Evaluation of Microbial Colonisation on Healing Caps of Implants in Full Arch Rehabilitations Using Culture Media - An in vitro microbial analysis

Ganesh S*[1], Dr. Vaishnavi Rajaraman[2], Dr. Dhanraj M Ganapathy[3], A. S. Smiline Girija[4]

[1]Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600 077, Tamil Nadu, India.

[2]Assistant Professor, Department of Prosthodontics, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600 077, Tamil Nadu, India.

[3]Professor and Head, Department of Prosthodontics, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600 077, Tamil Nadu, India.

[4]Associate Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600 077, Tamil Nadu, India.

Corresponding Email: 151901083.sdc@saveetha.com

Abstract:

Background: Dental implants are a common treatment option for replacing lost teeth and edentulous instances, but their success depends on the peri-implant tissues being free of inflammation. It is important to keep in mind that some characteristics of dental implants, such as roughness, are essential for the growth of bacterial biofilms, the main pathogen source for peri-implantitis. **Aim:** This study aimed to investigate the polymicrobial flora inhabiting the healing caps of Full Arch Rehabilitations and their potential implications for the overall healing process and implant success. **Material & Methods:** For this study, 100 swabs were collected from the healing caps of complete denture implants placed patients under the age group of 20-40. The swabs were cultured in blood agar, MacConkey agar and nutrient agar. Then the plates were incubated at 37°C for 24 hours. **Results:** Microscopic examination of the bacterial samples revealed that the organisms were spherical in shape, consistent with the morphology of cocci. **Conclusion:** From the study we conclude that the most prevalent organisms on healing cap is streptococcus species. Knowing the load of bacteria colonized in the oral cavity in dental implant patients can improve and optimize the maintenance care and warrant the survival and longevity of the dental implant.

Keywords: Blood agar, Full-Arch Rehabilitations, Implant Healing Caps, MacConkey agar, Nutrient agar, Streptococcus species.

INTRODUCTION

Dental implants have revolutionized oral rehabilitation, offering a predictable and effective solution for patients with edentulous jaws. In implant therapy, the healing phase is crucial for successful osseointegration and long-term implant stability (1). During this phase, healing caps are

used to protect the implant fixtures and promote tissue healing. However, the oral environment is inherently rich in microorganisms, which can colonize the healing caps and potentially affect the surrounding tissues, leading to complications such as peri-implant mucositis or peri-implantitis (2). Understanding the microbial flora in this context is essential for improving clinical outcomes.

The oral cavity harbors a complex polymicrobial community, including both commensal and pathogenic species (3). Among these, Gram-positive cocci, particularly Streptococcus species, and Gram-negative rods are commonly encountered (4). The use of selective culture media, such as MacConkey agar for Gram-negative bacteria, aids in isolating and identifying specific microbial species. Evaluating the microbial composition on healing caps provides insights into potential risk factors for infection and helps in devising strategies for implant maintenance and infection control (5).

In vitro studies are pivotal for analyzing microbial colonization under controlled conditions. By simulating the oral environment, these studies allow researchers to investigate the growth patterns, diversity, and interactions of microbial species (6). The use of culture media such as MacConkey agar enables precise identification of organisms and their behavior on implant surfaces. This approach not only provides a better understanding of microbial dynamics but also helps in assessing the effectiveness of antimicrobial treatments and modifications in implant designs (7).

This study aims to evaluate the microbial growth on healing caps of implants placed in completely edentulous jaws for full arch rehabilitations, with a focus on Streptococcus species and Gram-negative bacteria . Using in vitro methods and selective culture media, the research seeks to identify key microbial players during the healing phase and their potential implications for implant therapy. The findings of this study will contribute to developing targeted strategies for minimizing microbial colonization and ensuring the success of full-arch rehabilitations.

MATERIALS AND METHODS

Study design

This is an in vitro observational study aimed at evaluating the microbial growth on healing caps of dental implants placed in full arch rehabilitations.

Sample Collection

For this study, 100 swabs were collected from the healing caps of complete denture implants placed patients under the age group of 20-40 years of age from the Department of Implantology, Saveetha Dental College and Hospitals, Chennai, India.

Plaque samples were collected in swabs from the healing caps of implants placed in edentulous patients undergoing full-arch rehabilitations. These samples were taken aseptically to prevent contamination and stored in sterile transport media until processing.

Inclusion Criteria

1. Patients with completely edentulous jaws who received implant-supported full-arch prostheses.
2. Implants with healing caps in place for at least 7 days.
3. No active signs of peri-implantitis or other oral infections during sample collection.

Exclusion Criteria:

1. Patients with systemic conditions affecting microbial composition (e.g., diabetes or immunosuppression).
2. Recent use of antibiotics or antiseptic mouthwashes within the past 3 months.
3. Implants with failed osseointegration or mobility.

Sample Testing

The swabs were cultured in blood agar, MacConkey agar and nutrient agar.

1. Nutrient Agar: Used for baseline microbial analysis It acts as a foundational medium to assess the overall microbial diversity and abundance in a given sample. This provides an overview of bacterial growth before applying more selective media like blood agar or MacConkey agar.
2. Blood Agar: Used to assess hemolytic activity and isolate beta-hemolytic Streptococcus species.
3. MacConkey Agar: Used to detect Gram-negative bacteria commonly associated with oral infections.

The plates were then incubated at 37°C for 24 hours. The grown colonies were gram stained and viewed under microscope. The microbial colonies were examined microscopically to identify bacterial morphology. Gram staining was performed to classify bacteria as Gram-positive or Gram-negative. Beta-hemolysis observed on blood agar was used as an indicator of pathogenicity.

Data Analysis

The type and prevalence of microorganisms were recorded. Descriptive analysis was used to present the microbial profile. Comparisons were drawn to highlight the presence of any specific pathogen species and their potential implications in peri-implant health.

RESULTS

The study identified microbial colonization on the healing caps of implants in completely edentulous jaws with implant supported full arch rehabilitations using culture-based (Figure 1) and microscopic methods (Figure 2).

1. Microscopic Analysis: Microscopic examination of the bacterial samples revealed that the organisms were spherical in shape, consistent with the morphology of cocci (Figure 2A).

2.Gram Staining: It confirmed that these bacteria were Gram-positive (Figure 1B), suggesting their potential identity as members of the Streptococcus genus, a common group of oral bacteria.

2. Hemolytic Activity:

The bacterial colonies were cultured on blood agar to assess hemolytic properties. The colonies exhibited beta-hemolysis, characterized by a clear zone around the colonies due to the complete lysis of red blood cells (Figure 1C). Beta-hemolysis is a hallmark of pathogenic Streptococcus species, further supporting the identification of the bacteria.

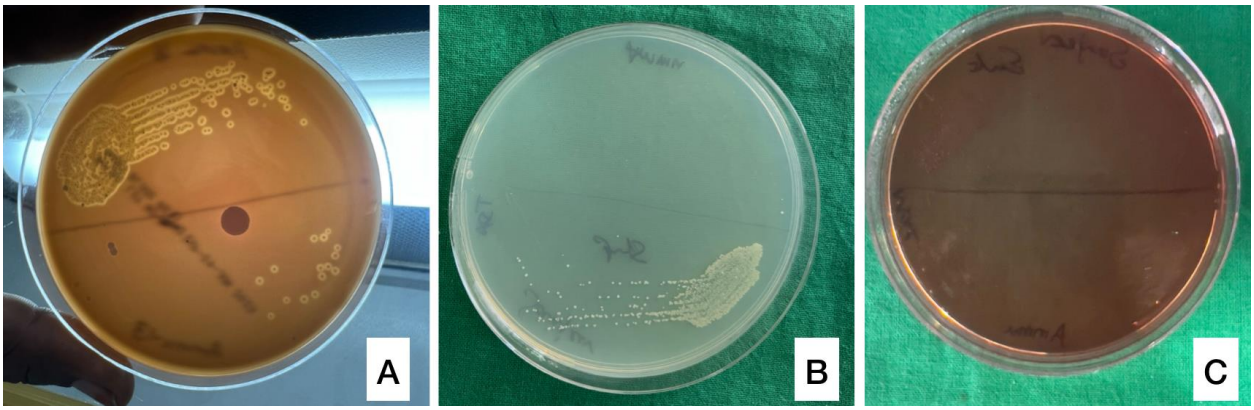

Figure 1: Colonies grown on different culture media: A- Blood agar; B-nutrient agar; C-MacConkey agar

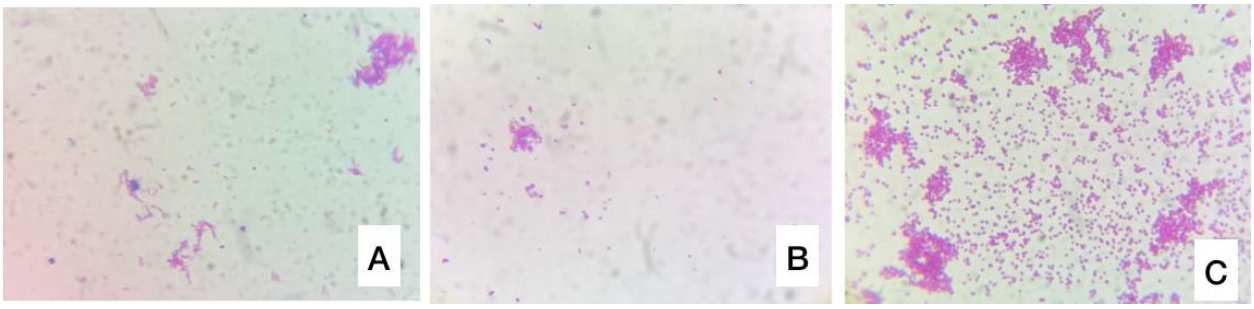

Figure 2: Gram staining shows gram positive Streptococcus in chain suggestive of Streptococcus species: A- Nutrient agar; B-Mac Conkey agar; C- Blood agar

The microbial colonization analysis revealed a predominance of Streptococcus species, accounting for 80% of the total isolated bacteria. Other microbial species identified included Actinomyces 9%, Porphyromonas 4%, Fusobacterium 2%, Prevotella 2%, Miscellaneous Gram-positive cocci

3%. The high prevalence of Streptococcus species suggests their critical role in initial biofilm formation on healing caps. While many Streptococcus species are commensal, their dominance may facilitate the secondary colonization of pathogenic anaerobes like Porphyromonas and Fusobacterium, which could potentially lead to peri-implant complications.

DISCUSSION

The identification of spherical, Gram-positive bacteria observed microscopically, coupled with their beta-hemolytic properties on blood agar, strongly suggests the presence of Streptococcus species in the oral cavity and on the surface of healing caps in dental implants (8). These bacteria are known for their significant role in the oral microbiome, often being part of the commensal flora. However, under certain conditions, Streptococcus species can become opportunistic pathogens, leading to peri-implant infections. The ability of these bacteria to colonize implant surfaces emphasizes the importance of microbial management during the healing phase of implant therapy (9).

The results highlight the importance of the absence of inflammation in the oral cavity and peri-implant tissues for the success of implant treatments. Peri-implant tissues are particularly vulnerable to colonization by opportunistic microorganisms, which can compromise the healing process and long-term success of the implant (10). The beta-hemolytic activity observed indicates the potential virulence of the bacterial strain, as hemolysis is a hallmark of pathogenicity in certain Streptococcus species. This reinforces the need for dentists to closely monitor microbial colonization during and after implant placement.

Peri-implant infections are often linked to microbial plaque biofilms formed on implant surfaces. The study underscores the role of plaque in initiating peri-implantitis, a condition that could significantly impair implant longevity (11). The presence of Streptococcus species in plaque samples further points to the importance of regular plaque control and oral hygiene measures. Understanding the microbial composition of the oral cavity and its dynamic changes during implant treatment can help in early detection and prevention of infections, thereby enhancing treatment outcomes (12).

From a clinical perspective, these findings provide valuable evidence for dentists to implement targeted strategies in post-implant care (13). Regular microbiological assessments, along with appropriate oral hygiene instructions for patients, could minimize the risk of bacterial colonization on implant surfaces. Additionally, the use of antibacterial agents or coatings on implant materials may further reduce the risk of peri-implant infections. Such interventions would directly contribute to ensuring the longevity and stability of the implants (14).

However, this study has certain limitations. Firstly, as the investigation was conducted in vitro, it does not fully replicate the complexities of the oral environment in vivo, such as salivary flow, host immune responses, and microbial interactions within biofilms (15). Secondly, the reliance on culture media such as blood agar and MacConkey agar may not detect all microbial species, particularly anaerobic bacteria or fastidious organisms that require specialized conditions for growth. Future studies should incorporate advanced molecular techniques, such as 16S rRNA sequencing, to provide a more comprehensive understanding of the microbial communities involved (16).

Finally, the study emphasizes the broader significance of understanding the microbial patterns in patients undergoing implant therapy. Evidence-based conclusions derived from microbial investigations equip dentists with critical insights for personalized patient care. By focusing on the specific pathogens involved, clinicians can adopt a preventive approach to address potential complications, thus safeguarding the success of implant treatments. Future research could explore advanced diagnostic tools and antimicrobial strategies to further optimize post-implant maintenance and improve patient outcomes.

CONCLUSION

From the study we conclude that the most prevalent organisms on healing cap is streptococcus species. Knowing the load of bacteria colonized in the oral cavity in dental implant patients can improve and optimize the maintenance care and warrant the survival and longevity of the dental implant. Future research should focus on validating these findings in larger cohorts, exploring additional microbial components, and investigating the functional implications of the observed shifts for improved clinical outcomes in implant dentistry.

AUTHOR CONTRIBUTION

Concept and design: Dr. Vaishnavi Rajaraman, Dr. Dhanraj M Ganapathy.
Acquisition, analysis, or interpretation of data: Ganesh S, A. S. Smiline Girija
Drafting of the manuscript: Ganesh S, Dr. Vaishnavi Rajaraman.
Critical review of the manuscript for important intellectual content: Dr. Vaishnavi Rajaraman

CONFLICT OF INTEREST: The author declares that there is no conflict of interest in the present study.

ACKNOWLEDGEMENT

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

REFERENCE

1. Lang NP, Berglundh T, Giannobile WV, Sanz M. Lindhe's Clinical Periodontology and Implant Dentistry, 2 Volume Set. John Wiley & Sons; 2021. 1369 p.
2. Rajasekar A, Varghese SS. Frequency of red complex microorganisms in dental implants with sandblasted acid etched and anodized surface by polymerase chain reaction. J Osseointegr. 2023 Oct 23;15(4):228–32.
3. Rajasekar A, Varghese SS. Microbiological Profile in Periodontitis and Peri-Implantitis: A Systematic Review. JLT [Internet]. 2022 [cited 2024 Dec 31];32(4). Available from: https://www.dl.begellhouse.com/journals/1bef42082d7a0fdf,2cfe86057422e688,4efd68d00a5a8b7d.html
4. Shahabouee M, Rismanchian M, Yaghini J, Babashahi A, Badrian H, Goroohi H. Microflora around teeth and dental implants. Dent Res J (Isfahan). 2012 Mar;9(2):215–20.
5. Sushma B, Rajaraman V, Ariga P, Vellingiri M, Sekaran S. Assessing dental implant biomaterial awareness across Indian sectors: A multi-city survey with emphasis on hafnium utilization. LPI. 2024 Oct 22;44(3):15175–82.
6. Leonhardt A, Berglundh T, Ericsson I, Dahlén G. Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. Clin Oral Implants Res. 1992 Sep;3(3):112–9.
7. Guarnieri R, Rappelli G, Piemontese M, Procaccini M, Quaranta A. A Double-Blind Randomized Trial Comparing Implants with Laser-Microtextured and Machined Collar Surfaces: Microbiologic and Clinical Results. Int J Oral Maxillofac Implants. 2016 Sep-Oct;31(5):1117–25.
8. Rajasekar A, Varghese SS. Bacterial Profile Associated with Peri-Implantitis: A Systematic Review. JLT [Internet]. 2023 [cited 2024 Dec 31];33(3). Available from: https://www.dl.begellhouse.com/journals/1bef42082d7a0fdf,0d41c3904f13435f,4ac5e8965566cd26.html
9. Wadhwani V, Ganapathy D, Rajaraman V, Maiti S. Knowledge, Attitude and Perception Regarding the Financial Aspects of Prosthetic Rehabilitation via Complete Denture. 2023 Jan 19 [cited 2024 Dec 30]; Available from: https://hdl.handle.net/10481/79958
10. Herekar M, Sethi M, Prithviraj DR, Bhat K, Fernandes A, Patil V. A Clinical Study Evaluating Changes in the Microbial Flora Around Dental Implants During Various Stages of Implant Restoration. Implant Dent. 2015 Oct;24(5):527–32.
11. Vickery K. Microbial Biofilms in Healthcare: Formation, Prevention and Treatment. MDPI; 2020. 166 p.
12. Yalavarthi S, Nallaswamy D, Jain A. Resistance to oral pathogens among dentures wearers. J Pure Appl Microbiol. 2018 Jun 30;12(2):961–7.
13. Swetha S, Jain A. Assessment of microbial growth and salivary pH in patients wearing fixed partial denture. J Pure Appl Microbiol. 2017 Dec 30;11(4):1925–8.
14. Ogata Y. Risk Factors for Peri-implant Diseases. Springer Nature; 2020. 119 p.
15. Son M, Song Y, Yu Y, Kim SY, Huh JB, Bae EB, et al. The oral microbiome of implant-abutment screw holes compared with the peri-implant sulcus and natural supragingival plaque in healthy individuals. J Periodontal Implant Sci. 2023 Jun;53(3):233–44.
16. Arora O, Ahmed N, Siurkel Y, Ronsivalle V, Cicciù M, Minervini G. A comparative evaluation of physical properties of CAD/CAM complete denture resins- an in vitro study. BMC Oral Health. 2024 Jan 10;24(1):1–7.