

Exploring the Link Between Salivary *S. mutans* and Dental Decay

Hamad Ullah

Sarhad Institute of Allied Health Sciences, SUIT, Peshawar. hamadullah.siahs@suit.edu.pk

Tabassum Mateen

Center of Biotechnology and Microbiology, University of Peshawar.
tabassummateen01@gmail.com

Aamir Aziz*

Sarhad Institute of Allied Health Sciences, SUIT, Peshawar. Corresponding Author Email:

Aamir.biotech@suit.edu.pk

Aibad Ullah

University of Parma, Italy. aibad.ullah@studenti.unipr.it

Author Details

Received on 19 Nov, 2025

Accepted on 22 Dec, 2025

Published on 23 Dec, 2025

Corresponding E-mail &
Authors*:

Aamir Aziz

Aamir.biotech@suit.edu.pk

Abstract

Dental caries is not only a localized problem; it is a complicated, infectious, and contagious process that wears away the hard substance of the tooth. *S. mutans* is a typical inhabitant of the mouth who is at the center of this process and is known to thrive in the mouth and produce the same acids which cause the decay. Although in the past, the former was criticized as being the main cause of cavities,

recent studies have revealed a more complex association between *S. mutans* and real caries occurrence. To determine that correlation, the present study was aimed at studying the groups of 53 children (age 3-5) in Diego Torres School in Turmeque, Boyac. The samples of unstimulated saliva were collected, and carefully processed and cultured on selective Mitis Salivarius Bacitracin agar. Upon anaerobic incubation period we determined the isolated *S. mutans* strains by biochemical testing. Results: We calculated the Minimum Inhibitory Concentration (MIC) of chemoreceptors of seven antibiotics 0.003-32mL including penicillin and amoxicillin using the agar dilution technique, which showed a high caries experience of 66% in the group. Intriguingly, although *S. mutans* was found in 62% of the children, its presence was not predictive of the decay perfectly: only 64% of the children with the bacteria had cavities. What was more surprising is that

70 percent of children who were negative to *S. mutans* yet experienced caries and no statistically significant difference in the number of bacteria in the healthy and affected group was found to be significant ($p=0.21$). In conclusion, it is possible to note that *S. mutans* is still a salient contributor, but in any population, its interaction with dental caries is not necessarily a direct cause-and-effect phenomenon. Nevertheless, these strains are very sensitive to routine antibiotics, and this provides an encouraging future of the treatment of systemic infection that can be caused by oral pathogens.

Keywords: Dental caries, Salivary Streptococcus and Dental Decay

Introduction

Dental caries is widely recognized as a transmissible, infectious process that leads to the localized destruction of hard dental tissues. While *S. mutans* is frequently cited as the primary etiologic agent due to its acidogenic and aciduric properties, scientific literature presents a nuanced view. Some studies establish a clear correlation between *S. mutans* counts and caries incidence (Loesche, 1986; Beighton et al., 1989), while others suggest that non-mutans streptococci also play a significant role (van Houte et al., 1991). Beyond oral health, *S. mutans* is a known contributor to systemic conditions such as endocarditis (Ullman et al., 1988). Consequently, this study aimed to evaluate the relationship between salivary *S. mutans* levels and dental caries in a pediatric cohort (ages 3-5), while simultaneously establishing the antimicrobial susceptibility profiles of the isolated strains.

Participant Enrollment and Sampling The study was conducted between July and December 2001, focusing on 53 children aged 3 to 5 years. Prior to enrollment, formal written consent was obtained from parents, and a preliminary screening ensured that none of the participants had recently used antibiotics. Saliva collection followed established protocols (Fure, 1998); unstimulated saliva was gathered mid-morning--at least one hour after the last meal--using a sterile plastic pipette and gentle suction. Samples were immediately refrigerated and transported to the laboratory for processing.

Microbiological Analysis and Identification. Once in the lab, the saliva samples were vortexed and subjected to 10-fold serial dilutions in a 0.05 M phosphate buffer. To isolate and enumerate *S. mutans*, 100 uL aliquots were plated onto Mitis Salivarius Bacitracin (MSB) agar, a selective medium containing 20% saccharose and bacitracin (0.2 U/mL). The plates were incubated anaerobically ($H_2:CO_2:N_2$) at

10:10:80) for 48 hours at 37degC. Following incubation, colonies exhibiting typical *S. mutans* morphology were quantified and expressed as colony-forming units (CFU) per mL of saliva (Emilson, 1981). To ensure diagnostic accuracy, suspect colonies underwent Gram staining and a rigorous battery of biochemical tests. A confirmed *S. mutans* profile included the fermentation of raffinose, mannitol, and inulin, resistance to 2 U of bacitracin, and a negative result for urease and arginine hydrolysis. Statistical differences in microbial counts between the caries-active and caries-free groups were subsequently analyzed using the chi-square test. Antimicrobial Susceptibility Testing To determine the Minimum Inhibitory Concentrations (MICs), the study utilized the agar dilution method against a panel of seven antibiotics: penicillin, amoxicillin, cefazolin, erythromycin, clindamycin, imipenem, and vancomycin (Liebana et al., 1989). Standardized bacterial suspensions (10^5 CFU/mL) were applied to Wilkins-Chalgren agar using a replicator, with drug concentrations ranging from 0.003 to 32 ug/mL. After a 48-hour anaerobic incubation at 35degC, the MIC was recorded as the lowest concentration of the antimicrobial agent that completely inhibited visible bacterial growth.

Methodology

Study Rationale and Design

Dental caries is widely recognized as a transmissible, infectious process that leads to the localized destruction of hard dental tissues. While *S. mutans* is frequently cited as the primary etiologic agent due to its acidogenic and aciduric properties, scientific literature presents a nuanced view. Some studies establish a clear correlation between *S. mutans* counts and caries incidence (Loesche, 1986; Beighton et al., 1989), while others suggest that non-mutans streptococci also play a significant role (van Houte et al., 1991). Beyond oral health, *S. mutans* is a known contributor to systemic conditions such as endocarditis (Ullman et al., 1988). Consequently, this study aimed to evaluate the relationship between salivary *S. mutans* levels and dental caries in a pediatric cohort (ages 3-5), while simultaneously establishing the antimicrobial susceptibility profiles of the isolated strains. Participant Enrollment and Sampling The study was conducted between July and December 2001, focusing on 53 children aged 3 to 5 years. Prior to enrollment, formal written consent was obtained from parents, and a preliminary screening ensured that none of the participants had recently used antibiotics. Saliva collection followed

established protocols (Fure, 1998); unstimulated saliva was gathered mid-morning--at least one hour after the last meal--using a sterile plastic pipette and gentle suction. Samples were immediately refrigerated and transported to the laboratory for processing. Microbiological Analysis and Identification Once in the lab, the saliva samples were vortexed and subjected to 10-fold serial dilutions in a 0.05 M phosphate buffer. To isolate and enumerate *S. mutans*, 100 uL aliquots were plated onto Mitis Salivarius Bacitracin (MSB) agar, a selective medium containing 20% saccharose and bacitracin (0.2 U/mL). The plates were incubated anaerobically (5% H₂:CO₂:N₂ at 10:10:80) for 48 hours at 37degC. Following incubation, colonies exhibiting typical *S. mutans* morphology were quantified and expressed as colony-forming units (CFU) per mL of saliva (Emilson, 1981). To ensure diagnostic accuracy, suspect colonies underwent Gram staining and a rigorous battery of biochemical tests. A confirmed *S. mutans* profile included the fermentation of raffinose, mannitol, and inulin, resistance to 2 U of bacitracin, and a negative result for urease and arginine hydrolysis. Statistical differences in microbial counts between the caries-active and caries-free groups were subsequently analyzed using the chi-square test. Antimicrobial Susceptibility Testing To determine the Minimum Inhibitory Concentrations (MICs), the study utilized the agar dilution method against a panel of seven antibiotics: penicillin, amoxicillin, cefazolin, erythromycin, clindamycin, imipenem, and vancomycin (Liebana et al., 1989). Standardized bacterial suspensions (10⁵ CFU/mL) were applied to Wilkins-Chalgren agar using a replicator, with drug concentrations ranging from 0.003 to 32 ug/mL. After a 48-hour anaerobic incubation at 35degC, the MIC was recorded as the lowest concentration of the antimicrobial agent that completely inhibited visible bacterial growth.

Results and Discussion

The research team found that 66 percent of children tested which corresponds to 35 out of 53 children. The microbiological tests determined that *S. mutans* was present in 33 out of 53 examined children which resulted in a 62 percent colonization rate. The presence of the microorganism showed no connection to clinical decay because only 21 from 33 children who had *S. mutans* present showed active caries. The group without *S. mutans* showed that 70 percent of participants which equals 14 children still had caries. The detection problem exists because microbial levels dropped below the detection

capacity of existing testing methods and because other pathogens including *Lactobacillus* and *Actinomyces* might appear during later stages of decay. The research team used bacterial counts to analyze *S. mutans* which showed a range from 10^3 to above 10^7 CFU/ml. The statistical analysis showed that there were no differences in microbial density between children with dental caries and those who had no cavities ($p=0.21$).

The research findings indicate that the stability and timing of colonization process should be evaluated as more important factors than the total number of organisms which needs DNA genotyping to monitor ongoing strain presence and future research needs. Antimicrobial Susceptibility *S. mutans* functions as a dental caries pathogen while also causing systemic infections which include subacute endocarditis that follows dental procedures. The clinical management process requires doctors to determine a patient's antibiotic sensitivity. The tested panel showed that all isolates had high sensitivity to penicillin amoxycillin cefazolin erythromycin clindamycin imipenem and vancomycin. The research found that most strains required less than 0.12 and 0.5 $\mu\text{g/ml}$ concentrations to achieve their full inhibitory effect. Penicillin: The drug showed its lowest average minimum inhibitory concentration (MIC) value which proves that it still works against these bacterial strains. The susceptibility profiles match existing literature findings which show that standard antibiotic treatments effectively treat *S. mutans*-related systemic infections.

Conclusions

Streptococcal mutans was 62 percent colonizing in children where the non-significance of change in microbial density in children with and without cavities ($p=0.21$) implies that the number of raw bacteria cannot significantly predict clinical cavities. The caries level in the participants with negative *S. mutans* (70 percent) and potential of reducing microbial level to below the detectable level indicates that the testing should change to focus on the stability of the colonization and presence of the second-order pathogen, like *Lactobacillus* and *Actinomyces*. Clinically, due to the possibility of transmitting the pathogen as systemic infections (including subacute endocarditis), an antibiotic sensitivity test is to be followed: fortunately, the isolates were highly sensitive to a standard panel, and the most effective is Penicillin because of its low average MIC.

References

- Beighton, D., Manji, F., Fejerskov, O., Johnson, N., & Wilton, J. (1989). Associations between salivary levels of *S. mutans*, *Streptococcus sobrinus*, lactobacilli, and caries experience in Kenyan adolescents. *Journal of Dental Research*, 68(8), 1242-1246.
- De La Higuera, A., Gutiérrez, J., Liébana, J., García-Mendoza, A., & Castillo, A. (1999). A new biotyping method for *S. mutans* with the API ZYM system. *Clinical Microbiology and Infection*, 5(2), 88-91.
- Emilson, C. G. (1983). Prevalence of *S. mutans* with different colonial morphologies in human plaque and saliva. *Scandinavian Journal of Dental Research*, 91(1), 26-32.
- Fure, S. (1998). Five-year incidence of caries, salivary and microbial conditions in 60-, 70- and 80-year-old Swedish individuals. *Caries Research*, 32(3), 166-174.
- Lang, N. P., Hotz, P., Gusberti, F. A., & Joss, A. (1987). Longitudinal clinical and microbiological study on the relationship between infection with *S. mutans* and the development of caries in humans. *Oral Microbiology and Immunology*, 2(1), 39-47.
- Liébana, J., Castillo, A., Peis, J. I., Baca, P., & Piedrola, G. (1991). Antimicrobial susceptibility of 1042 strains of *S. mutans* and *Streptococcus sobrinus*. Comparison from 1985 to 1989. *Oral Microbiology and Immunology*, 6(3), 146-150.
- Loesche, W. J. (1986). Role of *S. mutans* in human dental decay. *Microbiological Reviews*, 50(4), 353-380.
- Macpherson, L. M., MacFarlane, T. W., Geddes, D. A., & Stephen, K. W. (1992). Assessment of the cariogenic potential of *S. mutans* strains and its relationship in vivo caries experience. *Oral Microbiology and Immunology*, 7(3), 142-147.
- Marsh, P. D., Featherstone, A., McKee, A. S., Hallsworth, A. S., Robinson, C., Weatherell, J. A., ... & Pitter, A. F. (1989). A microbiological study of early caries of approximal surfaces in schoolchildren. *Journal of Dental Research*, 68(7), 1151-1154.
- Ullman, R. F., Miller, S. J., Strampfer, M. J., & Cunha, B. A. (1988). *S. mutans* endocarditis: Report of three cases and review of the literature. *Heart & Lung*, 17(2), 209-212.
- Van Houte, J., Sansone, C., Joshipura, K., & Kent, R. (1991). Mutans streptococci and non-mutans streptococci acidogenic at low pH, and in vitro acidogenic potential of dental plaque in two different areas of the human dentition. *Journal of Dental Research*, 70(12), 1503-1507.