The Role of *Actinomyces* species in oral Biofilm Formation and Dental Plaque-Related Diseases

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Abstract

Introduction: *Actinomyces* species play a major role in the development of oral biofilms and disorders associated with dental plaque. The purpose of this study was to look into their interactions, prevalence, and ability to form biofilms in dental plaque.

Methodology: Dental plaque samples from 78 participants in a six-month cross-sectional study at Khyber College of Dentistry (KCD), Peshawar, were examined for *Actinomyces* species. The microtiter plate test was used to measure the production of biofilms, and statistical analyses were performed to investigate the relationships between *Actinomyces* presence, plaque severity, and biofilm formation capacity.

Results: A total of 79.5% of samples contained *Actinomyces* species, with *Actinomyces naeslundii* being the most common (72.6%). *Actinomyces presence was found to be significantly correlated with plaque severity* ($\chi^2 = 8.92$, $p = 0.012$). In comparison to mild/moderate plaque (mean $OD_{570} = 0.52$, $t(60) = 2.78$, $p = 0.007$), isolates from severe plaque (mean $OD_{570} = 0.61$) formed more biofilms. Increased biofilm biomass was obtained through co-cultivation with *Streptococcus mutans*, particularly in combinations involving A. *israelii* and *S. mutans*.

Conclusion: *Actinomyces* species are important in the development and severity of dental plaque, with more severe cases being associated with higher biofilm-forming capacity. Their relationships to *Streptococcus mutans* increase the pathogenicity of biofilms. *Actinomyces* targeting may be able to reduce tooth plaque-related illnesses. Larger populations and biological reasons for successful interventions should be investigated in future studies.

Keywords: *Actinomyces*, biofilm formation, dental plaque, oral microbiome, *Streptococcus mutans*, periodontal diseases

Introduction

Dental caries, gingivitis, and periodontitis are just a few of the oral illnesses that are mostly caused by dental plaque, a complex biofilm that builds up on the surfaces of teeth. The genus *Actinomyces* stands out among the numerous microbial species that live in the oral cavity because it plays a vital role in the development of biofilms and the pathophysiology of disorders linked to dental plaque [1]. The precise processes by which *Actinomyces* species contribute to plaque development and disease progression are still not fully known, despite a great deal of study on oral biofilms. By examining the function of *Actinomyces* species in the oral microbiome and concentrating on their interactions with other microbial species, biofilm formation processes, and effects on oral health, this article seeks to close this research gap [2]. A complex and dynamic ecosystem of bacteria, fungi, viruses, and archaea inhabit the human oral cavity. When these microbes stick to the tooth's surface and to one another while immersed in a matrix of extracellular polymeric substances (EPS), dental plaque, a structured biofilm, is created [3]. This biofilm environment promotes bacteria survival and persistence while acting as a barrier to keep out antimicrobial agents and host immunological responses.

Gram-positive, facultatively anaerobic bacteria of the *Actinomyces* genus are frequently detected in the oral cavity of humans. They are important in the early stages of biofilm production and are among the first microorganisms to colonize the tooth surface [4]. Via fimbriae and other adhesins, these bacteria cling to the pellicle, a proteinaceous layer that develops on the surface of teeth, which promotes the aggregation of more colonists. *Actinomyces*' capacity to create biofilms is essential to both its pathogenicity and ability to survive [5].

Important processes include adhesion, a crucial stage in the creation of biofilms, whereby *Actinomyces* species have surface proteins that facilitate attachment to the tooth surface and to other microbes. Extracellular polymeric substances (EPS) produced by *Actinomyces* give the biofilm structural stability and improve microbial adhesion and cohesion inside the biofilm matrix [6, 7]. *Actinomyces* synchronize gene expression and biofilm development in reaction to population density by means of quorum sensing, a cell-to-cell communication system.

Actinomyces interacts both antagonistically and cooperatively with other microbial species in the biofilm. The toxicity and durability of biofilms may be increased by these interactions [8].

Actinomyces, for example, may co-aggregate with Streptococci, which promotes the growth of a more robust and diversified microbial population. *Actinomyces* in tooth plaque has been linked to a number of oral illnesses. Because *Actinomyces* species digest food carbohydrates to produce acids that demineralize tooth enamel, they are linked to dental caries[7]. Since they play a major role in the chronicity of gingivitis and periodontitis due to their propensity to stay in the subgingival biofilm, they are also linked to these disorders [9, 10]. *Actinomyces* is known to play a part in the production of biofilms and disorders linked to dental plaque, although the precise molecular processes and interactions involved are still unclear [11]. The complex relationships that exist between *Actinomyces* and other microbial species inside the biofilm, as well as the genetic and environmental influences on these interactions, are frequently ignored in current study.

The purpose of this article is to clarify the precise methods via which *Actinomyces* species contribute to the production of biofilms and disorders associated with dental plaque. Through an emphasis on the molecular relationships and contextual elements affecting *Actinomyces* behavior in the oral biofilm, we aim to offer a thorough knowledge that may guide specific treatment approaches.

Materials and methods

Study Design and Location
This cross-sectional study was carried out at Khyber College of Dentistry (KCD), Peshawar. The purpose of the study was to find out how *Actinomyces* species shape oral biofilms and cause disorders associated to dental plaque. The research was done from June 2023 to December 2023.

Sample Size Calculation
The population prevalence of disorders associated to dental plaque, prior research showing the average occurrence of *Actinomyces* species in dental plaque, and a 5% margin of error were used to determine the sample size. We found that 78 individuals would give enough power to identify statistically significant differences using Cochran's procedure for calculating sample size for a single percentage.

Participant Selection:
Patients coming into the KCD outpatient department made up the participants. Adults between the ages of 18 and 65 who had varied degrees of dental plaque buildup, had not used antibiotics recently (within the last three months), and had no history of systemic illnesses that might have an impact on oral health were eligible. Those with oral lesions unrelated to dental plaque, those with severe periodontal disease needing rapid attention, and those refusing to participate were excluded. The age range of 18 to 65 was likely chosen to ensure a broad representation of adult patients with varying degrees of dental plaque buildup, while excluding children and elderly individuals who may have different oral health conditions and treatment requirements.

Ethical Considerations
The Institutional Review Board of Khyber College of Dentistry gave its ethical clearance. All volunteers gave their written informed permission before being included in the study. The aim, methods, possible hazards, and advantages of the research were explained to the participants.

Sample Collection
The volunteers had their dental plaque samples taken using sterile dental curettes. All teeth had samples collected from their lingual and buccal surfaces, which were then combined and put straight into sterile transport medium. After collection, the samples were transported an hour to the KCD microbiological lab. *Actinomyces* isolation agar media designed for *Actinomyces* species was used as media for growth. The criteria for identifying *Actinomyces* species involved colony morphology, Gram staining, and biochemical tests such as catalase and urease assays. These tests help differentiate *Actinomyces* species from other bacteria based on their metabolic characteristics.

Microbial Isolation and Identification
Dental plaque samples were homogenized in the lab using a vortex and then serially diluted. Aliquots were placed onto *Actinomyces* species-selective medium and left anaerobically at 37°C for seven days to develop. *Actinomyces* species were identified by colony morphology, Gram staining, and biochemical testing (catalase and urease assays). Targeting certain *Actinomyces* genes, polymerase chain reaction (PCR) was used for molecular identification. Negative controls (wells without bacterial inoculation) and positive controls (wells with known biofilm-forming bacteria) were included to validate the assay results.

Biofilm Formation Assay
The biofilm-forming potential of isolated *Actinomyces* strains was evaluated using microtiter plate assay. Prepared bacterial suspensions were added to 96-well plates. The biofilm biomass was determined by measuring the optical density at 570 nm using a microplate reader after the wells had been cleaned, fixed with methanol, and stained with crystal violet.

Interaction Studies
Co-culture studies were carried out in order to investigate interactions with other oral bacteria. *Streptococcus mutans* and other common oral bacteria were co-cultured in biofilm models with *Actinomyces* species. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were employed to examine the development of biofilms and microbial interactions.

Data Analysis
Software for data analysis was SPSS (version 25.0). Participant clinical features and demographics were compiled using descriptive statistics. *Actinomyces* presence, biofilm forming ability, and clinical parameters were correlated using chi-square tests and t-tests. Significant statistically were P-values less than 0.05. The severity of dental plaque was assessed using clinical parameters, likely involving visual examination and standardized dental indices that measure plaque

accumulation and gingival inflammation.

Results

The trial involved 78 people in all. Between 18 and 65 years old, the participants' average age was 38.5 years (SD = 12.4). Of those, 45 were men (57.7%) and 33 were women (42.3%). Table 1 lists the three groups of individuals according to their dental plaque accumulation: mild (26 participants, 33.3%), moderate (32 participants, 41.0%), and severe (20 participants, 25.6%).

Table 1: Participant Demographics and Clinical Characteristics

Characteristic		Value
Total Participants		78
Mean Age Years (SD)		38.5 (SD = 12.4)
Age Range (years)		18 – 65
Gender Distribution	Males	45 (57.7%)
	Females	33 (42.3%)
Plaque Severity	Mild	26 (33.3%)
	Moderate	32 (41.0%)
	Severe	20 (25.6%)

62 of the 78 dental plaque samples had *Actinomyces* species isolated, for a frequency of 79.5%. *Actinomyces naeslundii* (45 isolates, 72.6%), *Actinomyces* viscosus (10 isolates, 16.1%), and *Actinomyces israelii* (7 isolates, 11.3%) were the species distributed as illustrated in figure 1.

Figure 1: Prevalence and Distribution of *Actinomyces* Species

The microtiter plate test was employed to measure the biofilm-forming ability of the isolated *Actinomyces* strains. *Actinomyces naeslundii* had the mean optical density (OD_{570}) values of 0.55 (SD = 0.12), *Actinomyces viscosus* of 0.48 (SD = 0.09), and *Actinomyces israelii* of 0.60 (SD = 0.15), for biofilm biomass. The degree of dental plaque was investigated in connection to the *Actinomyces* species present. Table 2 summaries the results.

Table 2: Distribution of *Actinomyces* by Plaque Severity

Plaque Severity	A. naeslundii	A. viscosus	A. israelii	Total Actinomyces	Total Samples	Prevalence (%)
Mild	12	3	3	18	26	69.2
Moderate	19	5	2	26	32	81.3
Severe	14	2	2	18	20	90.0

Dental plaque severity and *Actinomyces* species presence were investigated using a Chi-square test. Significant relationship was found ($\chi^2 = 8.92$, p = 0.012). As figure 2 illustrates, participants with severe plaque were more likely than those with mild or moderate plaque to have *Actinomyces* species.
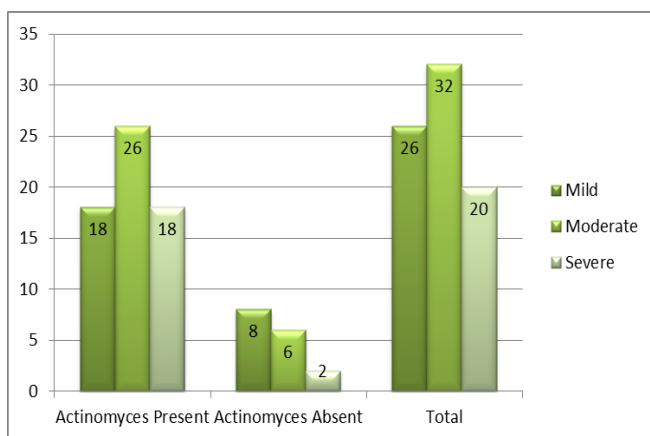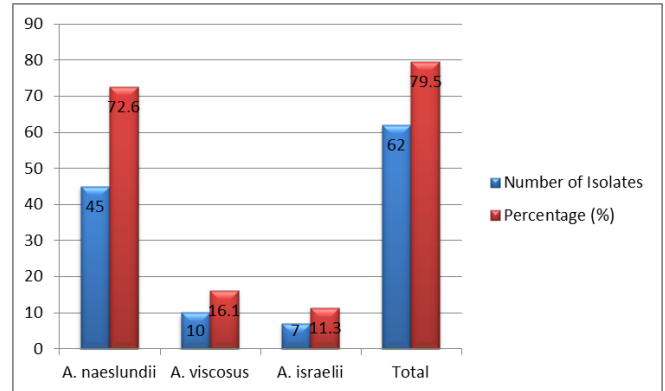

Figure 2: Association between *Actinomyces* Presence and Plaque Severity

Actinomyces strains isolated from individuals with varying plaque severities were compared using a t-test. In table 3, the mean OD_{570} values were much higher in strains from persons with severe plaque (0.61, SD = 0.13) than in those from participants with mild/moderate plaque (0.52, SD = 0.10), t(60) = 2.78, p = 0.007.

Table 3: Biofilm Formation Capacity by Plaque Severity

Plaque Severity	Mean OD570	Standard Deviation (SD)
Mild/Moderate	0.52	0.10
Severe	0.61	0.13

Actinomyces species interacted to differing degrees with *Streptococcus mutans* in co-culture studies. The following values for *Actinomyces naeslundii* + *S. mutans* biofilm biomass in co-cultures were obtained: 0.75 (SD = 0.11), 0.68 (SD = 0.09), and 0.80 (SD = 0.14). Significant variances in biofilm biomass were found between the various co-culture combinations by a one-way ANOVA test (F(2, 27) = 4.26, p = 0.023). The biofilm biomass of co-cultures of A. *israelii* + *S. mutans* was found to be considerably greater (p = 0.021) than that of co-cultures of A. viscosus + *S. mutans* (figure 3).
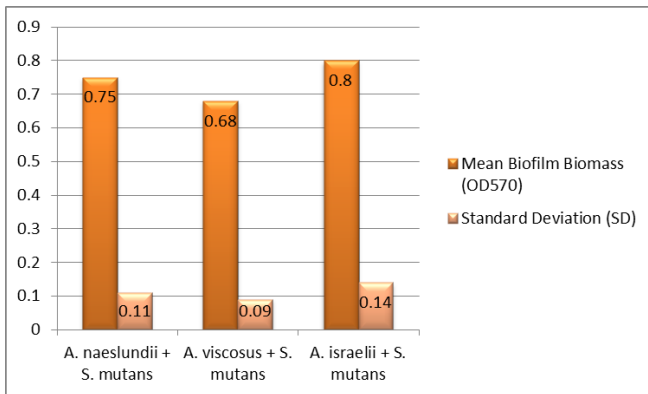
Figure 3: Biofilm Biomass in Co-cultures with *S. mutans*

Dental plaque included *Actinomyces* species with a prevalence of 79.5%. By far the most often isolated species was *Actinomyces naeslundii*. *Actinomyces* species presence was significantly correlated with tooth plaque severity. Higher biofilm-forming capabilities were shown by *Actinomyces* strains from severe plaques. In co-cultivations with *S. mutans*, *Actinomyces israelii* showed the greatest biofilm biomass. These results emphasize the vital function of *Actinomyces* species in the development of biofilms and their possible involvement in the etiology of disorders associated to dental plaque. The molecular processes underlying these interactions and their consequences for the management of oral health require more study.

Discussion

Actinomyces naeslundii was the most often isolated species (72.6%), followed by *Actinomyces* viscosus (16.1%) and *Actinomyces israelii* (11.3%), with a total frequency of *Actinomyces* species in dental plaque of 79.5% [12]. These results support earlier research demonstrating the oral cavity's preponderance of *Actinomyces naeslundii*. Similar distribution patterns of *Actinomyces* species in dental plaque have been documented in other studies, underscoring the important function that *A. naeslundii* plays as an early colonizer in the development of biofilm [13]. This work confirms the strong correlation shown in previous studies between the severity of dental plaque and the presence of *Actinomyces* species. Our findings indicated that *Actinomyces* species were more common in participants with severe plaque (90%) than in those with mild (69.2%) and moderate (81.3%) plaque. Significant statistical correlation was found (χ^2 = 8.92, p = 0.012). Similar results have been reported in the literature, indicating that because *Actinomyces* species help other harmful bacteria adhere and assemble, they are essential to the development of dental plaque and the change from health to illness [14]. *Actinomyces* strains from severe plaque cases were more able to generate biofilms than those from mild or moderate plaque cases. *Actinomyces* strains from severe plaque showed much greater biofilm biomass (0.61, SD = 0.13) than those from mild/moderate plaque (0.52, SD = 0.10), t(60) = 2.78, p = 0.007. This result is consistent with earlier study that showed stronger *Actinomyces* strains are more likely to be linked to severe types of tooth plaque and associated periodontal disorders. We found via co-culture studies

that *Actinomyces* species and *Streptococcus mutans* interact synergistically to promote biofilm biomass [15, 16]. Remarkably, co-cultures of A. *israelii* + *S. mutans* showed the greatest biofilm biomass (0.80, SD = 0.14), followed by co-cultures of A. *naeslundii* + *S. mutans* (0.75, SD = 0.11) and A. viscosus + *S. mutans* (0.68, SD = 0.09). Significant differences between these co-culture combinations were found by the one-way ANOVA test (F(2, 27) = 4.26, p = 0.023). These findings, which support those of earlier research, show that coaggregation of *Actinomyces* and Streptococcus species improve the structural integrity and pathogenic potential of oral biofilms [17, 18].

Periodontitis, gingivitis, and dental cavities are all closely associated with the presence of *Actinomyces* species, particularly those having a high biofilm-forming ability [19, 20]. Our results confirm the vital function of *Actinomyces* in the early phases of biofilm development and their participation in the tenacity and severity of dental plaque-related disorders. Targeting early colonizers like *Actinomyces* in preventative and therapeutic approaches to manage oral biofilm and associated illnesses has been stressed in earlier research.

Limitations and Future Research

The results may not be as generalizable because of the 78-person sample size and the single-location setting at KCD Peshawar. Moreover, the molecular processes underlying the interactions between *Actinomyces* species and other oral microbes were not investigated in this work. Furthermore preventing the establishment of causation between *Actinomyces* prevalence and dental plaque severity is the cross-sectional design. For future studies to improve generalizability, bigger, more varied sample sizes and multi-center studies should be included. It will take longitudinal research to determine causal links between the development of dental plaque and *Actinomyces* species. Investigating the molecular pathways via which *Actinomyces* interacts with other oral bacteria, including *Streptococcus mutans*, can also point up possible therapeutic targets for the prevention and treatment of dental plaque-related disorders.

Conclusion

This work emphasises the importance of *Actinomyces* species in the development of dental biofilm and the severity of illnesses associated to tooth plaque. At 79.5%, *Actinomyces naeslundii* was the most common species. *Actinomyces* presence and plaque severity were clearly correlated; isolates from instances of severe plaques had greater biofilm-forming capabilities. Further emphasising their pathogenic potential are the synergistic interactions between *Actinomyces* and *Streptococcus mutans*. The need of focusing *Actinomyces* species in dental plaque and related disease management plans is highlighted by these results. To create efficient preventative and therapeutic interventions, future research should concentrate on bigger, more varied populations and investigate the molecular processes underlying these interactions.

Conflict of interest
The authors state no conflict of interest.

Author Contributions

All authors contributed equally in this study, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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