Analysis of Caries Associated Acidogenic Bacteria in Subjects with Dental Caries

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Abstract
Cariogenic Microorganisms Can Be Described By Their Ability To Colonize On Tooth Surfaces, Causing A Marked Reduction Of Ph In Presence Of A Sugar Substrate And Consequently Induce Dental Caries. Streptococcus Mutans, Streptococcus Sanguinis, Lactobacillus Casei And Actinomyces Viscosus Fulfil Most Of These Criteria. This Study Looked At The Relationship Between Mutans Streptococci, Lactobacillus, Actinomyces In Saliva Collected From Tooth Sites In Three Ethnic Groups, With Rampant Caries, Individual With Single Caries And Without Caries. All Samples Were Collected And Cultured Onto Selective Media. Colonies Were Enumerated And Identified Using Biochemical Tests. ‘Descriptive Statistical Analysis’ (I.E. Mean, Standard Deviation And Standard Error) Was Carried Out For All The Groups In This Study. A Higher Mean Microbial Count Was Observed In Individuals With Rampant Caries, Than Individuals With Single Caries And Was Least In The Control Group. Hence, Oral Microbes Are In Constant Flux But Have Interrelationships In Children With Caries.
Keywords: Streptococcus, Lactobacilli, Actinomycyes and Dental Caries.

Introduction
Dental Caries Is A Ubiquitous Disease. It Is An Irreversible Microbial Disease Of Calcified Tissues Of The Teeth, Characterized By Demineralization Of The Inorganic Portion And Destruction Of The Organic Substance Of The Tooth, Which Often Leads To Cavitations. Even Though The Prevalence Of Dental Caries In Developed Nation Is On A Decline; The Infection Is On A Rise In Developing Nations Including India. The Etiology Of Dental Caries Is Generally Agreed To Be A Complex Process Shrouded By Many Indirect Factors That Obscure The Direct Causes. Bacteria Are Thought To Play An Integral Role In Etiology Of Dental Caries. The Oral Cavity Is Home To An Assortment Of Microorganisms And Several Of Them Have Been Illustrated As Having High Cariogenic Potential Particularly The Genus Streptococcus, Lactobacillus And Actinomyces. The Role Of Candida As A Causative Organism Has Also Been Documented.

The Present Study Was Carried Out In The Department Of Oral Pathology And Microbiology, Sawangi, Wardha, Maharashtra, India On A Total Number Of 150 Patients; Aged 6-14 Years To Estimate The Total Salivary Count Of Streptococcus, Lactobacillus And Actinomycyces.

Material and Methods
This Microbial Study Was Carried Out For Quantitation Of Streptococcus, Lactobacilli, And Actinomycyces In Rampant Caries, Single Caries And Caries Free Individuals. Statistical Analysis For The Comparison Of Total Streptococcal, Lactobacillus And Actinomycyces Count In Rampant Caries/ Single Caries/ Caries Free Individuals Was Carried Out. The Saliva Sample Was Collected In The Department Of Oral Pathology And Microbiology Sharad Pawar Dental College, Wardha From Patients Ranging From 6-14 Years Of Age. The Study Samples Consisted Total of. 150 Subjects; 50 Each With Rampant Caries (Group I), Single Carious Lesion (Group II) And Caries Free Individual (Group III) Respectively. The Microbial Assessment Of Saliva In The Respective Groups Was Carried Out By Selectively Culturing Of Microorganisms In Their Selective Medium. Mitis Salivarius Hiveg Agar Base, Actinomycyces Agar, Rogosa Sl Hivegtm Agar / Broth The Quantitative Microbial Assessment Was Done By A Colony Counter.

Result
The Results Were Presented In Three Sections With Detailed Analysis Of Streptococcus, Lactobacillus, And Actinomycyces Counts In Individual Groups As Descriptive Statistics And Their Significant Differences In The Groups. The Statistical Tests Used For The Analysis Of The Result Were One Way Anova, Multiple Comparison Tukey Test, Chisquare Test.
Table 1: Comparison of Streptococci Count In Saliva Of Group I, Group II And Group III
Age Of Groups – 6 – 14 Years

Table 2: Comparison of Lactobacilli Count In Saliva Of Group I, Group II And Group III
Age Of Groups – 6 – 14 Years

Table 3: Comparison Of Actinomyces Count In Saliva Of Group I, Group II And Group III
Age Of Groups – 6 – 14 Years

Table 4: Comparison Of Mean Microbial Count In Group I / Group II / Group III
Table 5: Comparison of Identification Of Bio Types Of Streptococci In Different Groups


Table 6: Comparison of Identification Of Biotypes Of Actinomyces In Different Groups

*Interpretation:*
**Interpretation:** Predominant Actinomyces Biotype Found In Group III Was A. Odontolyticus, A. Viscosus Followed By A. Naeslundii And A. Radicidentis Was Found To Be Predominant In Group I.

**Discussion**

Both Cariogenic And Non-Cariogenic Bacteria Are Present As Commensal Microflora In The Mouth. Among Large Number Of Bacterial Species Present In Dental Plaque And Saliva; Streptococcus Mutans, Lactobacilli, Actinomyces Have Been Positively Correlated With Dental Caries. Therefore, The Purpose Of The Current Study Was To Quantify The Streptococci, Lactobacilli And Actinomyces Counts In Saliva Of Subjects With Varying Number Of Teeth Affected By Dental Caries.

In The Present Study The Colony Counts Of Streptococci In Individuals With Rampant Caries And Individuals With Single Carious Lesion Was High (i.e. Above 1,00,000 CFU/MI Of Saliva) As Compared To Caries Free Individuals. However, When The Mean Streptococcal Count Was Compared Between The Three Groups, Rampant Caries Subjects Had Higher Counts Than In Single Caries Subjects Followed By The Control Group. This Result Can Be Attributed To The Fact That The Ms Organisms Contain A Wide Range Of Traits Such As Acidogenicity And Acidurance That Confer Them With An Ecological Advantage Over Other Oral Bacteria. The Major Virulence Factor Of Ms Which Is Important In Colonization Is Their Ability To Adhere To Host Surfaces.2,3 This Property Involves A Sucrose-Independent Initial Adherence To The Acquired Salivary Pellicle, Followed By Sucrose Dependent Cellular Accumulation. Adhesins From S. Mutans (Antigen I/Ii) And S. Sobrinus (Spa A) Interact With Salivary Proteins Of The Acquired Pellicle On The Tooth Surface To Promote Bacterial Adherence.

The Lactobacillus Counts Were Observed To Be Highest In Rampant Caries, As Compared To Individuals With Single Carious Lesion. This Result Was In Accordance With Holbrook Et Al, Who Documented Similar Results. This Result Can Be Attributed To The Fact That The Adherence Property Of Lactobacilli Is Due To S Layer Produced By It; This Protein Layer Has A Crystalline Structure And Is Responsible For The Surface Hydrophobicity. Therefore, They Are Considered Secondary Invaders Rather Than Initiators Of The Caries Process But Their Propagation Could Contribute To Further Spread Of Dental Caries.4

The Mean Actinomyces Counts In Caries Free Individuals Were Higher As Compared To Single Caries And Rampant Caries. The Result Could Be Attributed To The Reason That S. Mutans Prevents The Establishment Of Actinomyces Due To The Fact That S. Mutans Could Utilize Salivary Proteins As Source Of Nutrition Allowing It To Grow Rapidly Whereas In Case Of Actinomyces They Grow Slowly Because They Cannot Utilize It, Reflecting The Influence Of Microbial Interaction On Growth Rate.

To Increase The Specificity Of Our Study We Attempted To Identify The Biotypes Of Ms Based On The Principle Of Ph Change And Substrate Utilization By Kb005a Histrepth Identification Kit Which Is A Standardized, Colorimetric Identification System Utilizing Twelve Conventional Biochemical Tests. Results Indicated That Subspecies Of S. Mutans, S. Sobrinus And S. Sanguinis In Rampant Caries Subjects Was Statistically Different From Those Obtained In Subjects With A Single Carious Lesion. S. Mutans Was Detected In Increasing Order From 36% In Control Subjects To 52% In Individuals With Single Carious Lesion And 68% In Individuals With Rampant Caries. S. Sobrinus Was Detected In Increasing Order From 16% In Control Subjects As Compared To 34% In Rampant Caries Subjects And It Was 12% In Single Carious
Lesion. While there was a decrease observed in S. Sanguinis from 26% in control subjects to 10% & 6% in single carious lesion and rampant caries subjects respectively. The higher percentage of S. Mutans found in rampant caries as compared to single caries could probably be due to higher caries incidence and high total streptococcal count in rampant caries subjects.

These findings cause an alteration in biological environment in the oral cavity i.e. acidogenicity and aciduricity, which is important for the cariogenicity of the microorganisms especially Ms. high streptococcal count specifically S. Mutans and S. Sobrinus was seen to be positive in subjects with high caries index. S. Mutans and S. Sobrinus are usually seen to be existing together. Certain different morphological colonies were detected and categorized as others; our objective was to identify only bio-types of Ms present in human hosts only.

The distinction in isolation frequency between the two is important while considering their virulence mechanism. The relationship between S. Sobrinus, dietary sucrose, plaque and formation of dental decay can be associated with glucosyltransferase (Gtf) and their resulting in glucans formation. As a result, S. Sobrinus attaches minimally and in a non-specific manner to the aep followed by accumulation of glucan formation in the presence of sucrose.\(^5,6\) According to our study S. Sanguinis was found significantly higher (i.e. 26%) in caries free individual as compared to single caries individual (10%) caries free individual (6%), resulting in demonstrating S. Sanguinis that may play an antagonistic role against S. Mutans colonization having an interactive effect between the two playing an important role in children's caries experiences. Which was in accordance with the Loesche et al., 1975; Caufield et al., 2000, Ge Y et al 2008, who observed similar results.\(^7\)-\(^9\) In the present study, the total number of actinomyces species is found to be more in caries free individuals. Results indicated that subspecies of A. Odontolyticus, A. Naeslundii and A. Viscosus in rampart caries subjects was statistically different from those obtained in caries free subjects. A. Odontolyticus was detected in decreasing order from 56% in control subjects to 20% in individuals with single carious lesion and 6% in individuals with rampant caries. A. Naeslundii was detected in decreasing order from 36% in control subjects to 28% & 0% in single carious lesion and rampant caries subjects respectively. While there was a decrease observed in A. Viscosus from 46% in control subjects to 8% & 0% in single carious lesion and rampant caries subjects respectively. A. Radicidentis was found to be in higher percentage in rampant caries 30% in comparison to single caries 12% and caries free individual 0%. This result was in accordance with the study of Sarkonen N et al., (2001)\(^10\) who documented that actinomyces odontolyticus, actinomyces naeslundii and actinomyces viscosus are the primary actinomyces species in infant’s mouth as well as in early dental plaque and saliva in adults. This result can be attributed to the fact that actinomyces species display different patterns of colonization and distribution in mouth, which may be correlated with their different affinities and binding specificities to distinct surfaces. Some actinomyces species, such as A. Naeslundii, and A. Viscosus have a superior ability to adhere to a surface with their special surface structures, type 1 and 2 fimbriae. In the present study we had also seen that A. Naeslundii and A. Viscosus with 36% and 46% respectively in control subjects indicating its excellent adherence ability. The presence of which can be considered as an indicator of a healthy mouth.\(^11\),\(^12\)

The streptococcal counts correlated positively with dental caries experience and a negative correlation was observed with lactobacillus counts and dental caries experience. The
Actinomyces Counts Correlated Negatively With Dental Caries Experience And A Positive Correlation Was Observed With The Presence Of Candida And Increase Dental Caries Experience.

**Conclusion**

Dental Caries Continues To Be A Significant Public Health Problem In Many Parts Of The World. It Can Occur In Many Forms And May Affect Single Or Multiple Teeth. Epidemiological Studies Have Shown Majority Of Human Population’s World-Wide Experiencing Dental Caries And Reports Have Been Published Indicating That Prevalence Of Dental Caries Is Associated With Increase In Certain Number.

**The Conclusions Drawn From The Study Are:**

- A Higher Mean Microbial Count Was Observed In Individuals With Rampant Caries, Than Individuals With Single Caries And Was Least In The Control Group.
- The Streptococcal And Lactobacilli Count Was Found To Be Higher In The Subjects With Rampant Caries As Compared To Subjects With Single Caries Lesion.
- The Actinomyces Count Was Significantly Higher In Caries Free Individual As Compared To Subjects With Caries.
- Candida Was Higher In Number In Rampant Caries Subjects As Compare To Others.
- High Caries Incidence Was Found To Be Associated With Higher Count Of S.Mutans And S. Sobrinus.
- S.Sanguinis Was Found More Frequently In Caries Free Individuals As Compared To Individuals With Caries.
- A.Odontolyticus, A.Naeslundii And A.Viscosus Was Found To Be In Significantly Higher Numbers In Caries Free Individual Than In Individuals With Caries.
- Among All The Actinomyces Species, A.Odontolyticus, A.Naeslundii And A.Viscosus More Frequently Isolated Than The Other Subtypes.

Therefore, On The Basis Of These Results It Can Be Concluded That The Streptococcal Counts And The Incidence Of Candida Correlated Positively With Dental Caries Experience. A Negative Correlation Was Observed With Lactobacilli Counts And An Inverse Correlation With Actinomyces Counts.

**References**