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Understanding Multispecies Biofilm development on Dental Prostheses and its impact on Systemic Health: A Preliminary Investigation.

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Abstract

Background: Dental health is intricately linked to overall systemic well-being, with microbial imbalances in the oral cavity often associated with systemic disorders. Multispecies biofilm development on dental prostheses is a potential reservoir for pathogenic microorganisms, exacerbating oral health complications in individuals with systemic conditions. The study aimed to investigate the correlation between multispecies biofilm development on dental prostheses and systemic disorders, including diabetes, hypertension, cardiovascular diseases (CVD), and anxiety, using scanning electron microscopy (SEM) analysis.

Methodology: A cohort of 55 patients participated in the study, allowing for comprehensive isolation of dental prostheses followed by microbial analysis.

Results: Our findings revealed a heightened microbial presence in diabetic patients, with notable percentages in Aerobic Plate Count (APC) (80%) and Yeast Count (YC) (73%). However, correlation analysis indicated a weak association between microbial presence and diabetes. Notably, diabetic patients exhibited dominance in microbial species, including *Candida* (69.4%), *Streptococcus* (S) (68%), *Mutans* (70.2%), *Staphylococcus* (70.8%), *Actinomyces* (68.1%), and *Pseudomonas (P) aeruginosa* (74%). Hypertensive patients displayed a moderate microbial presence (APC: 12%, YC: 9.70%), with varied percentages for different microbes, correlating negatively with hypertension. Conversely, anxiety patients exhibited a modest microbial presence (APC: 4.46%, YC: 3.70%) with a negative correlation. Furthermore, cardiovascular patients demonstrated minimal microbial colonization (APC: 0.17%, absent yeast) with varying percentages for different microbes. SEM analysis of biofilms unveiled dense structures on prosthesis surfaces, comprising cocci, coccobacilli, and bacilli-shaped bacteria.

Conclusion: This research significantly advances our understanding of the microbial landscape associated with diverse health conditions, providing valuable insights for developing targeted interventions in oral health management.

Keywords

Bacteria, Biofilms, Dental Prostheses, Diabetes, Hypertension.

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Introduction

Dental health is integral to physical well-being and significantly impacts psychological conditions in individuals. Bacterial infections within the oral cavity, particularly those affecting dental implants, can profoundly disrupt systemic health. Hence, comprehending these infections' etiology, causes, and consequences is paramount for devising effective strategies to manage oral, dental, and overall health[1]. The oral microbiota typically maintains a non-pathogenic or opportunistic pathogen status, existing in a commensal relationship with the host[2,3]. However, various factors can disturb this delicate balance, fostering a transition to a pathogenic association. Suboptimal oral hygiene practices, compromised immune function, dietary habits, and underlying medical conditions are among the factors known to disrupt this commensal relationship[4,5]. Such disruptions can lead to the proliferation of pathogens, resulting in conditions such as dental caries, periodontitis, and peri-implant infections[6,7].

Opportunistic pathogens within the oral cavity, including *Pseudomonas aeruginosa*, exploit these conditions and are recognized for their diversity and adaptability in different environments[8]. Furthermore, these pathogens have the capability to form biofilms, a survival strategy enabling them to persist in a sessile state on dental crowns and other implants. Biofilms are intricate bacterial communities encased in a matrix of extracellular substances, providing protection and resilience against antibacterial agents[8-10]. Various pathogens, such as *Escherichia coli*, *Enterococci, Bacillus*, and *Streptococcus mutans*, are reported to cause biofilm-associated dental infections, highlighting the diverse range of oral pathogens posing a threat[11].

Studies have implicated these bacteria in root canal infections, endodontic failures[15,16], and other dental complications. Additionally, gram-positive pathogens like streptococci, particularly *Streptococcus mutans*, are notorious for causing dental caries across all age groups[12-15]. These bacteria metabolize dietary sugars to produce acid, leading to enamel demineralization and cavity formation.

Given the multifaceted nature of oral infections and their potential consequences, this research aims to investigate multispecies biofilms on dental prostheses sourced from diverse patient populations. Such insights are crucial for devising effective control measures to mitigate oral hygiene threats and enhance dental care practices.

Methodology

Study Design

The study employed a laboratory-based experimental design focusing on the microbial analysis of dental prosthesis samples collected aseptically from plaque-prone areas.

Sample collection

The Dental Prosthesis was aseptically isolated from adjacent tissues with the patient's consent. Using sterile dental instruments, a total of 57 samples were meticulously collected from plaque-prone areas, immediately transferred to a sterile transport medium, and transported to the lab under controlled conditions. Rigorous processing, including microbial culture and tests, yielded vital insights into oral health. Stringent hygiene measures were maintained throughout to uphold analysis integrity.

Total Bacterial Count

Tryptone Soya Agar (TSA) (OXOID) and the pour plate method were employed for total bacterial count. Aseptically collected samples were serially diluted (101 to 106) in a sterile saline solution. Diluted samples (typically 1 mL) were mixed with molten TSA, poured into sterile Petri dishes, and gently swirled for even distribution. After incubation at 35°C for 48 hours, bacterial colonies were counted, and the total bacterial count was calculated as colony-forming units per milliliter (CFU/mL) in the original sample.

Segregation of Different Isolates

The collected swabs were used to inoculate different culture media to facilitate the growth of various types of bacteria. Mannitol Salt Agar (MSA)

(OXOID) was used to identify *S. aureus*, while the TSA was used to identify the total bacterial count. Mannitol egg Yolk Polymyxin (MYP) agar (OXOID) was used for L. monocytogenes, Xylose Lysine Deoxycholate (XLD) agar (MERCK) for *Salmonella spp*, Eosin methylene blue (EMB) agar (OXOID) for *E. coli*, and Pseudomonas/cetrimide agar for *P. aeruginosa*. DRBC was used for fungi and yeast.

Identification of bacterial Isolates

The bacterial isolates were characterized and identified using standard microbiological techniques, including Gram staining, morphological examination of colony and cell shape, and cultural and biochemical tests. Biochemical tests performed included catalase, coagulase, oxidase, methyl red, Voges Proskauer, citrate reduction, indole, and sugar fermentation tests. The identification of fungal isolates involved the use of lactophenol blue stain, which was applied to a clean glass slide containing mycelia growth. The mold isolates were then examined macroscopically and microscopically to identify their characteristic features.

Scanning electron microscopy (SEM)

The Dental Prosthesis sample was carefully prepared by first fixing it in a solution, e.g., 2.5% glutaraldehyde in phosphate buffer, to preserve its structure and prevent degradation. After fixation, the sample was rinsed with phosphate buffer to remove excess fixative and then dehydrated through a graded series of ethanol solutions (e.g., 30%, 50%, 70%, 90%, and 100% ethanol). The dehydrated sample was critical, and it was dried using carbon dioxide to prevent the collapse of the delicate biofilm structures. After dehydration, samples were platinum coated by the auto-fine coater (JEC-3000FC) at 20 mA current in a vacuum for 30 s. Images were acquired using JSM IT 100 JEOL (Japan) Electron Microscope using the following parameters. The tungsten is an electron beam source with high vacuum conditions for high-resolution and visualization purposes. The images were obtained at 5 to 20 KV electron volts (depending on the sample) at a 5–10 mm working distance from the pole piece. Modes of imaging were acquired using Secondary electron detector (SEI) images.

Statistical Analysis

The statistical analysis employed the Pearson correlation coefficient (r) to quantify relationships between microbes recovered from dental prostheses and systemic disorders, ensuring a robust and quantitative examination of the data.

Ethical Considerations

The aseptic isolation of dental prosthesis from adjacent tissues was conducted solely in vitro, with no direct involvement of human subjects or patients. Prior to the commencement of the study, informed consent was not sought from individuals as no direct human participation was required. Therefore, patient confidentiality and privacy concerns are not applicable to this research. The study's focus remains on laboratory-based experimentation and does not extend to direct clinical interventions or interactions with human subjects. Any results or findings arising from this study will be presented with utmost accuracy and integrity while ensuring the protection of human subjects' rights and welfare.

Results

In the current study, 55 patients were included to collect Dental Prosthesis samples for bacterial analysis. Among these participants, 21 were male, and 36 were female (Table 1). Patient history revealed that 8.8% of them reported experiencing anxiety. Additionally, 71% of the patients had a history of diabetes, and out of these, 13.3% also had heart problems.

Distribution of APC & YC and their correlation with systemic disorders

Notably, diabetic patients exhibited the highest microbial presence, constituting a substantial 80% of the APC and 73% of the YC, as illustrated in figure 1. Correlation analysis further elucidates a weak association with both APC (p=0.191) and YC (p=0.016), indicating a statistically insignificant connection between diabetes and an elevated microbial presence on the dental prosthesis. In contrast, hypertensive patients demonstrated a

moderate microbial presence, accounting for 12% in APC and 9.70% in YC (Figure 1).

Interestingly, hypertension exhibits a negative correlation with both APC (r=-0.240) and YC (r=-0.126), suggesting a negligible but inverse relationship between hypertension and microbial colonization.

Furthermore, anxiety patients displayed a more modest microbial presence, with 4.46% in APC and 3.70% in YC, as shown in figure 1. Correlation analysis indicates that anxiety negatively correlates with APC (r=-0.153) and YC (r=-0.072), underscoring a negligible but inverse association. Intriguingly, cardiovascular patients exhibited minimal microbial colonization, with an Aerobic Plate Count of 0.17% and an absence of detectable yeast, as depicted in figure 1.

Correlation analysis for cardiovascular disease reveals negative associations with both APC (r=-0.194) and YC (r=-0.055), suggesting an insignificant inverse relationship.

Table 1: Demographic and health profile of study participants.

Variables		**N(%)**
Gender	Male	21(36.8)
	Female	36(63.2)
Age (Years); Mean±SD		44.0±13.0
Co-morbidities	Hypertension	13(22.8)
	Diabetes	41(71.9)
	CVD	3(5.3)
	Anxiety	5(8.8)

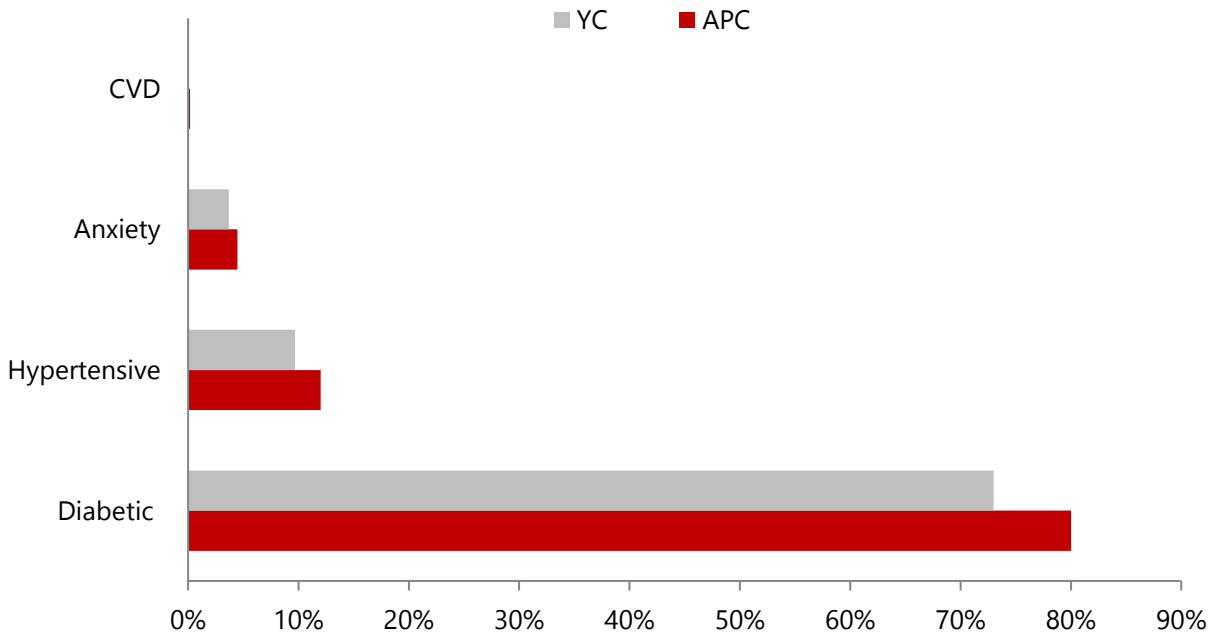

Figure 1: Distribution patterns of Aerobic Plate Count (APC) and Yeast Count (YC) in relation to systemic disorders

Detection of *Candida spp* & bacterial spp and their correlation with various disorders

Figure 2 provides a detailed overview of the microbial composition of dental prostheses among patients with distinct health conditions. In anxiety patients, varying percentages of microbial species were observed, including 13.8% *Candida*, 12.7% *Streptococcus*, 10.6% *Mutans*, 8.3% *Staphylococcus*, 11.3% *Actinomyces*, and 11.1% *P. aeruginosa*. The correlation analysis indicated non-significant positive correlations for *Candida spp*, *Streptococci spp*, and *S. mutans* (r=0.108, 0.143, 0.143, respectively), while *Staphylococci spp* and *Actinomycetes spp* showed non-significant negative and positive correlations (r=-0.036, 0.021, respectively). Notably, *P. Aeruginosa* exhibited a statistically insignificant negative correlation (r=-0.046).

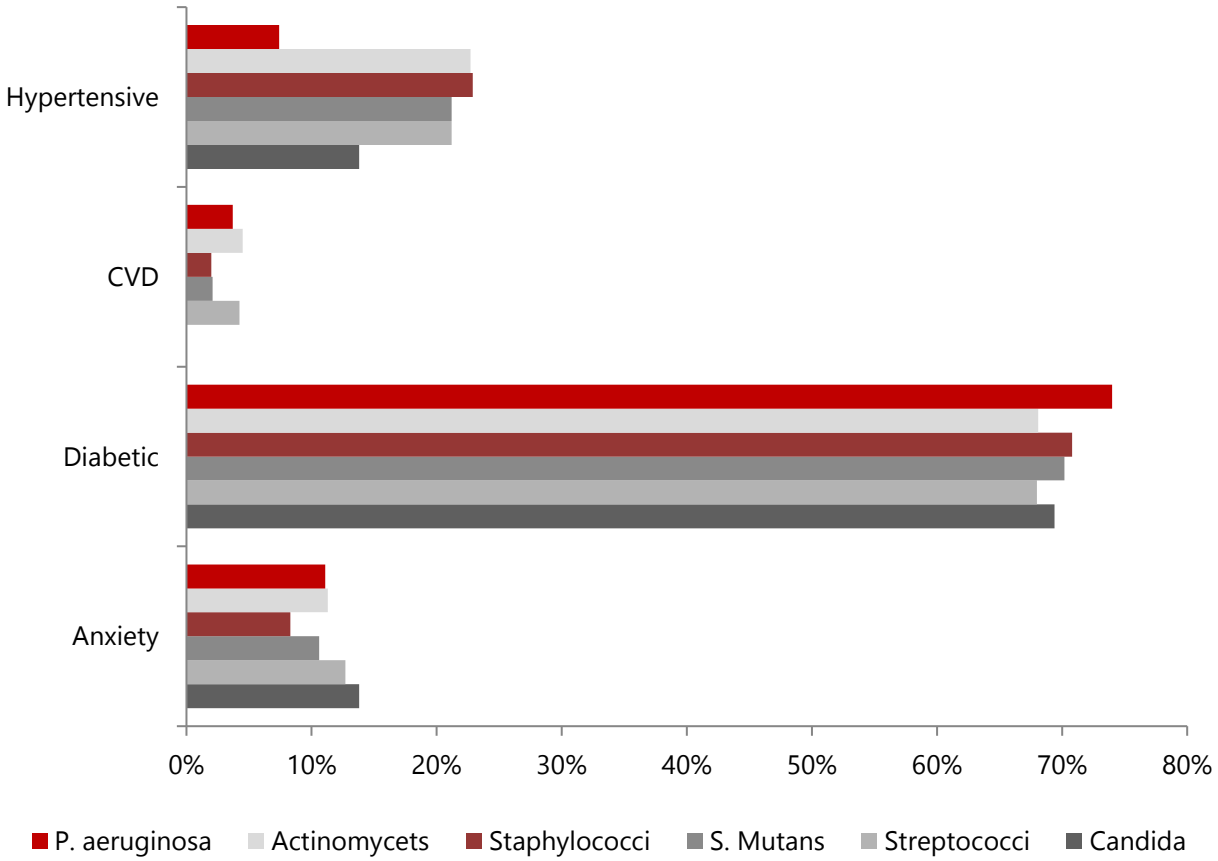

Figure 2: Distribution of *Candida spp, Actinomycetes, P. aeruginosa, Staphylococci, S. mutans* & *Streptococci* in dental prosthesis individuals

Shifting to diabetic patients, a substantial dominance of microbial species was evident, with 69.4% for *Candida*, 68% for *Streptococcus*, 70.2% for *Mutans*, 70.8% for *Staphylococcus*, 68.1% for *Actinomyces*, and 74% for *P. aeruginosa*. *Candida spp* displayed a negligible positive correlation (r=0.009), indicating an increased prevalence in diabetic patients. Conversely, *Streptococci spp, S. mutans*, and *Actinomycetes spp* exhibited insignificant negative correlations (r=-0.083, 0.020, -0.060, respectively). *Staphylococci spp* and *P. aeruginosa* showed non-significant positive and negative correlations, respectively, adding complexity to the microbial landscape associated with diabetes. Examining cardiovascular disease (CVD) patients revealed an absence of *Candida* (0.00%), along with 4.25% for *Streptococcus*, 2.10% for *Mutans*, 2.00% for *Staphylococcus*, 4.50% for *Actinomyces*, and 3.70% for *P. aeruginosa*. *Candida spp, S. mutans,* and *Staphylococci spp* in CVD

patients exhibited significant negative correlations (r=-0.309, -0.304, -0.329, respectively). On the other hand, *Streptococci spp, Actinomycetes spp*, and *P. aeruginosa* showed non-significant negative correlations (r=-0.098, -0.059, -0.066, respectively), reflecting a unique microbial profile associated with cardiovascular conditions. Hypertensive patients displayed varying percentages, with 13.8% for *Candida*, 21.2% for *Streptococcus*, 21.2% for *Mutans*, 22.9% for *Staphylococcus*, 22.7% for *Actinomyces*, and 7.40% for *P. aeruginosa*. In this group, *Candida spp* showed a non-significant negative correlation (r=-0.105), while *Streptococci spp* displayed a positive correlation (r= 0.031). *S. mutans, Staphylococci spp,* and *Actinomycetes spp* exhibited non-significant negative, positive, and positive correlations, respectively. Notably, *P. aeruginosa* demonstrated a statistically significant negative correlation (r=-0.264), suggesting potential implications for hypertensive-related microbial dynamics.

SEM analysis of Biofilms on Dental Prosthesis

In this study, SEM was employed to conduct a detailed analysis of the biofilm structure, providing valuable insights into the spatial arrangement, morphology of microbial constituents, and the extracellular polymeric matrix that collectively constitutes the biofilm architecture. A closer look at the scanning electron microscopy (Figure 3) of these implants revealed that their surfaces were covered with dirt and debris, along with the presence of cocci, coccobacilli, and bacilli-shaped bacteria. All these elements together formed dense biofilm structures on the Dental Prosthesis.

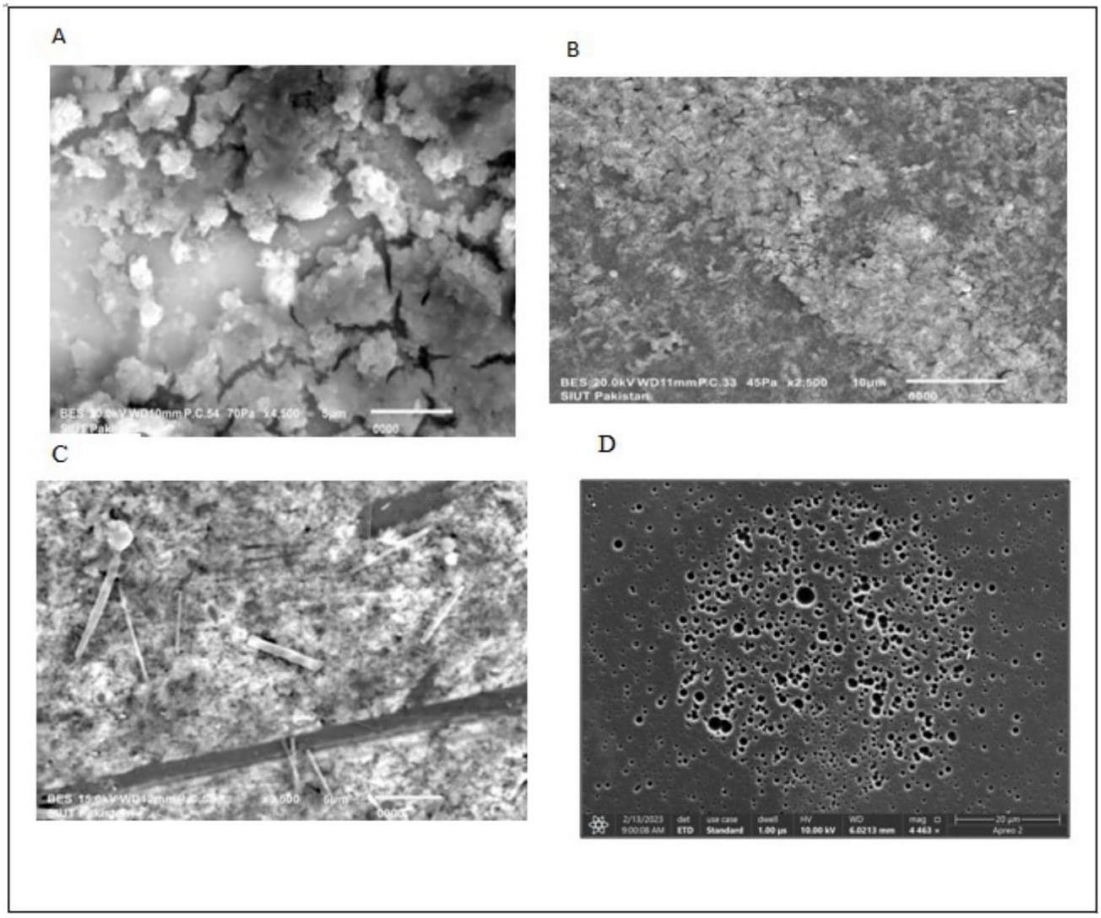

Figure 3: A, B, and C images show scanning electron micrographs of biofilm on dental prosthesis. Image D depicts the clean dental prosthesis.

Discussion

The findings of this study shed light on the association between microbial presence on dental prostheses and systemic disorders, revealing notable insights into the oral microbiome in various health conditions. Notably, diabetic patients exhibited the highest microbial presence, particularly evident with an 80% APC and a 73% YC. While correlation analysis indicated a weak association, the significant dominance of *Candida* underscores the relevance of specific microbial entities in the context of diabetes-related oral health complications[23-25]. These findings are consistent with prior literature linking oral health to diabetes complications, thereby contributing to the growing body of evidence supporting a link between diabetes and altered oral microbial composition.

Moreover, the substantial prevalence of *S. mutans*, *Staphylococcus, Actinomycetes,* and *P. aeruginosa* on dental prostheses, as evidenced by our study, aligns with previous research[26-28], indicating a distinct microbial landscape associated with diabetes. This observation further underscores the potential influence of diabetes on oral microbial dynamics.

In contrast, hypertensive patients displayed moderate microbial presence, with an inverse correlation with hypertension, adding complexity to our understanding of the oral microbiome in hypertensive conditions. This finding complements earlier studies suggesting a potential interplay between hypertension and oral microbial dynamics, highlighting the intricate relationship between systemic health and oral microflora.

Our study also revealed variations in microbial prevalence among anxiety patients, with a modest microbial presence and a negative correlation, emphasizing the interconnectedness of mental health and oral microbiota[26-28]. Additionally, CVD patients displayed relatively low bacterial microbial colonization and an absence of detectable yeast, suggesting a potential protective effect of cardiovascular health or medications on oral microbial colonization.

Furthermore, SEM analysis provided valuable insights into the biofilm structure of dental prostheses, elucidating dynamic associations among cocci, coccobacilli, and bacilli-shaped bacteria within multispecies cell aggregates. These findings underscore the importance of doctor-patient coordination in preventing oral health complications and emphasize the need for tailored oral hygiene measures in patients with systemic disorders.

Overall, this study contributes to our understanding of the complex interactions between systemic health conditions and the oral microbiome, highlighting the need for interdisciplinary approaches to oral health management.

Conclusion

It is concluded that systemic disorders have a significant impact on oral microbiota, infectious diseases, and biofilm development. Consequently, patients with such conditions are prone to developing other complications, such as multispecies biofilm on dental prostheses.

Conflicts of Interest

The Author(s) declare no conflicts of interest.

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