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Evaluation and comparison of streptococcus mutans and scardovia wiggsiae from the predentate to permanent dentition using polymerase chain reaction

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ABSTRACT

Background: Streptococcus mutans has been long associated with initiation of dental caries. Nevertheless, dental caries is also seen in its absence, indicating the role of another micro-organism.

Aim: To evaluate and compare the presence of Streptococcus mutans (S. mutans) and Scardovia wiggsiae (S. wiggsiae) in children from predentate to permanent dentition period using polymerase chain reaction.

Materials and Methods: 80 children aged between 0 to 14 yrs., were divided into 8 groups of 10 each. Unstimulated salivary samples were collected from children of predentate to permanent dentition period with and without dental caries, which served as experimental and control groups respectively. The samples were compared and evaluated for S. wiggsiae & S. mutans. 2ml of unstimulated saliva samples were collected in a sterile 50ml graduated container & swab method was used to collect saliva from infants below 6-months of age & swabs were placed in MTM medium & stored at 20° C followed by DNA extraction procedure.

Results: The present study showed that both S. mutans and S. wiggsiae were present in the predentate period & gradually increased upto mixed dentition period. However, both the organisms decreased in permanent dentition group. In primary, mixed & permanent dentition control groups, S. mutans counts was significantly greater than S. wiggsiae, whereas in the experimental group S. wiggsiae was significantly higher than S. mutans.

Conclusion: S. mutans could be the commensal and S. wiggsiae could be the major etiological organism in progression of dental caries.

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1. Introduction

Dental caries is a complex and multifactorial chronic disease that is heavily influenced by numerous biomedical factors (e.g.: bacteria, host, genetics and diet) and by social determinants of health. The microbiota of dental caries is complex and includes acidogenic and aciduric organisms. Dental caries as an infectious, transmissible disease was first demonstrated by Keyes (1960).¹ A group

of phenotypically similar bacteria, collectively known as the Mutans Streptococci (MS), has been indicated as the primary bacterial component involved in development of dental caries in humans.^{2,3} S. mutans was more sensitive to traditional methods of detection like selective agar plate culturing, S. mutans and Lactobacillus count test and calorimetric testing. However, with the advent of PCR, many novel presumed unculturable taxa in oral infections have been identified. Recent studies have revealed that S. mutans was detected in caries free populations and was not detected in all cases of childhood caries,^{4,5} suggesting

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that there could be other species that could have cariogenic potential. Molecular methods have identified a novel pathogen that may alter the current understanding of dental caries, which is *Scardovia wiggisiae* (*S. wiggisiae*). It is anaerobic, gram positive, non-motile, non-sporing bacillus with acidogenic and aciduric properties⁶ in Bifidobacterium group, found to be associated with initiation and progression of dental caries. *Scardovia wiggisiae* has been associated with severe early childhood caries even in the absence of *S. mutans*.⁷ There is a lacunae of studies showing the trend of these organisms across various dentition periods. In addition, no study states the time of initial acquisition of this micro-organism. Understanding the acquisition and colonization of these species may be essential for developing strategies to prevent dental caries. Hence this study was aimed to evaluate and compare the presence of *S. mutans* and *S. wiggisiae* from the pre-eruptive to permanent dentition period.

2. Materials and Methods

A randomised case-control study was conducted in Bangalore (Karnataka) among 80 children aged between 0 to 14 years. They were divided into 8 groups of 10 each. Clinical examination was performed by a single examiner using mouth mirror and straight probe under the natural light. Decayed, extracted and filled surfaces (def) in deciduous teeth & Decayed, Missing and Filled surfaces in permanent teeth were counted in each subject. (according to Gruebell. A.O in 1944).

2.1. Study design

1. Group 1 included children before the eruption of tooth.
2. Group 2 included children during the first tooth eruption.
3. Group 3 included children with no caries in primary dentition with def score 0.
4. Group 4 included children with early childhood caries in primary dentition with a def score of 5 or above
5. Group 5 included children with no caries in mixed dentition with def and DMF score 0.
6. Group 6 included children with dental caries in mixed dentition with a def and DMF score of 5 or above.
7. Group 7 included children with no caries in permanent dentition with DMF score 0.
8. Group 8 included children with dental caries in permanent dentition with DMF score of 5 or above.

2.2. Sample collection

The sample was collected by one of the following methods depending upon the age of the patient.

2.2.1. Swab method

1. Saliva sample was collected in cotton swabs for infants.
2. The swabs were swept across the facial, lingual, palatal and occlusal surfaces of the maxillary and mandibular arches.
3. The swabs were stored in MTM medium and stored at 20° Celsius until taken for DNA extraction.

2.2.2. Spitting method

1. The subject was asked to accumulate unstimulated saliva in the floor of the mouth and then spit into a sterile 50ml graduated container. 2ml of saliva was collected from each subject and was stored at 20°C until taken for DNA extraction.

2.3. Microbial analysis using real-time polymerase chain reaction

1. Total genomic DNA from the saliva samples was isolated using N-Cetyl-N, N, N- trimethyl ammonium bromide (CTAB) method. The extracted DNA was qualified using Spectrophotometer (Sartorius) for standardizing the PCR.
2. The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (Vivaspec Biophotometer, Germany). The primers for quantification analysis were designed using Perkin Elmer Primer.
3. Express[®] software. The Melting temperature (T_m) was calculated and the synthesized primers were purified by HPLC. The quantified DNA was used to detect the presence of *S. mutans* and *S. wiggisiae* using the specific primers below and primer optimization was done in a gradient PCR and found the annealing temperature as 60°C (Table 1). Quantification was performed in Applied Biosystems StepOne Real Time PCR (Foster City, CA). A standard curve with highest R² value was constructed based on the values generated by the qPCR and the quantity of *S. mutans* and *S. wiggisiae* in each sample was calculated against the standard values.

2.4. Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was performed for statistical analyses. Descriptive analysis includes expression of *Scardovia wiggisiae* and *Streptococcus mutans* levels using mean and standard deviation.

3. Results

The present study showed that both *S. mutans* and *S. wiggisiae* were present in the pre-eruptive group. However, *S. mutans* was significantly higher than *S. wiggisiae* in both

the pre-dentate and first tooth eruption group at $P < 0.001$ (Table 2)

S. mutans was significantly higher in control groups than in experimental groups, whereas *S. wiggisiae* were significantly higher in experimental groups than the control groups (Table 3, Figure 1). There was a steady increase in both the organisms from pre-dentate to mixed dentition period followed by a decrease in permanent dentition (Table 3, Figure 1)

Table 1: Species specific primers

Primer		Sequence (5' - 3')
<i>S. mutans</i>	FP	GCCTACAGCTCAGAGATGCTATTCT
	RP	GCCATACACCACTCATGAATTGA
<i>S. wiggisiae</i>	FP	GTGGACTTTATGAATAAGC
	RP	CTACCGTTAAGCAGTAAG

4. Discussion

Dental caries has been included in a group of conditions like cancer or diabetes which has multifactorial etiology.⁸ The factors that can vary prevalence pattern and severity of dental caries are age, sex, race, socio-economic status, geographical location, food habit and habits of oral hygiene.⁹ The three major hypothesis that explains the etiology of dental caries are: the specific, the non-specific and the ecological plaque hypothesis.^{10–12} The specific plaque hypothesis states that only few specific species are actively involved in the disease like streptococcus mutans and streptococcus sobrinus, and on the other hand the nonspecific plaque hypothesis proposes that the dental caries is a result of overall activity of entire plaque microflora which contains numerous bacterial species.¹¹ The ecological plaque hypothesis suggests that dental caries occurs when there is a shift in the balance of resident microflora which is driven by changes in local environmental conditions.¹²

Although *Streptococcus mutans* was isolated from carious lesion by James Kilian Clarke in 1924, the genuine interest in this bacterium was generated in 1960s when researchers began studying dental caries.¹³ Ability to initiate and maintain microbial growth, continue producing acid at low pH values, rapid metabolism of sugars to lactic and other organic acids, ability to attain the critical pH for enamel demineralization more rapidly than other common plaque bacteria and ability to produce Intracellular Poly-Saccharides (IPSs) as glycogen, are some of the factors responsible for cariogenicity of *Streptococcus mutans*.^{14,15} Milgrom et al in 2000, found that children with increased levels of *S. mutans* were five times more prone to have dental caries than children with lower levels of *S. mutans*.¹⁶ However, today *S. mutans* and its involvement in dental caries is debatable. Recent studies have shown the association of *S. wiggisiae* in dental caries in the absence of

S. mutans.

Results of the present study showed both *S. mutans* and *S. wiggisiae* were present in all the study groups (Tables 2 and 3 and Figure 1). *S. mutans* was significantly higher than *S. wiggisiae* in the pre-dentate period. Though an early study did not report the presence of MS in young children before tooth eruption,¹⁷ Milgrom et al. (2000) stated that 25% of 6-month-old infant had MS colonization,¹⁶ however Wan et al (2001) found that 50% of 6-month-old pre-dentate children had MS colonization.¹⁸ The present study showed that all children in the pre-dentate group had both the organisms. The wide range of MS prevalence found among investigations may be due to differences in the sensitivity of MS detection of the various media used.¹⁹ The present study showed that *S. wiggisiae* was present in the pre-dentate period as early as 1 month. Xu et al in 2015 showed that phyla Actinobacteria had colonized all neonate's mouths by 3 or 4 days after delivery. *Bifidobacterium* belongs to phyla Actinobacteria and *Scardovia* species belong to genus *Bifidobacterium*.²⁰ However further studies are required to substantiate the time of initial acquisition of *S. wiggisiae*.

Although *S. mutans* are present in the pre-dentate period, it favors hard, non-desquamating surface for establishment of permanent colonies. Eruption of tooth provides new binding site and new ecological events take place in the oral environment, *Streptococcus mutans* accelerates its colonization at this stage,²¹ *S. wiggisiae* too had increased in number with the eruption of tooth but it was significantly lesser than *S. mutans*.

Present study also showed the trend of both the organisms across different dentition period in both caries-free and caries associated children, which revealed that both organisms increased from pre-dentate to mixed dentition period followed by a decrease in permanent dentition (Table 3, Figure 1). During the mixed-dentition phase, the oral ecosystem undergoes several changes related to the eruption of permanent teeth and onset of puberty.²² Adolescence is characterized by major hormonal changes, which is associated with nutritional enrichment of the oral environment. This event results in increase of some groups of oral microorganisms, including gram-negative anaerobes and spirochetes.²³ However, the count of both the organisms decreased in permanent dentition, this is because, as per ecological plaque hypothesis the changes in ecological factors requires different bacterial qualities and stimulates alterations in composition of the bacteria.⁴ In addition, there is improvement in oral hygiene practice in older children as compared to the younger ones, as tooth brushing skills improve with age.²⁴

The present study showed that *S. mutans* counts was significantly higher than *S. wiggisiae* in control groups across the various dentition period (Table 3, Figure 1) whereas, *S. wiggisiae* was significantly higher than *S. mutans* in experimental groups (Table 3, Figure 1). Tanner et

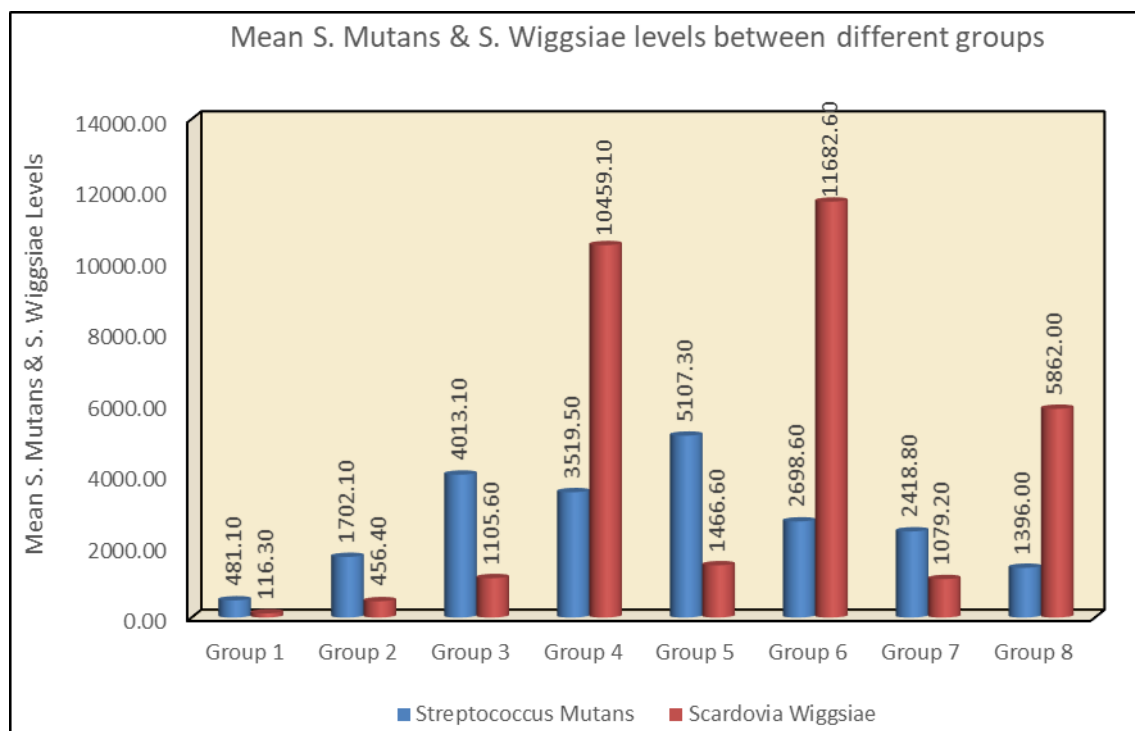
Table 2: Comparison of mean *S. mutans* & *S. wiggisiae* levels [in CFU] in Pre-dentate group and 1st Tooth Eruption

Group	Organism	N	Mean	SD	Mean Diff	P-Value
Pre-dentate	<i>S. mutans</i>	10	418.10	320.83	364.80	0.001*
	<i>S. wiggisiae</i>	10	116.30	103.05		
1 st Tooth Eruption	<i>S. mutans</i>	10	1702.10	652.99	1245.70	<0.001*
	<i>S. wiggisiae</i>	10	456.40	133.11		

*Statistically Significant

Table 3: Comparison of mean streptococcus and scardovia wiggisiae levels in control and test groups across dentitions

Dentition	Group	Organism	N	Mean	SD	Mean Diff	P-Value
Primary	Control	<i>S. mutans</i>	10	4013.10	1446.39	2907.50	<0.001*
		<i>S. wiggisiae</i>	10	1105.60	521.49		
	Test	<i>S. mutans</i>	10	3519.50	2488.59	-6939.60	<0.001*
		<i>S. wiggisiae</i>	10	10459.10	2670.35		
Mixed	Control	<i>S. mutans</i>	10	5107.30	1033.88	3640.70	<0.001*
		<i>S. wiggisiae</i>	10	1466.60	377.98		
	Test	<i>S. mutans</i>	10	2698.60	1312.79	-8984.00	<0.001*
		<i>S. wiggisiae</i>	10	11682.60	4227.02		
Permanent	Control	<i>S. mutans</i>	10	2418.80	723.28	1339.60	<0.001*
		<i>S. wiggisiae</i>	10	1079.20	155.83		
	Test	<i>S. mutans</i>	10	1396.00	667.40	-4466.00	<0.001*
		<i>S. wiggisiae</i>	10	5862.00	2581.16		

**Fig. 1:**

al in 2011 stated that *S. wiggisiae* was associated with severe ECC irrespective of the presence or absence of *S. mutans*.⁷ Vacharaksa et al in 2015, stated that the colonization with *S. wiggisiae* was related to ECC; and many caries-free children had a detectable level of *S. mutans* as part of their commensals.²⁵ Hence, presence of *S. mutans* alone may not precisely represent caries status. Present study is also in accordance with studies done by Unsal G et al 2017,²⁶ Pretika Chandna et al 2018²⁷ and Sucheta Prabhu Matondka et al 2019²⁸ which concluded a positive association of *S. wiggisiae* with ECC.

Mello de Matos- 2017 through dual-species biofilm model containing bifidobacteria and *Streptococcus mutans* revealed that Bifidobacteria in combination with *S. mutans* not only produced significant amount of acids but also accentuated acidogenicity of biofilm, as *S. mutans* metabolizes carbon sources at a higher rate, producing acids faster than bifidobacteria and lactobacilli. Both bifidobacteria and lactobacilli favors lower pH to produce acids, thus *S. mutans* promotes a favorable environment for these species to thrive. Bifidobacteria metabolizes raffinose to a higher extent than *L. acidophilus* and *S. mutans*. This is a significant finding as bifidobacteria do not require the consumption of sweets or snacks for acid production, since raffinose is present naturally in healthy foods.²⁹ In addition, *S. wiggisiae* primarily being an anaerobic bacterium can survive and continue to produce acid under more mature biofilm with low oxygen unlike *S. mutans* which is a facultative anaerobic bacterium.³⁰

Mai Kameda et al in 2020 showed that *S. wiggisiae* produces acetic acid as their major end product from a unique metabolic pathway known as F6PPK (fructose-6-phosphate pathway) shunt, whereas *S. mutans* and other caries associated bacteria produces lactic acid as their main end product.³⁰ Hoppenbrouwers et al in 1998 stated that when compared to lactic acid, acetic acid is non-ionized in low pH environments, and penetrates the enamel faster and decalcify it than lactic acid.³¹ Hence it may be concluded that *S. wiggisiae* which produces acetic acid is involved in caries progression than *S. mutans* which produces lactic acid which aids in lowering the pH and favoring other organisms like *S. wiggisiae* to thrive.

Through the bulk of evidence, it can be concluded that the relation of *S. mutans* and dental caries can be associative but not causative.³² Acid production by dental plaque is not solely dependent upon the presence of mutans streptococci; caries can occur in the absence of these species and their presence does not necessarily indicate caries activity. Other oral bacterias like non-mutans streptococci, *Actinomyces* spp. and *Bifidobacterium* spp., are acidogenic and aciduric and they outnumber mutans streptococci in dental caries.³³

The present study showed that *S. wiggisiae* was significantly higher in experimental groups whereas *S. mutans* was higher in controls suggesting that *S. wiggisiae* has a major role in development and progression of dental

caries and *S. mutans* could be the commensal.

5. Conclusion

S. mutans and *S. wiggisiae* were present in predentate period and counts of both the organisms increased from predentate to mixed dentition period and decreased in permanent dentition period. *S. wiggisiae* was significantly higher in experimental groups suggesting a major role in development of dental caries whereas, *S. mutans* was higher in control groups indicating that it could be a commensal in the oral cavity.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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