ISSN (O) : 2582-3271
PubMed-National Library of Medicine - ID: 101766917

International Journal of Dental Science and Clinical Research (IJDSCR)

Dr. Angel Vaidic Publication

Available Online at: http://www.ijdscr.org

Volume - 7, Issue – 5, October - 2025, Page No. : 23 – 39

Effectiveness of Probiotics as an Adjunct to Standard of Care on Streptococcus Mutans Levels, Streptococcus Sanguinis Levels and Quality of Life in Children with Severe Early Childhood Caries: A Randomized Controlled Trial

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Citation of this Article: Dr. Ritika Raviraj Shetty, Dr. Ann Thomas, Dr. Sowmya B Shetty, Dr. Vaibhavi Bhat, Dr. Amrutha Pai, "Effectiveness of Probiotics as an Adjunct to Standard of Care on Streptococcus Mutans Levels, Streptococcus Sanguinis Levels and Quality of Life in Children with Severe Early Childhood Caries: A Randomized Controlled Trial.'' IJDSCR – October – 2025, Vol. – 7, Issue - 5, Page No. 23-39.

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

ABSTRACT

Aim

This study aimed to evaluate the effectiveness of probiotic therapy as an adjunct to standard care in managing S-ECC.

Methodology

A randomized controlled trial was conducted involving 40 children with S-ECC aged between 3-5 years; divided equally into an intervention group (standard care + daily probiotic for 30 days) and a control group (standard care alone). The saliva sample collection was carried out at baseline, 7th day, 1 month and 3 month follow up and microbiologically S. mutans was assessed at all 4 intervals and S. sanguinis only at baseline and 3 month follow up. A self-administered questionnaire with ECOHIS scores was used to assess the oral health related quality of life at baseline and 3 month follow up. Data was analysed statistically.

Results

A statistically significant reduction in S. mutans level in the intervention group at 1 month ($p<0.001$) and 3

month follow up ($p<0.001$) and a significant increase in the S. sanguinis levels at 3 months ($p<0.001$) was found compared to control group. No significant difference was found between the groups in the oral health related quality of life ($p = 0.09$) at 3 month follow-up.

Conclusion

Our findings suggest a substantial effectiveness microbiologically with use of probiotics as an adjunct to standard of care. A longer research duration is recommended to extrapolate the clinic evidence.

Keywords

Dental Caries, S-ECC, Probiotics, Streptococcus mutans, Streptococcus sanguinis, Oral Health, Microbiota, Quality of Life.

INTRODUCTION

Early Childhood Caries (ECC) is a global epidemic affecting infants and preschool-aged children. Its onset is predominantly linked to a dysbiotic shift in the oral microbiome often triggered by inappropriate feeding behaviors which includes the frequent intake of fermentable carbohydrates.[1] The disease has a multifactorial origin, shaped by an intricate interplay of biological, medical, behavioral, psychological, cultural, and lifestyle factors. In addition, social determinants such as parental education, socioeconomic status, and poor access to preventive dental care greatly influence the risk, onset, and severity of ECC.[2]

The AAPD defines S-ECC as any signs of smooth-surface caries in a child younger than three years. For children aged three to five, it includes one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth, or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5).[3] Severe early childhood caries (S-ECC) can significantly affect a child's overall health and development beyond the oral cavity.[4] In terms of cognitive and academic development, the discomfort from S-ECC can cause disturbed sleep, irritability, and poor concentration, affecting learning and school performance.[5] Frequent absenteeism due to pain or treatment needs contributes to poor academic outcomes, while impaired immunity increases susceptibility to systemic infections such as respiratory and gastrointestinal illnesses, leading to unplanned hospital admissions. These episodes disrupt family routines, cause parental work absences, and add financial and emotional strain.[6]

Management of ECC involves a comprehensive approach combining therapeutic measures to address the disease and its causes, preventive strategies to halt progression, and restorative care to restore form and function.[7]Beyond improving oral health, effective management of ECC plays a crucial role in enhancing the child's overall immunity and supporting holistic development. In recent years, probiotics have gained attention as an adjunctive intervention in the management of early childhood caries (ECC), particularly for their potential role in enhancing preventive strategies and supporting overall oral health in young children.[8] In children with ECC, probiotics can be delivered via lozenges, chewable tablets, or gels.[9] They act against Streptococcus mutans through competitive exclusion, antimicrobial production (e.g., bacteriocins, hydrogen peroxide, organic acids), pH modulation, inhibition of glucan synthesis, and disruption of quorum sensing.[10,11,12,13] They may also enhance immune defenses by increasing secretory IgA and supporting anti-inflammatory responses.[14] Regular use of safe, multi-strain probiotics can help establish a healthier oral

microbiome, lower cariogenic risk, and reduce ECC recurrence in children.

Assessment of *S. mutans* levels before and after intervention offers a reliable measure of microbial control and guides follow-up protocols to reduce relapse risk.[15] Understanding its role in S-ECC is essential for evaluating probiotic efficacy and developing long-term preventive strategies to maintain microbiome balance. Persistently high *S. mutans* counts are linked to early lesion recurrence post-rehabilitation, reflecting unresolved biological and behavioral risk factors.[16] The interplay between *S. mutans* and *S. sanguinis* is well documented. *S. sanguinis* acts as a protective commensal, producing antimicrobial agents and competing for ecological niches, thereby limiting *S. mutans* growth.[17] Higher *S. sanguinis* levels correlate with healthier dentitions and lower caries prevalence. Tracking its levels pre- and post-treatment can indicate microbial rebalancing and treatment success in restoring a caries-resistant environment.

While clinical management of Severe Early Childhood Caries (S-ECC) remains central, this study also examines its broader impact on children and caregivers. Recognizing that S-ECC affects emotional, social, and developmental domains, oral health-related quality of life (OHRQoL) will be assessed using the validated Early Childhood Oral Health Impact Scale (ECOHIS). This enables evaluation of treatment effects on pain, eating and sleeping patterns, communication, emotional distress, parental worry, and family functioning.[18]

Documenting these changes supports a more holistic understanding of outcomes, linking clinical success with meaningful, patient-centered care in young children with S-ECC.

This study seeks to evaluate the impact of combining standard care with probiotic supplementation by examining changes in S. mutans and S. sanguinis levels and oral health-related quality of life. The research aims to contribute to a more comprehensive understanding of the role probiotics as an adjunct to standard of care in management of S-ECC.

AIM AND OBJECTIVES

Aim of the Study

The aim of the study is to evaluate the effect of one month probiotic therapy as an adjunct to standard of care in children with Severe Early Childhood Caries.

Objectives of the Study

1. To assess and compare the effect of probiotic as an adjunct to standard of care and only standard of care on streptococcus mutans levels in children with S-ECC at 7 days, 30 days and 3 months from baseline
2. To assess and compare the effect of probiotic as an adjunct to standard of care and only standard of care on streptococcus sanguinis level in children with S-ECC at 3 months from baseline
3. To compare and evaluate the effect of probiotic as an adjunct to standard of care and only standard of care on quality of life in children with S-ECC at 3 months from baseline

MATERIALS AND METHODS

Source of Data

Children aged 3-5 years visiting the department of pediatric and preventive dentistry at AJ Institute of Dental Sciences, Mangalore.

Materials used for the study

- Plastic sterile containers (for sample

collection).

- Mutans sanguis agar medium
- Fluoridated toothpaste (Pediflor)
- Commercially available (0.12%) chlorhexidine mouth rinse.(Chlohex)
- Probiotic powder sachet (Lactogut kidz)- (Lactobacillus reuteri - 0.1 billion cfu, Bacillus caogulans- 0.2 billion cfu, Lactobacillus rhamnosus- 0.2 billion cfu, Bifidobacteriurn longum-0.06 billion cfu, Bifidobacterium bifidum- 0.1 billion cfu, Bifidobacterium infantis- 0.1 billion cfu, Saccharomyces boulardii- 0.14 billion cfu, Streptococcus thermophilus- 0.1 billion cfu, Fructooligosaccharides- 20 gm, Lactitol -10 mg) (figure 6)

METHODOLOGY

This single-blinded, parallel-group randomized controlled trial was conducted among 40 children aged 3–5 years diagnosed with Severe Early Childhood Caries (S-ECC) after obtaining the approval from the Institutional Ethics Committee. Children reporting to the department who fulfilled the inclusion and exclusion criteria and whose parents or legal guardian gave written informed consent to participate in the study were enrolled for the study.

Participants were randomly allocated into two equal groups of 20 using a block randomization method i.e. Group A- intervention group and Group B- control group. Group assignment was performed by an independent researcher, and the allocation sequence was concealed until assignment. Due to the nature of the intervention, blinding of participants and caregivers was not feasible; however the data analyst was blinded to group identity.

The standard of care for S-ECC was met for all the participants additional to which the intervention group was supplemented with 1gm sachet of Lactogut Kidz probiotic daily for 30 days. The sachet was mixed with 200 mL of water and before swallowing the participant swished the solution in the mouth for 30 sec following nighttime oral hygiene routine. Caregivers were advised not to provide any additional water for 30 minutes following administration. Caregivers were requested to share videos of their child consuming the probiotic to monitor compliance and ensure proper intake. Adherence was monitored by distributing sachets for the entire course and collecting unused sachets at follow-up visits.

All the participants underwent microbiological assessment of S.mutans and S.sanguinis counts and a standardized questionnaire to assess the quality of life of children. Data collection was conducted at four intervals: baseline, the 7th day, one month, and three months from baseline. At baseline and 3 month follow-up the participants were subjected to saliva sample collection for microbiological assessment of S. mutans and S. sanguinis and the parents were subjected to the standardised questionnaire. Subsequently, saliva sample was collected on the seventh day and one month from baseline to assess the shift in S. mutans count. All the data collected were subjected to statistical analysis.

A standardized protocol for management of S-ECC was used to provide an unbiased standard of care for all the participants. At the time of enrolment, each participant underwent a comprehensive dietary assessment followed by individualized counselling. Throughout the study period, caregivers and children were consistently guided and encouraged to implement dietary modifications. All the participants

were provided with a soft-bristled toothbrush and fluoridated toothpaste (Pediflor) and instructed on home oral hygiene measures (figure 9) immediately after enrolment. They were also provided with Chlorhexidine mouthwash (Chlohex) and were advised to rinse with 5 ml after brushing at night for the first seven days to reduce the bacterial load. The comprehensive dental treatment was started after collection of baseline data; as the first line of treatment all the cavitated lesions were temporised to reduce the bacterial load. Further each of the affected teeth based on the extent of the carious lesion the definitive treatment was carried out, so as to achieve full mouth rehabilitation within a month.

The definitive treatment carried out included restoration of Class I lesions were managed with preventive resin restorations (PRR), while Class II lesions were restored with RmGIC or stainless steel crowns. Class III and IV anterior lesions were restored with composite resin. Teeth with pulpal involvement were treated with pulpotomy or pulpectomy followed by stainless steel crowns (SSCs) in posteriors and strip crowns in the anteriors. Teeth which cannot undergo rehabilitation were extracted. As preventive measures topical fluoride application was performed using acidulated phosphate fluoride (APF) gel, and pit and fissure sealants were applied to high-risk caries-free molars.

Saliva Collection and Microbiological Assessment

Participants were asked to refrain from eating and drinking one hour before saliva collection in order to obtain a relatively constant baseline. They were seated in the coachman's position (upright, with heads tilted slightly down) to pool saliva in the mouth and were asked not to swallow or move their tongue/lips during the collection period.

The first expectorant was discarded to eliminate food debris that could contaminate the sample and cause analytical inaccuracy. The subsequent sample was expectorated into a pre-labelled sterile container, and approximately 2 mL of saliva was collected. The samples were immediately transported for microbiological analysis. They were tested for colony-forming units using Mutans sanguinis agar medium. During the study, the participants were instructed to carry out routine oral hygiene practices.

Preparation of Agar medium: 49.5g of agar powder was suspended in 500ml of distilled water, which was boiled to dissolve the medium completely (figure 13). It was sterilized by autoclaving at 15lbs pressure (121℃) for 15 minutes (figure 14). It was let to cool for 2hours. When the solution reached 50℃-55℃, the solution was poured into sterile petri plates (figure 15). Finally, it was let to solidify.

Inoculation of Salivary Sample

The saliva sample was homogenized by a vortex mixer for 2 minutes. Saliva was diluted using ten fold dilution technique by withdrawing 0.01 ml of saliva and 0.09ml of saline using a micropipette into a microcentrifuge tube. This mixture was homogenised by a vortex mixer for 30 seconds. 0.001ml of saliva from 1:10 dilution was withdrawn using a calibrated sterile inoculating loop then streaked on the Petri dishes of Mutans-Sanguis agar under aseptic conditions. The whole inoculation procedure was done under aseptic conditions to avoid contamination.

Incubation of Inoculated Media:

The inoculated plates were placed in incubation chamber and incubated aerobically at 37°C for 48 hours. Following incubation, colonies were identified by the morphological characteristics of S. mutans and S. sanguinis on Mutans Sanguis agar medium. S.

sanguinis colonies appeared smooth or rough, hard, and rubbery in consistency, either gray, white, or colorless which adhere strongly to the agar medium making them difficult to remove with a inoculating loop and can be differentiated from S. mutans colonies which are irregular, rough, white or yellow in color, resembling granular frosted glass and can be easily picked off or easily detachable from the agar medium. Bacterial count was done using the conventional plate count method. The colony count was expressed as number of colony forming units per milli litre (CFU/ml) of saliva. The colony counts were reassessed by another investigator to check of any count discrepancies.

Questionnaire

At baseline and at 3 months, the parents were asked to fill out a questionnaire consisting of two parts, the demographic data and the Early Childhood Oral Health Impact Scale (ECOHIS) developed by Bhavna Talekar Pahel, R Gary Rozier & Gary D Slade consisting of 13 questions divide into two sections- the child impact section containing 8 questions and family impact section containing 4 questions and a question on pain ; 2 additional questions based on literature search.

The Early Childhood Oral Health Impact Scale (ECOHIS) was based on a 5-point Likert scale.The response options were coded: 0 = never; 1 = hardly ever; 2 = occasionally; 3 = often; 4 = very often. The values ranged from 0 to 52 for the total scale (0–36 for the child section and 0–16 for the family section). A higher score expressed a higher negative impact, whereas a lower score expressed a lower negative impact on the quality of life. All the answers were subjected to statistical analysis.

Statistical Analysis

The data was collected, coded and fed in SPSS IBM version 23, for statistical analysis. the outcomes between the two groups were compared using appropriate statistical methods. A two-sample t-test was employed to assess whether the observed mean difference between groups is statistically significant, given the chosen power (80%) and confidence level (95%). Normality and homogeneity of variance assumptions were checked, and if violated, non-parametric alternatives were used.

Secondary analysis included confidence intervals for the mean difference to quantify precision. All analyses were conducted using statistical software (e.g., R, SPSS, or STATA), with a two-sided significance threshold of $\alpha = 0.05$. Sensitivity analyses assessed the impact of missing data or outliers, ensuring robust and reliable conclusions.

RESULTS

Table 1: Intragroup Comparison of S. Mutans Counts at Different Time Intervals in Both the Groups (Anova Test)

Group	Time intervals	Mean (Cfu/ml) x 10^5	SD	P-Value
Intervention group	Baseline	154.3	30.61	<0.001(HS)
	7th Day	108	20.51	
	1 Month	63	13.7	
	3 Month	71.8	13.94	
Control group	Baseline	156	31.27	<0.001(HS)
	7th Day	112.5	31.00	
	1 Month	86.3	21.87	
	3 Month	120.1	27.67	

Table 1: depicts that intragroup comparison of S. mutans counts at different time intervals in both the groups**.** In the intervention group, the baseline mean count of S. mutans was 154.3 ± 30.61, which significantly decreased to 108 ± 20.51 by the 7th day and at 1 month 63 ± 13.7 followed by a slight increase at 3 months 71.8 ± 13.94; however, levels remained significantly lower than baseline. This reduction over time was statistically significant ($p < 0.001$). Whereas in the control group, the baseline mean S. mutans count was 156 ± 31.27 which decreased to 112.5 ± 31.00 by the 7th day and further to 86.3 ± 21.87 at 1 month. However, by 3 months, a rebound increase to 120.1 ± 27.67 was noted. Despite the increase, overall changes across time points were statistically significant ($p < 0.001$).

Table 2: Intragroup Comparison of the Mean Difference in S. Mutans Counts between the Time Intervals in Both the Groups (Repeated Measures Anova)

Groups	Intragroup time intervals		Mean (Cfu/ml) x 10^5	SD	p-value	F value
Intervention group	baseline	7th day	46.3	13.53	<0.001 (HS)	218.24
		1 month	91.2	20.90	<0.001 (HS)	
		3 month	82.5	26.82	<0.001 (HS)	
	7th day	1 month	45	11.65	<0.001 (HS)	
		3 month	36.2	17.89	<0.001 (HS)	
	1 month	3 month	-8.8	9.97	<0.01 (SS)	
Control group	baseline	7th day	43.5	16.03	<0.001 (HS)	110.46
		1 month	69.7	16.83	<0.001 (HS)	
		3 month	35.9	17.59	<0.001 (HS)	
	7th day	1 month	26.2	14.61	<0.001 (HS)	
		3 month	-7.6	20.82	0.1168 (NS)	
	1 month	3 month	-33.8	17.35	<0.001 (HS)	

Table 2: presents the intragroup comparisons of S. mutans colony-forming units per millilitre (Cfu/ml) at multiple time intervals at baseline, 7th day, 1 month, and 3 months. In the intervention group, there was a highly significant reduction in S. mutans counts from baseline to the 7th day, with a mean difference of 46.3 ± 13.53 and a p-value less than 0.001. From baseline to 1 month, the mean difference was 91.2 ± 20.90, and from baseline to 3 months, it was 82.5 ± 26.82, both showing p-values less than 0.001. Further, statistically significant reductions were noted from the 7th day to 1 month with a mean difference of 45 ± 11.65, and from the 7th day to 3 months with a mean difference of 36.2 ± 17.89, both with p-values less than 0.001. A minor but statistically significant increase in S. mutans count was observed between 1 month and 3 months, with a mean difference of −8.8 ± 9.97 and a p-value less than 0.01, suggesting early recolonization.

In the control group, significant reductions in S. mutans counts were observed from baseline to the 7th day with a mean difference of 43.5 ± 16.03, from baseline to 1 month with a mean difference of 69.7 ± 16.83, and from baseline

to 3 months with a mean difference of 35.9 ± 17.59, all showing p-values less than 0.001. both with p-values less than 0.001. However, the change from the 7th day to 3 months with a mean difference of −7.6 ± 20.82 was not statistically significant as the p-value was 0.1168, indicating that with only standard of care met there was a pronounced recolonization.

Table 3: Intragroup Comparison of S. Sanguinis Counts at Different Time Intervals in Both the Groups (Paired Samples T-Test)

Groups	Time intervals	N	Mean (Cfu/ml) x 10^3	SD	p-value
Intervention group	Baseline	20	1.45	1.28	<0.001(HS)
	3 Month	20	13.4	4.87	
Control group	Baseline	20	1.4	1.19	0.018(SS)
	3 Month	20	2.45	1.93	

Table 3: presents the intragroup comparison of S. Sanguinis counts at baseline and at the 3-month interval in both the intervention and control groups. The analysis was performed using the Paired Samples t-test to evaluate changes in the levels of this beneficial commensal organism over time.

In the intervention group, the mean S. Sanguinis count increased from 1.45 ± 1.28 at baseline to 13.4 ± 4.87 at 3 months. This increase was statistically highly significant ($p < 0.001$), indicating a substantial improvement in the colonization of beneficial bacteria, likely due to the probiotic intervention.

In the control group, the mean S. Sanguinis count rose from 1.4 ± 1.19 at baseline to 2.45 ± 1.93 at 3 months. Although this increase was more modest, it was still statistically significant ($p = 0.018$), suggesting that standard care alone had a limited effect on enhancing beneficial microbial populations compared to the intervention group.

Table 4: Intragroup Comparison of Ecohis Scores at Baseline and 3 Months in Both the Groups (Independent Samples T-Test)

Questions	Intervention group							Control group						
	Baseline			3 months			P-value	Baseline			3 months			P-value
	N	Mean	SD	N	Mean	SD		N	Mean	SD	N	Mean	SD	
Q1	20	3.35	0.58	20	0.35	0.48	<0.001 (HS)	20	3.85	0.36	20	0.25	0.44	<0.001 (HS)
Q2	20	3.35	0.71	20	0.60	0.50	<0.001 (HS)	20	2.6	0.94	20	0.45	0.51	<0.001 (HS)
Q3	20	2.95	0.68	20	0.40	0.50	<0.001 (HS)	20	2.4	0.82	20	0.50	0.51	<0.001 (HS)
Q4	20	2.8	0.89	20	0.35	0.48	<0.001 (HS)	20	2.31	0.94	20	0.50	0.51	<0.001 (HS)
Q5	20	2.6	1.14	20	0.30	0.47	<0.001 (HS)	20	2.2	0.76	20	0.45	0.51	<0.001 (HS)
Q6	20	2	1.12	20	0.40	0.50	<0.001 (HS)	20	2.4	0.59	20	0.40	0.50	<0.001 (HS)
Q7	20	2	0.97	20	0.35	0.48	<0.001 (HS)	20	2.2	0.52	20	0.60	0.50	<0.001 (HS)
Q8	20	2.3	1.30	20	0.60	0.50	<0.001 (HS)	20	2.35	0.74	20	0.35	0.48	<0.001 (HS)
Q9	20	2.35	1.22	20	0.45	0.51	<0.001 (HS)	20	2.4	0.88	20	0.45	0.51	<0.001 (HS)
Q2-Q9	20	2.54	1.23	20	0.43	0.73	<0.001 (HS)	20	2.52	1.20	20	0.46	0.58	<0.001 (HS)
Q10	20	1.95	0.68	20	0.25	0.44	<0.001 (HS)	20	1.75	0.85	20	0.35	0.48	<0.001 (HS)
Q11	20	1.75	0.85	20	0.35	0.48	<0.001 (HS)	20	1.4	0.68	20	0.50	0.51	<0.001 (HS)
Q12	20	1.75	0.85	20	0.35	0.48	<0.001 (HS)	20	1.6	0.75	20	0.55	0.51	<0.001 (HS)
Q13	20	1.85	0.74	20	0.90	0.85	<0.001 (HS)	20	1.7	0.65	20	0.65	0.48	<0.001 (HS)
Q10-Q13	20	1.83	1.23	20	0.46	0.47	<0.001 (HS)	20	1.61	0.98	20	0.51	0.44	<0.001 (HS)
Q1-Q13	20	30.55	5.45	20	5.65	1.30	<0.001 (HS)	20	29.05	2.74	20	6	1.17	<0.001 (HS)

Graph 1: Intragroup Comparison of Ecohis Scores at Baseline and 3 Months in the Intervention Group

Graph 2: Intragroup Comparison of Ecohis at Baseline and 3 Months in the Control Group

Table 4: Graphs 1 and 2 present the intragroup comparison of ECOHIS questionnaire scores at baseline and after 3 months in both the intervention and control groups. In the intervention group, the total ECOHIS score significantly decreased from a baseline mean of 30.55 ± 5.45 to 5.65 ± 1.30 at 3 months. In the control group, the score reduced from 29.05 ± 2.74 at baseline to 6.00 ± 1.17 at 3 months. These reductions were statistically highly significant in both groups ($p < 0.001$), indicating a marked improvement in overall oral health-related quality of life (OHRQoL) following treatment.

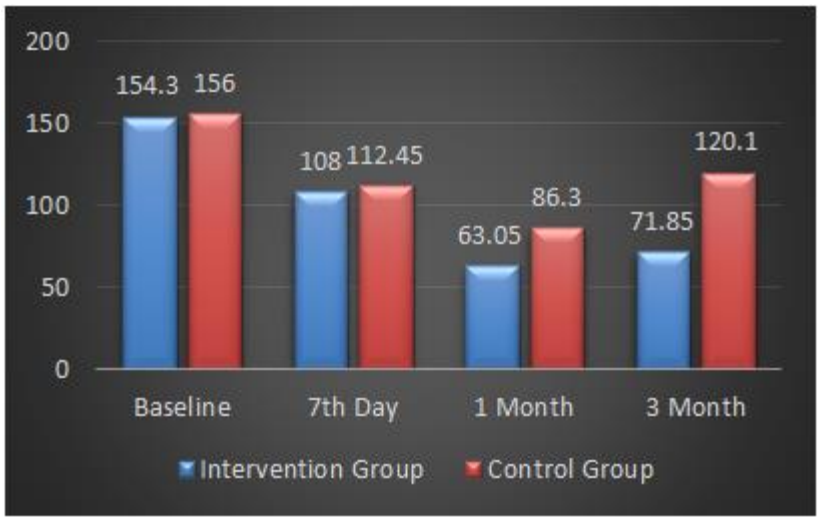
Statistically significant reductions were also observed across all individual ECOHIS items (Q1 to Q13). In the child impact section (Q2–Q9), the intervention group improved from 2.54 ± 1.23 to 0.43 ± 0.73, and the control group from 2.52 ± 1.20 to 0.46 ± 0.58 ($p < 0.001$ for both). In the family impact section (Q10–Q13), the intervention group showed a reduction from 1.83 ± 1.23 to 0.46 ± 0.47, and the control group from 1.61 ± 0.98 to 0.51 ± 0.44 ($p < 0.001$ for both), again demonstrating statistically significant improvements.

These findings reflect a meaningful and statistically significant enhancement in OHRQoL among children with severe early childhood caries. Although both groups benefited from treatment, the slightly greater reduction in ECOHIS scores in the intervention group suggests that the intervention offered additional improvement in child and family well-being.

Table 5: Intergroup Comparison of Mean Difference in S. Mutans and S. Sanguinis Counts at Different Time Intervals (Independent Samples T-Test)

Bacterial count	Time intervals		Group	N	Mean(Cfu/ml)	SD	p-value
S. Mutans Count	Baseline	7th Day	IG	20	46.3	13.53	0.863(NS)
			CG	20	43.55	16.03	
		1 Month	IG	20	91.25	20.90	0.008 (SS)
			CG	20	69.70	16.83	
		3 Month	IG	20	82.45	26.82	<0.001(HS)
			CG	20	35.90	27.67	
S. Sanguinis	Baseline	3 Month	IG	20	11.95	4.87	<0.001(HS)
			CG	20	1.05	1.93	

GRAPH 3 : **INTERGROUP COMPARISON OF S. MUTANS COUNTS AT DIFFERENT TIME INTERVALS**



GRAPH 4: **INTERGROUP COMPARISON OF S. SANGUINIS COUNTS AT DIFFERENT TIME INTERVALS**

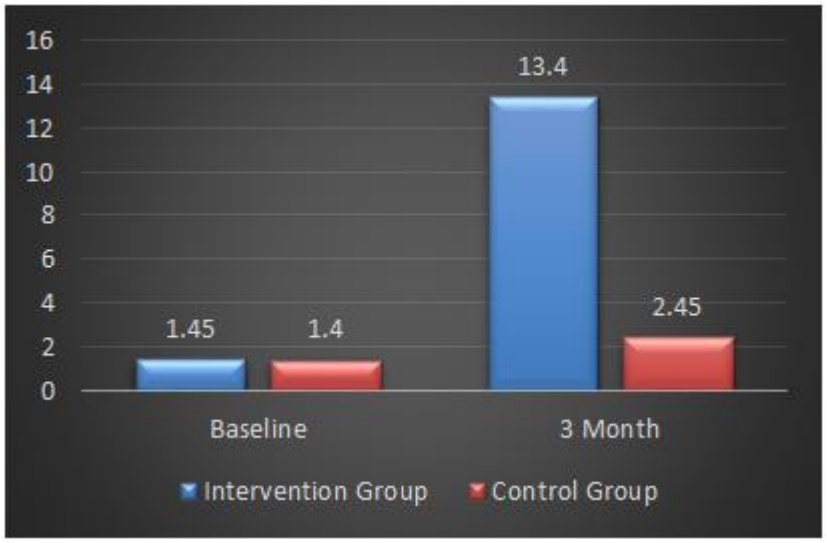


Table 5 Graph 3, 4 present the intergroup comparison of mean differences in S. mutans and S. sanguinis counts at various time intervals using the Independent Samples t-test.

For S. mutans, at the 7th day, the mean reduction was 46.3 ± 13.53 in the intervention group and 43.55 ± 16.03 in the control group. The difference between the groups was not statistically significant (p = 0.863), indicating a similar short-term effect of both interventions.

At 1 month, the intervention group showed a greater mean reduction of 91.25 ± 20.90, while the control group had a reduction of 69.70 ± 16.83. This difference was statistically significant (p = 0.008), indicating that the addition of probiotics provided superior bacterial suppression after one month.

At 3 months, the intervention group maintained a mean reduction of 82.45 ± 26.82, whereas the control group showed a reduction of 35.90 ± 27.67. This difference was highly statistically significant (p <

0.001), confirming the sustained efficacy of the probiotic intervention in reducing cariogenic bacterial levels over time.

Regarding S. sanguinis, both groups started with similar baseline levels. At 3 months, the intervention group showed a substantial increase with a mean difference of 11.95 ± 4.87, while the control group showed only a modest increase of 1.05 ± 1.93. This difference was highly statistically significant ($p < 0.001$), indicating the intervention's effectiveness in promoting colonization of beneficial bacteria.

Table 6: Intergroup Comparison of Mean Difference in Ecohis Score at Baseline and 3 Months (Welch's T-Test - An Independent Samples T-Test)

Questions	Intervention group		Control group		p-value
	Mean difference	SD	Mean difference	SD	
Q2- Q9	2.11	1.74	2.06	1.70	0.927(NS)
Q10-Q13	1.37	1.74	1.10	1.39	0.591(NS)
Q1-Q13	24.9	7.71	23.05	3.87	0.346(NS)

Table 6 presents the intergroup comparison of mean differences in ECOHIS scores between baseline and 3 months. For the child impact section (Q2–Q9), the mean difference in scores was 2.11 ± 1.74 in the intervention group and 2.06 ± 1.70 in the control group. This difference was not statistically significant ($p = 0.927$), indicating that both groups experienced similar improvements in child-related quality of life outcomes. In the family impact section (Q10–Q13), the intervention group showed a mean score difference of 1.37 ± 1.74, compared to 1.10 ± 1.39 in the control group. This difference was also not statistically significant ($p = 0.591$), suggesting comparable reductions in family distress or burden between the two groups.

The total ECOHIS score (Q1–Q13) demonstrated a mean difference of 24.9 ± 7.71 in the intervention group and 23.05 ± 3.87 in the control group. Although the intervention group showed a slightly higher numerical improvement, the difference between groups remained statistically non-significant ($p = 0.346$). These findings indicate that while both the intervention and control groups showed clinically meaningful improvements in oral health-related quality of life over the 3-month period, the differences between groups were not statistically significant.

DISCUSSION

The therapeutic use of probiotics in dentistry has garnered growing interest due to their potential to beneficially alter the oral microbiota and improve host immune response. Numerous studies have confirmed their efficacy in gastrointestinal health; however, their application in the context of oral diseases, particularly dental caries, is still under exploration. According to Devine and Marsh (2009)[19], probiotics may offer a novel approach to oral health by competing with

pathogenic bacteria, thereby helping maintain microbial homeostasis. While this has been well-studied in general oral hygiene and in cases of mild-to-moderate caries, there is limited research addressing their role in the management of Severe Early Childhood Caries (S-ECC). Hence, our study sought to bridge this gap, by evaluating the use of probiotics as an adjunct to standard care in children diagnosed with S-ECC.

The standard of care for managing early childhood caries (ECC) typically involves a comprehensive three-pronged approach: reduction of microbial load, improvement in dietary habits, and enhancement of tooth resistance through remineralization strategies along with complete oral rehabilitation. Even though these measures are considered clinically effective, multiple studies have highlighted the high recurrence rates of caries even after full-mouth rehabilitation in children.[20] Suggesting a drawback in the current treatment approach to fully manage the disease. This inadequacy could be because of the time required to unlearn a habit, namely the dietary habit. Achieving lasting dietary change is a major challenge in children often requiring months or even years for them to adopt and sustain it, however in this transitional period children could remain vulnerable to microbial recolonization and the continued progression of disease.

In the light of this scenario there is a need for adjunctive strategies that can help stabilize the oral environment until healthier habits are firmly established in S-ECC children. Hence in this study, probiotics was used as a novel adjunct to the standard of care aimed at addressing microbial dysbiosis in the transitional period, and its effectiveness was evaluated by assessing the S. mutans and S. sanguinis counts, progression of existing incipient lesions and occurrence of new carious lesions. These two microbes were assessed simultaneously to capture a more comprehensive picture of probiotic induced shifts in oral ecology and its potential for beneficial recolonization, as there is limited scientific literature.

Our study findings indicate that probiotic was an effective adjunct to standard of care in in reducing Streptococcus mutans levels in children with S-ECC; as a statistically significant reduction ($p < 0.01$) was found when compared to only standard of care. The only other study to have assessed the effectiveness of probiotics with standard of care in children with S-EEC is by Hasslof et.al[21] who also found a significant reduction in S. mutans counts. Multiple studies have assessed the effect of probiotics usage in children with ECC on S.mutans levels and have reported a significant reduction.[22] Yousuf et al. (2020)[23] had similar finding and additionally noted a mild rebound on discontinuation of the intervention. In contrast a randomized controlled trial by Taipale et al. (2013)[24] involving infants and toddlers aged 1–2 years, found no significant reduction in S. mutans or caries incidence following 6 month probiotic supplementation. This could be due to the use of a single, gut-associated strain like Bifidobacterium animalis as the probiotic intervention; which lacks oral niche adaptation, limiting the therapeutic impact in the oral cavity.

Our finding also shed light on the resilience of cariogenic flora to recolonize within a short period even after standard of care was met. The control group, which received only the standard of care, showed consistent decrease in *S. mutans* levels within the 1st month and a significant rebound at 3rd month. Multiple studies have shown that even after the

standard of care is met in children with ECC a significant rebound in the S.mutans counts and increment of caries.[25] A similar finding was reported by Rodriguez et al. (2016)[26], who found recolonization of S.mutans within a month after the initial reduction following full-mouth rehabilitation under GA. It is also important to note that, the intervention group also had a significant rebound of S. mutans though not comparable to the control group by 3 month follow up; which indicates the need for protocol of probiotic therapy periodically once in every 2-3 months after the completion of the oral rehabilitation along with the adjunctive probiotic therapy to maintain the bacterial levels.

S.sanguinis a normal commensal bacteria is reported to be in inverse relation to the cariogenic bacteria.36 Our study findings indicate that probiotic was an effective adjunct to standard of care in increasing the Streptococcus sanguinis levels in children with S-ECC; as a high statistically significant difference ($p < 0.001$) was found between the groups. Similar findings were found in a study conducted by Patidar et.al(2020)50 in children with ECC. Contradictory to our findings, Çaglar et al. (2008)[27] reported no significant increase in commensal bacteria after probiotic use and this could be attributed to the use of Bifidobacterium species, which are less adaptive to the oral environment than Lactobacillus species. Also, studies examining changes in S. sanguinis following probiotic supplementation in children are limited.

The interplay between Streptococcus sanguinis and S. mutans is central to understanding the microbial ecology in ECC. In a study by Patidar et al. (2020)[28], involving children aged 3–6 years, reported a significant post-treatment decrease in S. mutans and a corresponding increase in S. sanguinis (both $p < 0.001$), highlighting the latter's potential role as a biomarker of oral health. These findings align with our intervention group which demonstrated a marked reduction in S. mutans and a concurrent increase in S. sanguinis, suggesting a transition toward a more balanced and health-associated oral microbiome. This microbial shift is in compliance with the ecological plaque hypothesis, which views caries as a consequence of microbial imbalance rather than infection by a single pathogen.

The impact on oral health related quality of life of children with S-ECC assessed using ECOHIS scores revealed a statistically significant improvement in both groups at 3-month follow-up. However, there was no evidence to suggest greater effectiveness of probiotics as an adjunct to standard of care over only standard of care. Though the statistical evidence for this parameter is non-significant, it should not be considered as lacking; as the quality of life assessment measures pain, eating and sleeping difficulties, in the child and parental distress, guilt, and work disruption; the substantial alleviation of these clinical, emotional, and functional burden associated with severe early childhood caries is evident. Additionally, it can be assumed that given the initial high burden of the disease, its recurrence and disablement to the initial levels or near initial levels is almost an impossibility, hence achievement of a statistically significant difference between the groups is highly unattainable for this parameter. It's also important to note that quality of life is a broad and subjective measure, influenced by caregivers' perceptions, in contrast to more objective parameters like microbial counts.

While the study offers valuable insights and shows encouraging results, it is important to recognize certain limitations that may have influenced the

outcomes. The main limitation of our study was its short follow-up period of three months, which was insufficient to capture the subtle changes in quality of life with probiotic use. Additionally, the sample size may limit the statistical power needed to detect subtle differences.

CONCLUSION

In this randomized controlled trial our microbiological evidence support the effective use of probiotic as an adjunct to standard of care in children with S-ECC for it was found to effectively modulate oral microbial ecology by reducing cariogenic Streptococcus mutans levels and increasing Streptococcus sanguinis levels. However impact on oral health related quality of life was found in both groups with no statistically significant difference between them in this limited study period. So, further research of long-term duration is recommended to extraplorate these findings.

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