

Assessing Oral Mucosal Health Related to Secondary Bacterial Infections among Pediatric Patients Presenting with Type 1 Diabetes

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Abstract

Background: Juvenile diabetes or Type 1 Diabetes (T1D) mellitus is an autoimmune-mediated disease resulting in insulin deficiency, and it is the second most frequent disease in children. T1D also affects the oral health of a child. **Objectives:** This current work was undertaken to characterise and compare the salivary microbiome of children with Type 1 Diabetes (T1D) and healthy controls while evaluating the influence of oral environmental factors such as DMFT index, salivary flow rate, and buffering capacity. **Methodology:** The study was a comparative cross-sectional study conducted at the Department of Pediatric and Preventive Dentistry and Central Research Laboratory, Institute of Dental Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, during the period from October 2023 to March 2024. A total of 20 children (10 healthy individuals, 10 with T1D) were selected. Detailed case history and DMFT/DMFS index were recorded. The saliva sample was collected from all patients and was analysed for pH and flow rate. The samples were processed for microbiological evaluation using routine microbiological procedures, and the isolated bacteria were subjected to antibiotic sensitivity tests. **Results:** Among 20 children, 10 (50%) had Type 1 Diabetes and 10 (50.0%) were healthy controls. The mean DMFT score was higher in the diabetic group (3.8 ± 1.03) compared to controls (2.8 ± 1.03), with a significant difference ($p < 0.05$). Salivary flow rate, pH, and buffering capacity were lower in diabetic children ($p < 0.01$). The mean CFU count was higher in the diabetic group (20 ± 3.13) than in controls (11.4 ± 2.46), showing significant variation ($p < 0.05$). **Conclusion:** Children with Type 1 Diabetes showed altered salivary microbiota, reduced salivary flow rate, buffering capacity, and lower pH, resulting in higher DMFT scores and bacterial counts compared to healthy children. [Bangladesh Journal of Infectious Diseases, June 2025;12(1):141-150]

Keywords: Type 1 diabetes; pediatric patients; oral microbiome; salivary pH; salivary flowrate; antibiotic resistance

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Introduction

Type 1 diabetes (T1D) mellitus is an autoimmune-mediated disease that destroys beta cells of the pancreas, causing absolute insulin deficiency. It is also known as insulin-dependent diabetes and is idiopathic. It is one of the most common diseases in children, and it is generally associated with soft tissue abnormalities in the oral cavity, which compromises an individual's overall quality¹. It is one of the most frequent chronic diseases of children and adolescents, but it can occur at any age. Type 1 diabetes affects 5.0% to 10.0% of diabetes patients globally and is the second most prevalent autoimmune illness in children; its incidence has more than tripled²⁻³. T1D affects around 1.1 million kids and youngsters under twenty globally. The fact that it might take years between early cell damage and clinical diabetes illustrates Type 1 diabetes's complicated aetiology⁴.

The rising amount of data links mouth bacteria to various systemic disorders, including diabetes. The oral microbiome may negatively impact our overall and dental health, a significant part of the human microbiome. The host's genetic makeup can affect microbial composition and activity, as well as the activation of innate and adaptive immunity and susceptibility to certain illnesses⁵⁻⁶. Gingivitis and periodontitis are commonly associated with diabetes because of the increase in sugar levels. It forms a favourable and optimum condition for the bacteria to grow in the oral cavity by compromising the oral flora, causing accumulation of the bacteria in the gingiva, causing gingivitis, and then progressing to weakening of the periodontal ligament, causing periodontitis⁷⁻⁸. This leads to impairment of the bone structure and mobility of the teeth. As there is a decrease in saliva production, there is an increased risk of cavities and oral infections because of xerostomia. The flushing action of saliva does not take place in the average amount, and this attracts other opportunistic bacteria to harbour, such as oral thrush, white patches like leukoplakia, lichen planus, and so on⁹⁻¹⁰. The patient complains of alteration of taste, burning mouth and inability to eat and swallow because of recurrent aphthous ulcers and delayed wound healing¹¹.

Therefore, addressing the microbiome during the latent stage of diabetes may allow for early therapy and delay T1D development in children with cell autoimmunity¹²⁻¹³. The current work uses microbial techniques to characterise the salivary microbiome of children with T1D. The effect of salivary factors on the microbiota were investigated, including the

DMFT index, salivary flow rate, and buffering capacity.

Methodology

Study Settings: This was a comparative cross-sectional study conducted in the Department of Pediatric and Preventive Dentistry in collaboration with the Central Research Laboratory, Institute of Dental Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India. The study was carried out over a period of six months from October 2023 to March 2024.

Study Population and Sample Collection: Clinical samples (saliva) were collected from children attending the Department of Paediatrics and Preventive Dentistry, Institute of Dental Sciences, S 'O' A University, with T1D. These saliva samples were processed in the Central Research Laboratory to isolate and identify the microorganisms by standard staining, culturing, and biochemical techniques. Samples were collected from patients fulfilling the inclusion and exclusion criteria.

Selection Criteria: The study included children below 18 years of age diagnosed with Type 1 Diabetes and maintaining good oral health, along with healthy children under 18 years with good oral health serving as controls. Patients with existing oral infections, individuals above 18 years of age, and those unwilling to participate were excluded from the study.

Demographic Data: Detailed demographic data of the patients were collected and recorded in an Excel sheet. Salivary parameters such as the DMFT index and salivary flow rate were analyzed.

Salivary pH Analysis using pH Meter: The pH meter (Eutech) was initially standardized with pH 4.5 and pH 7 solutions, followed by measurement of the sample pH. The pH meter readings of the samples were then tabulated¹⁴.

Buffering Capacity of Saliva Samples: 1.5 ml of 5 mmol/l HCl was mixed with 0.5 ml of saliva. Using a digital pH meter, the liquid was agitated forcefully, centrifuged for one minute, and then let stand for ten minutes before the final pH was determined¹⁴.

Processing of Samples for Microbiological and Biochemical Investigations: Saliva samples mixed with holding medium were kept at room

temperature, and then 2 ml of the fresh 2 ml sample mixture was mixed with 8 ml of sterile normal saline and shaken to form a homogenous mixture. All samples were mixed by vigorous shaking and serially diluted¹⁵.

Enumeration of the Bacterial Population: All the bacterial population enumerations were carried out by pour plate techniques using a Nutrient agar (NA) medium. The total number of bacteria in the population was counted using a colony counter¹⁵.

Pure Culture Maintenance: The mixed bacterial culture was further streaked on different media, like Nutrient agar and blood Agar (BA), to detect the presence of cariogenic *Streptococcus mutans* and *Lactobacillus* species. Single colonies were picked up randomly by a sterile loop from plates and then re-streaked on individual NA and BA agar plates. The NA slants were incubated at 37⁰ C for 24 h and preserved for further use¹⁵.

Colony Morphological Identification: The initial identification of the isolated colonies was based on morphological observations. It includes some basic tests such as colony colour, reverse side colour, cell morphology and spore morphology, if any. For further identification, Gram staining was performed for all the isolated bacteria¹⁵.

Antibiotic Susceptibility Testing of the Clinical Isolates: All the identified bacterial strains were subjected to antibiotic sensitivity tests using Kirby-Bauer's/ disc-diffusion method. Blood agar and Mueller-Hinton agar were used for the method.^{15, 16}

Statistical Analysis: The collected data were analyzed using SPSS version 25.0. Descriptive statistics such as mean, standard deviation, and percentage were calculated. Comparison between diabetic and healthy groups was done using the Student's t-test. A p-value of less than 0.05 was considered statistically significant. Results were presented in tables and figures.

Ethical Consideration: This study was carried out after the approval of the Ethical Committee of the Institute of Dental Sciences via letter no. IEC-IDS/IDS.SOA/2023/I-43 dated 10 October 2023.

Results

The samples were collected under two categories. The two categories were GROUP A: saliva of children with T1D; GROUP B: healthy children (Control Group). Each group had ten samples. The samples were further processed for microbiological and biochemical results.

Table 1. Demographic Data (Age/Sex), DMFT Index of the Participants

GROUP A: Saliva of children with T1D				GROUP B: Healthy children with no diabetes			
Patient SI No	AGE (years)/ Sex	Diabetic Patient (Def /DMF)	Diabetic Patient (Def /DMF Total Scoring)	Patient SI No	AGE (years)/ Sex	Healthy Patient (def /DMF)	Healthy Patient (def /DMF Total scoring)
1	11M	d=2, e=0, f=2	4	11	10 M	d=1, e=2, f=0	3
2	12 F	d=3, e=1, f=1	5	12	12 M	d=1, e=2, f=1	4
3	10 F	d=2, e=0, f=1	3	13	13 F	d=1, e=1, f=0	2
4	14 F	d=2, e=2, f=1	5	14	11 F	d=1, e=2, f=1	4
5	13M	d=2, e=1, f=2	5	15	12 M	d=2, e=2, f=0	4
6	11 M	d=1, e=0, f=2	3	16	14 F	d=0, e=2, f=0	2
7	12 M	d=2, e=0, f=2	4	17	10 M	d=1, e=2, f=0	3
8	13 M	d=1, e=0, f=1	2	18	14 M	d=0, e=1, f=0	1
9	11 F	d=1, e=2, f=0	3	19	13 F	d=0, e=2, f=0	2

GROUP A: Saliva of children with T1D				GROUP B: Healthy children with no diabetes			
10	10 F	d=1, e=2, f=1	4	20	11 F	d=1, e=2, f=0	3
Statistic al values	- Mean DMFT score: 3.8 - Standard deviation: 1.03 - Median: 4; Range: 2-5			Statistic al values	- Mean DMFT score: 2.8 - Standard deviation: 1.03 - Median: 3; Range: 1-4		

Group 1 comprises healthy patients, and group 2 comprises Type 1 diabetic patients. The oral health status is calculated using this DMFT index to understand the oral health status. The DMFT index is categorised as Decayed (D), Missing (M) or Filled (F) tooth is a quantitative measurement tool. The index is calculated by summing each patient's total number of decayed, missing or filled teeth. This table shows there is an increase in the score of the diabetic group as the diabetic patient has reduced salivary flow and is viscous, thus accumulating more bacteria, causing more decaying of the teeth, resulting in more restoration (filled teeth) compared to that of the healthy group (Table 1, Figure I). Statistical Analysis DMFT Index indicates that Children with T1D (Group A) appear to have a higher average DMFT score compared to healthy children (Group B). The variability (standard deviation) is the same in both groups. The range of scores is slightly wider in Group A (2 to 5) compared to Group B (1 to 4). The bar graph compares the average DMFT/def index between the T1D and healthy groups. The T1D Group average is 3.8, whereas the Healthy Group Average is 2.8. This indicates that children with T1D have a higher incidence of dental problems compared to healthy children.

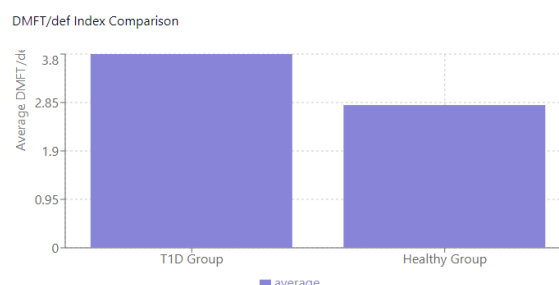


Figure I: DMFT/def Index Comparison

The mean salivary flow of Group A, where the children were diabetic patients, was 0.2503 ± 0.005 . This value was slightly less than Group B (healthy controls), which was 0.3102 ± 0.06 . Similarly, the salivary pH was slightly more acidic in Group A (mean value 6.478 ± 0.221) patients than that of Group B (mean value 7.49 ± 0.037), which consisted of the healthy controls. Likewise, the salivary buffering capacity of the diabetic children group was 5.56 ± 0.221 , comparatively less than the mean value of 6.746 ± 0.0252 (Table 2).

Table 2: Salivary flow rate, pH Values, Buffering Capacity

GROUP A: saliva of children with T1D				GROUP B: healthy children with no diabetes			
Saliva Sample no	Flow rate	pH values	Buffering Capacity	Saliva Sample no	Flow rate	pH values	Buffering Capacity
1	0.256	6.69	5.91	11	0.310	7.39	6.54
2	0.244	6.65	5.92	12	0.225	7.32	6.95
3	0.254	6.67	5.95	13	0.324	7.62	6.46
4	0.241	6.61	5.43	14	0.332	7.63	6.43
5	0.254	6.66	5.42	15	0.357	7.13	6.34
6	0.245	6.61	5.45	16	0.325	7.35	6.91
7	0.255	6.39	5.46	17	0.236	7.36	6.93
8	0.253	6.12	5.42	18	0.234	7.67	6.95
9	0.245	6.12	5.43	19	0.431	7.68	6.97
10	0.256	6.26	5.55	20	0.328	7.75	6.98
Mean $\pm$ Std. dev	0.2503 ± 0.005	6.478 ± 0.221	5.596 ± 0.221	Mean $\pm$ Std. dev	0.3102 ± 0.06	7.49 ± 0.037	6.746 ± 0.0252

Children with T1D show lower salivary flow rates, more acidic pH, and lower buffering capacity compared to healthy children (Figure III).

The T1D group generally shows higher variability in these parameters, except for flow rate. These differences could potentially impact oral health in children with T1D. This graph compares three salivary parameters: Flow Rate, pH Value, and Buffering Capacity (Figure II).

Salivary Parameters Comparison

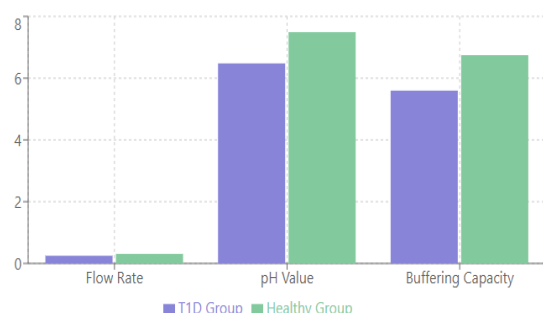


Figure II: Salivary Parameters

The Flow Rate is T1D (0.2503) < Healthy (0.3102), and the pH Value is T1D (6.478) < Healthy (7.49), whereas the buffering Capacity: T1D (5.596) <

Healthy (6.746). All three parameters are lower in the T1D group, suggesting that T1D affects salivary function, which could contribute to increased dental problems.

The saliva samples from both groups were processed using nutrient and blood agar. CFU of the dominant colonies counted, where the minimum count for a colony was 8 (Table 3).

More CFU was found in blood agar for both groups than in nutrient agar. This can be attributed to the specialized nature of blood agar, as it provides more nutrients than nutrient agar. When the CFUs of both groups were compared, Group A (saliva of children with T1D) yielded more bacterial colonies than Group B (saliva of healthy children). In both nutrient agar and blood agar, the average number of CFU was higher in Group A than in Group B. The dominant colonies were further processed to get a pure culture and a Gram stain. These colonies were identified based on their colony characteristics and Gram character, as shown in Table 5. Six major bacterial strains were isolated from both groups: *Enterococcus species*, *Lactobacillus species*, *Streptococcus mutans*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. All of them were gram-positive (Table 4).

Table 3: Microbiological Processing Of Saliva Samples

Sample distribution	Sample number	Growth on Nutrient Agar	Average No of CFU	Growth on Blood Agar	Average No of CFU	
GROUP A: saliva of children with T1D	1	+	20	+	25	Mean CFU: 20 Standard deviation: 3.13 Range: 15-25
	2	+	25	+	35	
	3	+	20	+	25	
	4	+	22	+	27	
	5	+	15	+	20	
	6	+	18	+	25	
	7	+	25	+	29	
	8	+	17	+	25	
	9	+	18	+	23	
	10	+	20	+	26	
GROUP B: saliva of healthy Children	11	+	8	+	4	Mean CFU: 11.4 Standard deviation: 2.46 Range: 8-15
	12	+	10	+	15	
	13	+	11	+	17	
	14	+	13	+	18	
	15	+	9	+	14	
	16	+	14	+	16	
	17	+	13	+	17	
	18	+	15	+	19	
	19	+	13	+	17	
	20	+	08	+	18	

Table 4: Gram Staining and Colony Characteristics

Bacteria	Nutrient Agar	Blood agar	Gram Stain
<i>Enterococcus</i> species	Spherical, Mucoid, opaque, round colonies	Gamma hemolytic, round, greyish-white colonies	Gram-positive coccus
<i>Lactobacillus</i> species	2-5 micrometres, convex, entire, opaque, and without pigment	NA	Gram-positive bacilli
<i>Streptococcus mutans</i>	White/light grey coloured round elevated colonies	α haemolytic, circular, convex, smooth, and non-pigmented colonies.	Gram-positive coccus
<i>Streptococcus pyogenes</i>	Round, greyish-white, elevated, small colonies	β -hemolytic colonies, dome-shaped with a smooth or moist surface and clear margins	Gram-positive cocci, arranged in chains
<i>Streptococcus salivarius</i>	Round, convex, white butyrous colonies	α hemolytic, Round, grey, elevated, small colonies	Gram-positive coccus
<i>Staphylococcus aureus</i>	Round Golden yellow colonies	β -hemolytic, Round, Golden yellow colonies	Gram-positive coccus
<i>Staphylococcus aureus</i>	10	Amx, Amx-CV, Az, Cd, Cf, Dx, Mt, P, Ery	No resistance

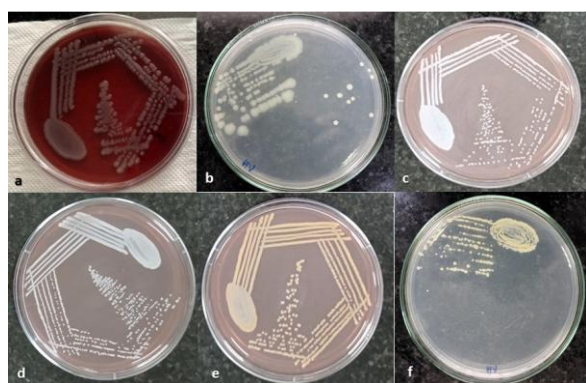
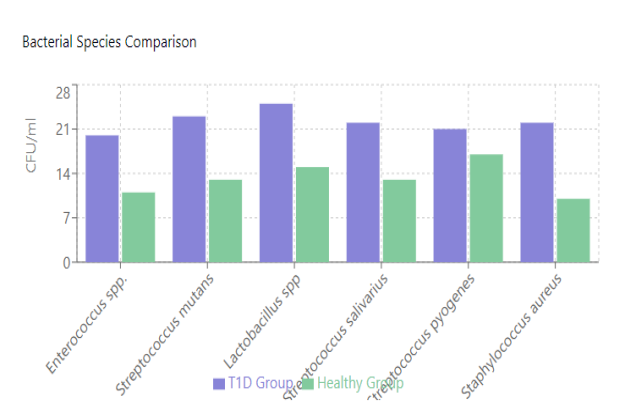


Figure IIIa: *Enterococcus* spp on blood agar; b. *Lactobacillus* on nutrient agar; c. *Streptococcus pyogenes* on blood agar; d. *Streptococcus mutans* on blood agar; e. *Staphylococcus aureus* on blood agar; f. *Staphylococcus aureus* on nutrient agar

**Figure IV: Bacterial Species Comparison**

The bacterial strains with colonies less than 10 CFU were not considered for further processing (Figure III a-f). The Difference in means CFU on NA was 8.6. This indicated that children with T1D show higher bacterial growth on nutrient agar. Similarly, the difference in means of CFU on blood agar was 10.5 CFU.

Bacterial growth is consistently higher in the T1D group compared to the healthy group. The variability (standard deviation) is similar between groups for nutrient agar, but slightly higher in the healthy group for blood agar. The range of CFU counts is wider in the T1D group, especially on blood agar (Figure IV).

The bar graph compares the presence of bacterial species in children with (T1D) and healthy children. All bacterial species are more prevalent in the T1D group compared to the healthy group. *Lactobacillus* species has the highest presence in both groups which was 25 CFU/ml in T1D and 15 CFU/ml in healthy. The largest difference is seen in *Staphylococcus aureus* which was 22 CFU/ml in T1D vs 10 CFU/ml in healthy.

This graph illustrates the number of bacterial species resistant to at least one antibiotic in each group. In the T1D group, all 6 bacterial species showed resistance to at least one antibiotic (Figure V).

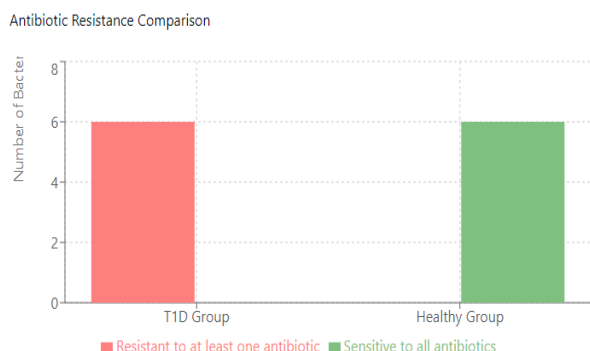


Figure V: Antibiotic Resistance Comparison

In the healthy group, none of the bacterial species showed resistance to any antibiotics (Table 5).

The average bacterial load of the T1D group is 22.17 CFU/ml, whereas in the healthy group, it is 13.17 CFU/ml. Furthermore, in the T1D group, the most common resistance was seen towards amoxicillin, penicillin, and azithromycin. Both groups showed sensitivity to amoxicillin-clavulanate, clindamycin, doxycycline, and metronidazole. These visualisations and statistics highlight significant differences in bacterial

presence and antibiotic resistance between children with T1D and healthy children (Figure VI).

The T1D group shows both higher bacterial loads and more antibiotic resistance, which could have implications for oral health management in these patients.



Figure VI: a. Bacterial Strains Isolated From Healthy Controls Showing Sensitivity To Prescribed Antibiotics; b. Bacterial Strains Isolated From Healthy Controls Showing Sensitivity To Prescribed Antibiotics

Table 5: Bacterial Species Identified From the Saliva Samples in Both Groups

Group	Bacterial species identified	Bacteria with minimum of 10 CFU/ml	Antibiotic sensitivity pattern	
			Sensitive Antibiotics	Resistant Antibiotics
GROUP A: saliva of children with T1D	<i>Enterococcus</i> species	20	Amx CV, Cd, Dx, Ery, Mt,	Amx, P, Az, Cf
	<i>Streptococcus mutans</i>	23	Amx-CV, Cd, Dx, Mt,	Amx, P, Az, Ery, Cf
	<i>Lactobacillus</i> species	25	Amx, Amx-CV, Az, Cd, Cf, Dx, Mt,	P, Ery
	<i>Streptococcus salivarius</i>	22	Amx, Amx-CV, Az, Cd, Cf, Dx, Mt,	P, Ery
	<i>Streptococcus pyogenes</i>	21	Amx-CV, Dx, Mt, Cf	Amx, P, Az, Ery, Cd
	<i>Staphylococcus aureus</i>	22	Amx-CV, Cf, Cd, Dx, Mt,	Amx, P, Az, Ery,
Healthy group	<i>Enterococcus</i> species	11	Amx, Amx-CV, Az, Cd, Cf, Dx, Mt,	P, Ery
	<i>Streptococcus mutans</i>	13	Amx-CV, Az, Cd, Cf, Dx, Mt, P, Ery	Amx,
	<i>Lactobacillus</i> species	15	Amx-CV, Az, Cd, Cf, Dx, Mt, P, Ery	No resistance
	<i>Streptococcus salivarius</i>	13	Amx-CV, Az, Cd, Cf, Dx, Mt, P, Ery	No resistance
	<i>Streptococcus pyogenes</i>	17	Amx-CV, Az, Cd, Cf, Dx, Mt, P, Ery	No resistance

Note: Amx: Amoxicillin; Amx-CV: Amoxicillin-Clavulanate; Az: Azithromycin; Cf: Cefixime; CD: Clindamycin; Dx: Doxycycline; Ery: Erythromycin; Mt: Metronidazole; P: Penicillin

Discussion

This study suggests that T1D directly impacts the oral microflora and the patient's overall oral health. The bacteria isolated from the diabetic group were more antibiotic-resistant, eventually affecting their health in the long run. The cleaning capacity of the saliva is reduced as there is an abnormality in the saliva flow rate and salivary buffering capacity. The pH of the saliva was slightly acidic due to sugar fermentation in the oral cavity, which will lead to cariogenic activities and dysbiosis and accumulation of more pathogenic organisms in the oral cavity. Many studies have been conducted on a similar problem, which is discussed in the following paragraphs.

Arrieta-Blanco et al¹⁷ evaluated 30 men and 40 women aged 11 to 81 years and reported increased dental problems and late complications among diabetic patients compared to non-diabetic individuals. Hilt et al¹⁸ observed a higher occurrence of buccal disorders in diabetic children due to metabolic disturbances and associated risk factors, while periodontal disease was more prevalent in diabetic individuals. Goteiner et al¹⁹ found that plaque scores and caries were more frequent in insulin-dependent diabetes mellitus patients, though gingival and plaque indices were similar in both diabetic and non-diabetic groups. Pachonski et al²⁰ investigated the relationship between caries, periodontal disease, and diabetes in 50 children aged 10–18 years and observed a significant increase in caries and periodontitis among diabetic patients. Similarly, Miralles-Jorda et al²¹ reported greater periodontal attachment loss in diabetic patients, though no significant difference was seen in caries, mucosal lesions, or salivary flow. Yuan et al²² found that the acute phase of T1D was marked by oral microbiota dysbiosis, which could improve with glycemic control. Babatzia et al²³ found significant increases in plaque and *Streptococcus mutans* levels in diabetic children, though no notable difference was observed in calculus, DMFS index, or *Candida albicans* counts. These findings were consistent with the present study, where diabetic children showed lower salivary flow rates and buffering capacity, along with higher levels of cariogenic organisms such as *Streptococcus mutans* and *Lactobacillus*, affecting their overall oral hygiene.

Ferizi et al²⁴ reported a significant rise in DMFT scores and a higher prevalence of *Lactobacillus* with reduced salivary flow among diabetic children. Singh et al²⁵ noted that *Lactobacillus casei* counts were higher than *Porphyromonas gingivalis* in

diabetic patients, with a slight increase in papillary bleeding index. Davidopoulou et al²⁶ found a direct correlation between altered oral microflora and increased risk of oral infections in T1D, leading to deterioration of dental and periodontal health. Pinducciu et al²⁷ studied 131 T1D patients and 20 healthy controls and observed significantly higher rates of gingivitis and periodontitis in diabetics with reduced salivary lysozyme levels. Jolanta et al²⁸ concluded that despite similar oral hygiene habits, diabetic children had increased gingivitis and periodontitis due to metabolic imbalance and predisposing factors. Mousa et al²⁹ isolated 32 new bacterial strains from 60 tongue scrapings and detected the *sea* gene in 64 isolates. Similar findings were seen in the present study, where diabetic children had higher DMFT scores, harboured more pathogenic organisms, and showed resistance to commonly used antibiotics.

Conclusion

In conclusion, patients with Type 1 Diabetes may directly influence their general dental health and oral microbiome. Over time, the diabetes group's health was negatively impacted by the bacteria that were isolated from them because they were more resistant to antibiotics. The saliva's cleaning ability is diminished because of an anomaly in the salivary buffering capacity and saliva flow rate. Due to sugar fermentation in the oral cavity, which causes cariogenic activity, dysbiosis, and the buildup of more pathogenic organisms there, the saliva's pH was somewhat acidic. Therefore, an appropriate treatment policy must be developed for children with Type 1 Diabetes for their dental problems. It should also accompany a suitable antibiotic policy should be developed to counter the antibiotic resistance problem.

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Conflict of Interest

None

Financial Disclosure

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Authors' contributions

SM supervised the study and approved the final manuscript. SP(1) handled sample collection, laboratory work, and data compilation. SR performed microbiological analysis, statistical

evaluation, and reviewed the manuscript. RD assisted in clinical assessment and data recording. SP(2) carried out bacterial culture and antibiotic testing. DS conducted biochemical analysis, interpreted results, and verified references. All the authors approved the final manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

This study was carried out after the approval of the Institute Ethical Committee of the Dental Sciences via letter no. IEC-IDS/IDS.SOA/2023/I-43 dated 10th October 2023.

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