colony-

forming units per milliliter (CFU/mL). For data analysis, colony counts were categorized into three groups: 10^5 CFU/mL, 10^6 CFU/mL, and 10^7 CFU/mL.

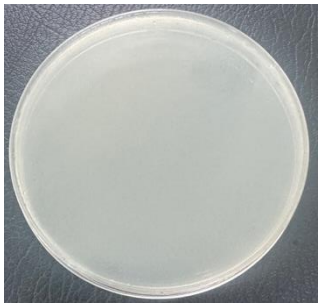

Figure 1. MRS agar plate for cultivation of *Lactobacillus* sp.

Data were analyzed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the distribution of variables. The relationship between *Lactobacillus* sp. abundance and caries severity was evaluated using the Spearman rank correlation test. To compare *Lactobacillus* sp. colony counts across the three caries severity levels, the Kruskal-Wallis test was performed, followed by the Mann-Whitney U test for pairwise post-hoc comparisons where a statistically significant overall difference was identified. A p-value <0.05 was considered statistically significant for all analyses.

RESULTS

A total of 28 female students with mean age 15,8 years (range 14-18 years old) participated in this study. Based on educational level, most respondents were senior high school students (SMA) with a total 20 respondents (71,4%) with remaining 8 respondents (28,6%) were junior high school students (SMP). Mild DMF-T was found in 10 respondents (35,7%), while moderate and severe DMF-T were each identified in 9 respondents (32,1%) as presented in Table 1.

Visual examination of inoculated MRS agar plates showed the numerous of bacterial colonies. The colonies were predominantly creamy white in color, circular in shape, smooth in surface appearance and exhibited entire margins. Most colonies appeared convex with variable diameters from 0.5 to 2 mm. The colonies were well distributed through the agar surface and were sufficiently separated to allow enumeration. The observed colony morphology was consistent with the typical characteristics of presumptive *Lactobacillus* sp. cultured on MRS agar (Figure 2).

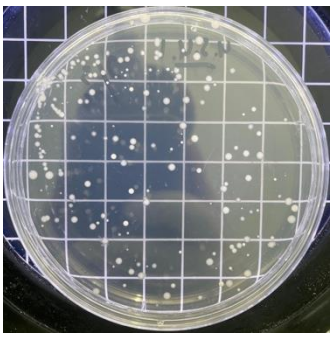

Figure 2. Representative growth of *Lactobacillus* sp. on MRS agar

To examine differences in *Lactobacillus* sp. colony count among those three caries levels, the Kruskal-Wallis test was conducted (Table 2). This test showed a statistically significant difference in *Lactobacillus* sp. colony counts among the three caries severity groups ($p = 0.018$). Post-hoc pairwise comparison using the Mann-Whitney U test was performed to identify which caries level resulted in significant difference (Table 2). The Mann–Whitney test showed no significant difference between the mild and moderate caries groups ($p = 0.718$). However, the severe caries group exhibited significantly higher *Lactobacillus* sp. colony counts than both the mild caries group ($p = 0.013$) and the moderate caries group ($p = 0.017$).

The correlation between DMF-T and *Lactobacillus* sp. colony counts were summarized in Table 3. In the severe group, 33,33% of students had *Lactobacillus* sp. count on 10^7, whereas in the mild and moderate group, there was none of *Lactobacillus* sp.

Table 1. Frequency distribution of respondent characteristics (n=28)

Educational level	Students n = 28	DMF-T Mild n=10	Moderate n=9	Severe n=9
SMP	8 (28,6%)	1	3	4
SMA	20 (71,4%)	9	6	5
Total	28 (100%)	10 (35,7%)	9 (32,1%)	9 (32,1%)

Table 2. Comparison of Lactobacillus sp. Colony Count Across Caries Level

DMF-T	*Lactobacillus* sp. count (CFU/ml)		*p-value* (Kruskal-Wallis)
	n	Median (min – max)	
Mild[a]	10	$6,5 \times 10^5$ ($1,7 \times 10^5$ – $8,9 \times 10^6$)	0,018
Moderate[a]	9	$4,5 \times 10^5$ (0 – $9,8 \times 10^6$)	
Severe[b]	9	$8,2 \times 10^6$ (3×10^5 – $14,7 \times 10^6$)	

Groups with different superscript letters (a,b) differ significantly according to Mann–Whitney post-hoc analysis.

Table 3. Correlation between *Lactobacillus* sp. colony count and DMF-T caries level

Lactobacillus sp. count (CFU/ml)	DMF-T			*p-value* (*Spearman rank test*)	r (*Spearman's rho*)
	Mild	Moderate	Severe		
	n (%)	n (%)	n (%)		
0	0 (0%)	2 (22,22%)	0 (0%)	0,018	0,443
10^5	6 (60%)	3 (33,33%)	1 (11,11%)		
10^6	4 (40%)	4 (44,44%)	5 (55,55%)		
10^7	0 (0%)	0 (0%)	3 (33,33%)		
Total	10 (100%)	9 (100%)	9 (100%)		

In the mild caries group, 60% of students had *Lactobacillus* sp. count on 10^5, but in the severe caries group, only 11,11% of children showed *Lactobacillus* sp. count on The Spearman's rho analysis showed a correlation coefficient of r=0.443 with a p-value of 0.018 ($p<0.05$) indicating there was significant positive correlation between *Lactobacillus* sp. count and these three groups of caries level. These findings indicate that higher *Lactobacillus* sp. colony counts were associated with greater caries severity among the study participants.

DISCUSSION

Dental caries can be defined as multifactorial disease that is caused by microorganisms, dietary factors and composition in saliva. Numerous microorganisms that might cause dental caries are known as cariogenic microorganisms, for example *S.mutans* and *Lactobacillus* sp[14]. *Lactobacillus* sp. function primarily as secondary colonizers in

carious lesions and dominate the deeper dentinal layers of severe carious lesions[15], whereas *S.mutans* initiates enamel demineralization[16]. The present study was carried out to determine the relationship between *Lactobacillus* sp. colony count and dental caries severity as measured by DMF-T index among female students at boarding school. DMF-T scores were categorized as mild, moderate and severe to analyze across the caries severity group. The salivary samples were used to assess *Lactobacillus* sp. then CFUs were calculated and tabulated.

inoculated plates, ranging from 0.5 to 2 mm. This heterogeneity may reflect differences in bacterial concentration among saliva samples, growth rates of different *Lactobacillus* species or strains, or competition among oral microorganisms during culture.

Post-hoc analysis showed no significant difference in *Lactobacillus* sp. colony counts between the mild and moderate DMF-T groups (p=0.718), whereas significant differences were observed between the severe DMF-T group and both mild (p=0.013) and moderate (p=0.017) groups. This finding may indicate that *Lactobacillus* colonization increases significantly when caries' severity reaches an advanced level in deep dentinal or pulp stages. *Lactobacillus* species may become more prevalent as the caries process advances because they are frequently linked to deep dentin lesions rather than initial enamel demineralization[17]. Thus, rather than being a sign of initial caries, *Lactobacillus* abundance may be a sign of caries development.

This study showed that Spearman's rank correlation test resulted in a statistically significant positive correlation (p=0.018), indicating that higher *Lactobacillus* sp. colony counts in saliva were associated with greater caries activity. These

The colonies were predominantly creamy-white, circular, smooth, and convex, with entire margins, which are consistent with the typical morphology of *Lactobacillus* sp. cultured on MRS agar. MRS agar is widely used as a selective medium for the isolation and enumeration of *Lactobacillus* because its nutrient rich composition and acidic pH favor the growth of acid tolerant lactic acid bacteria while inhibiting many competing microorganisms[13]. However, colony morphology alone cannot confirm species identity, so further biochemical or molecular identification is needed. Variation in colony size was observed among the results are in line with research confirming that salivary *Lactobacillus* burden and tooth caries experience are positively correlated. Fu et al stated that detection rate of lactobacilli is closely related to both the incidence and severity of dental caries, further reinforcing their role as microbial indicator of caries risk[17]. Another research showed that there is a significant difference between salivary *Lactobacillus* sp. count and dental caries in primary school students (p<0.001)[10], which indicates that higher microbial activity functioning as determinant of caries progression. The cariogenic activity of *Lactobacillus* sp. increases in the presence of existing carious lesions, because the pitted and retentive location provides a colonization niche for these bacteria, which are unable to adhere efficiently to smooth and intact surfaces[18,19].

This research also resulted in all three participants with *Lactobacillus* sp. counts at 10^7 CFU/ml only belonged to the severe caries group, while no participants in mild or moderate caries groups reached that number. This finding supports the ecological plaque hypothesis, in which repeated acid exposure to deep carious lesions creates an increasingly selective environment favoring aciduric species such as *Lactobacillus*. Frequent exposure to carbohydrates and prolonged acidic conditions

cause microorganisms such as *Lactobacillus* sp. can survive in low pH environments to become dominant, thus promoting enamel demineralization and caries development[18,20].

The population that is used in this study, which is female students residing in boarding schools, may represent a group with oral health vulnerability. Boarding school environments are associated with limited parental supervision of oral hygiene practices, communal dietary patterns with frequent consumption of carbohydrates, and limited access to dental care such as dental clinics or hospitals[9,21]. The distribution of caries severity in this study resulted across mild group (35.7%) and 32.1% for both moderate and severe groups indicate broad spectrum of oral health status present within a single boarding school and highlights need for preventive interventions in that school settings.

It is also found that two participants with undetectable *Lactobacillus* sp. colony count (0 colony) were classified in the moderate caries group. This could be an example where *Lactobacillus* colonizes existing caries lesions rather than initiating them, which is in line with Liu, et al finding[22]. These individuals may have had caries lesions in early enamel stage that were mostly caused by *S.mutans* or other early colonizers, with *Lactobacillus* not yet developed so this bacteria was undetectable.

Several limitations of this study should be considered. The sample that had been used in this study is relatively small, so it limits statistical analysis and generalization of the study. Additionally, the study did not assess other cariogenic variables such as *S.mutans* counts, salivary flow rate, dietary habits and oral hygiene practices, which are known to interact with *Lactobacillus* to burden in modulating caries risk. Future research with larger samples and multifactor variables would provide more complete understanding of microbial ecology in dental caries especially in boarding school population.

Overall, this study demonstrates that higher salivary *Lactobacillus* sp. counts is significantly associated with increasing DMF-T caries level among female boarding school students. This findings suggests that salivary Lactobacillus enumeration as indicator of caries severity and supports integration of microbiological screening into preventive oral health program for boarding school students in Indonesia.

CONCLUSION

Salivary *Lactobacillus* sp. colony count was significantly positive associated with dental caries severity among female boarding school students. Higher bacterial counts were observed in participants with higher DMF-T scores. These findings suggest that *Lactobacillus* sp. may serve as a useful microbiological indicator of caries severity and support the implementation of preventive oral health programs in boarding school settings.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to all who participated in this study. During the preparation of this manuscript, the authors used Claude and ChatGPT (OpenAI) solely for improving the English language and grammar of this manuscript. All scientific content, data analysis, and interpretation were developed by the authors.

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