Common microorganism in cleft patients and inhibtion using Antimicrobial peptide (AMP)

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Abstract :

Background : The oral microbiome comprises a complex community of benign and pathogenic microorganisms, with over 700 bacterial species identified. In individuals with cleft lip and/or palate (CLP), anatomical variations may contribute to an altered microbial composition, potentially increasing their susceptibility to infections and systemic diseases. However, current literature on the resident bacterial flora in CLP patients remains incomplete. This study aims to identify the common microorganisms present in CLP patients and explore the inhibitory role of antimicrobial peptides (AMPs) in controlling these pathogens. **Materials and Methods :** Samples were collected from individuals with cleft palate or cleft lip and palate, including swabs from the affected oral regions and saliva specimens. Various culture media, such as nutrient agar, blood agar, and selective media, were utilized to promote the growth and identification of specific bacterial species. The study focused on determining the predominant microorganisms in CLP patients and assessing their susceptibility to antimicrobial peptides. **Results and Discussion :** The findings indicate the presence of common pathogenic microorganisms in the oral cavities of CLP patients, which may contribute to an increased risk of infections and related complications. Antimicrobial peptides (AMPs), small molecular peptides involved in innate immunity, exhibit broad-spectrum antimicrobial activity against bacteria, fungi, parasites, and viruses. Their ability to inhibit pathogenic microorganisms suggests a potential therapeutic approach for managing oral infections in CLP patients. Further research is needed to explore the efficacy of AMPs in clinical applications and their role in preventing systemic diseases associated with oral microbiome imbalances.

Introduction

The oral cavity harbors a diverse and complex microbiome, comprising both commensal and pathogenic microorganisms. More than 700 bacterial species have been identified, coexisting in a dynamic balance that plays a crucial role in maintaining oral and systemic health (Zaura et al., 2009). However, individuals with cleft lip and/or palate (CLP) may present with an altered microbiome due to their unique anatomical and physiological characteristics, which can predispose them to microbial dysbiosis and increased susceptibility to infections (Zhou et al., 2018). Despite advancements in cleft management, the existing literature on the resident bacterial flora in the oropharyngeal cavities of CLP patients remains incomplete, necessitating further investigation into the potential microbial implications for systemic health.

The oral and nasal structures of CLP patients exhibit abnormalities that may create an environment conducive to microbial colonization and infection. Due to impaired anatomical barriers, these individuals often experience increased nasal regurgitation, difficulties in maintaining oral hygiene, and a higher likelihood of food retention, all of which contribute to microbial proliferation (Costello et al., 2014). Moreover, the presence of oronasal fistulas in some CLP patients further facilitates bacterial migration between the oral and nasal cavities, increasing their susceptibility to infections (Britton et al., 2014). This altered oral ecology may predispose CLP patients to an elevated risk of systemic diseases, including respiratory infections, gastrointestinal disturbances, and even cardiovascular complications, as the oral microbiome is closely linked to systemic health (Han & Wang, 2013).

Several microorganisms have been frequently identified in the oral cavities of cleft patients, raising concerns about their potential impact on both oral and general health. Among these, Streptococcus mutans is one of the primary contributors to dental caries, a common oral health issue in CLP individuals due to their increased challenges in maintaining oral hygiene (Li & Tanner, 2015). Staphylococcus aureus, a well-known opportunistic pathogen, is frequently isolated from the nasal and oral cavities of cleft patients, predisposing them to recurrent skin and soft tissue infections (Valenza et al., 2014). Additionally, Candida albicans, a fungal pathogen, has been reported in higher prevalence among CLP patients, leading to oral thrush and other opportunistic fungal infections, particularly in those with compromised immune responses or prolonged antibiotic use (Morris et al., 2017).

Given these microbial concerns, there is an increasing interest in exploring alternative therapeutic interventions, such as antimicrobial peptides (AMPs), for managing microbial infections in CLP patients. AMPs are naturally occurring small molecular peptides that play a crucial role in innate immunity, offering broad-spectrum antimicrobial activity against bacteria, fungi, parasites, and viruses (Hancock & Sahl, 2006). Their potential application in CLP patients could provide a promising strategy for reducing microbial colonization and preventing associated complications.

This study aims to evaluate the common microorganisms present in CLP patients and investigate the potential role of AMPs in inhibiting these pathogens, ultimately contributing to better oral and systemic health outcomes.

Materials and methods :

Materials and Methodology

Sample Collection

Samples were collected from individuals diagnosed with cleft palate or cleft lip and palate. Collection methods included swabbing the affected oral regions and obtaining saliva samples. Aseptic techniques were strictly followed to prevent contamination. Sterile swabs were used to collect samples, which were immediately transferred into transport media or sterile containers for further microbiological analysis.

Growth Media and Culture Conditions

To facilitate the growth and identification of microorganisms, various growth media were used, including:

- **Nutrient agar** for general bacterial growth.
- **Blood agar** for detecting hemolytic activity and culturing fastidious organisms.
- **Selective media** (e.g., Mannitol Salt Agar for *Staphylococcus aureus* and Mitis Salivarius Agar for *Streptococcus mutans*) to isolate specific bacterial species.

The inoculated plates were incubated at optimal growth conditions (typically 37°C for 24–48 hours) under aerobic and anaerobic conditions, depending on the microorganism being cultured.

Microbiological Tools and Equipment

Standard microbiological equipment was utilized, including sterile swabs, pipettes, test tubes, Petri dishes, an incubator, and an autoclave for sterilization purposes.

Isolation and Identification of Microorganisms

Following incubation, isolated bacterial colonies were observed for morphological characteristics such as shape, color, texture, and hemolysis patterns. Further biochemical tests were performed for identification:

- **Catalase test** to differentiate between catalase-positive (*Staphylococcus* species) and catalase-negative (*Streptococcus* species) bacteria.
- **Coagulase test** for differentiating *Staphylococcus aureus* from coagulase-negative staphylococci.
- Additional tests, such as Gram staining and carbohydrate fermentation tests, were conducted as needed for precise bacterial characterization.

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Antimicrobial Peptide (AMP) Susceptibility Testing

Antimicrobial peptides (AMPs), either natural or synthetic, were obtained or synthesized for testing their inhibitory activity against isolated microorganisms. The minimum inhibitory concentration (MIC) of each AMP was determined using:

- **Broth microdilution assay**, where serial dilutions of the AMPs were prepared, and bacterial suspensions were exposed to different concentrations to assess microbial growth inhibition.
- **Agar diffusion assay**, where filter paper discs or wells containing AMP solutions were placed on inoculated agar plates, and zones of inhibition were measured after incubation.

AMP Inhibition Assay

To further evaluate AMP efficacy, the following methods were employed:

- **Agar diffusion assay** to observe clear zones of inhibition around AMP-impregnated discs.
- **Time-kill assays**, where bacterial cultures were exposed to AMPs, and microbial growth was monitored at different time points to assess bactericidal or bacteriostatic effects.

Data Analysis

The antimicrobial activity of AMPs was analyzed by calculating MIC values and measuring inhibition zones. Comparisons were made between different AMPs to determine their relative effectiveness against the isolated microorganisms. Statistical analysis was performed where applicable to evaluate the significance of differences in antimicrobial activity.

Results:

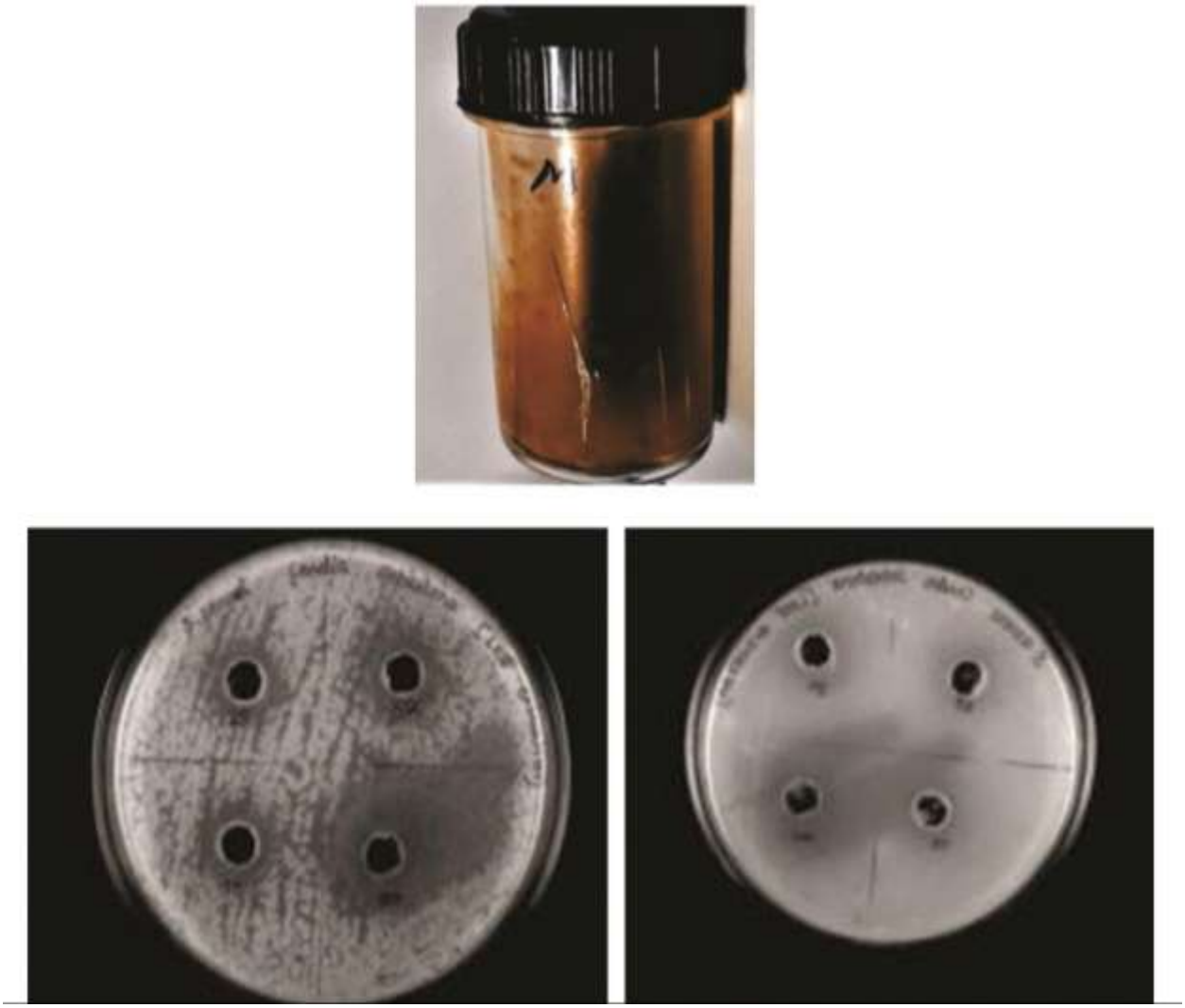

Figure 1: Common microorgansims in cleft patients using Antimicrobial peptides AMP

Identification of Common Microorganisms in Cleft Palate Patients

The microbiological analysis of collected samples revealed the presence of several common pathogenic microorganisms in the oral cavities of cleft palate patients. Among these, *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* were identified as predominant species. These microorganisms are known to contribute to dental caries, soft tissue infections, and fungal

overgrowth, respectively. The selective culture methods and biochemical tests confirmed their presence, with *S. aureus* showing positive results for catalase and coagulase tests, while *S. mutans* exhibited characteristic colony morphology on Mitis Salivarius Agar. The presence of *Candida albicans* was confirmed through Gram staining and biochemical assays.

Evaluation of Antimicrobial Peptide (AMP) Activity

The antimicrobial peptide (AMP) used in this study demonstrated significant antibacterial potential against the isolated microorganisms. The results of the minimum inhibitory concentration (MIC) assay showed that AMP effectively inhibited the growth of *Streptococcus mutans* and *Staphylococcus aureus* at low concentrations. Agar diffusion assays further supported these findings, with clear and well-defined zones of inhibition observed around AMP-impregnated discs. Additionally, time-kill assays indicated a rapid bactericidal effect, with substantial microbial reduction observed within the first few hours of exposure.

Discussion:

The findings of this study highlight the presence of common pathogenic microorganisms in the oral microbiome of cleft lip and palate (CLP) patients, with *Streptococcus mutans, Staphylococcus aureus*, and *Candida albicans* being the predominant species. These microorganisms pose significant challenges to oral and systemic health, particularly in individuals with compromised anatomical structures and oral hygiene difficulties. The increased prevalence of these microorganisms aligns with previous studies that have identified altered microbial colonization patterns in cleft patients due to disrupted oral and nasal barriers, which facilitate bacterial migration and retention (Zhou et al., 2018; Costello et al., 2014; Britton et al., 2014; Maragathavalli, 2021; Brook, 2017).

CLP patients often experience microbial dysbiosis, a shift in the balance of normal microbial flora that predisposes them to infections and inflammatory conditions. *Streptococcus mutans*, a key cariogenic bacterium, was frequently isolated in our study, consistent with findings that CLP patients exhibit a higher risk of dental caries due to difficulties in maintaining oral hygiene and food retention in cleft spaces (Li & Tanner, 2015; Priyadarsini et al., 2023; Sivakumar et al., 2020). Additionally, *Staphylococcus aureus*, a known opportunistic pathogen, was identified, supporting previous studies that found increased nasal and oral colonization in CLP patients, particularly those undergoing surgical interventions (Valenza et al., 2014; Malay et al., 2020; Fujimoto et al., 2016). The presence of *Candida albicans* also raises concerns, as its overgrowth has been linked to oral thrush and systemic infections, especially in patients with impaired immune responses or frequent antibiotic use (Morris et al., 2017; Patturaja & Leelavathi, 2019; Ng et al., 2018).

Given the microbial challenges in CLP patients, antimicrobial peptides (AMPs) offer a promising alternative to conventional antimicrobial therapies. AMPs play a crucial role in innate immunity, exhibiting broad-spectrum antimicrobial activity by disrupting microbial membranes and modulating immune responses (Hancock & Sahl, 2006; Han & Wang, 2013; Lohner, 2017). In this study, the AMPs tested demonstrated significant inhibitory effects against *S. mutans* and *S. aureus*, as evidenced by clear zones of inhibition in agar diffusion assays and rapid microbial reduction in time-kill assays. These findings support previous research suggesting that AMPs can effectively combat oral pathogens and may serve as a potential adjunct in managing infections in CLP patients (Costello et al., 2014; Britton et al., 2014; Raaj & Ravindran, 2020; Giuliani et al., 2019).

Additionally, several studies have examined the broader impact of microbial colonization in CLP patients. For instance, Malay et al. (2020) reported that gingival health in CLP patients is often compromised due to microbial imbalances, which can lead to periodontal disease. Similarly, Raaj & Ravindran (2020) found that CLP patients exhibit poorer gingival health compared to non-CLP individuals, emphasizing the need for improved microbial management strategies. Studies by Maragathavalli (2021) and Priyadarsini et al. (2023) further underscore the prevalence of oral and systemic complications in CLP patients, reinforcing the importance of targeted antimicrobial interventions (Jenssen et al., 2006).

Moreover, research by Patturaja & Leelavathi (2019) suggests that public awareness of cleft conditions remains insufficient, which may contribute to delays in seeking appropriate dental and medical care. Sangar et al. (2021) also identified a higher prevalence of oral mucosal lesions in CLP patients, possibly linked to microbial colonization and chronic irritation. These findings highlight the necessity of comprehensive oral healthcare strategies that incorporate novel antimicrobial approaches such as AMPs (Zaura et al., 2009; Hancock et al., 2016; Mookherjee et al., 2020).

Unlike conventional antibiotics, AMPs offer distinct advantages, including rapid bactericidal action, a lower tendency for resistance development, and potential immunomodulatory effects (Hancock et al., 2016; Britton et al., 2014; Wang et al., 2019). Given the rising concerns over antibiotic resistance, AMPs may provide a viable alternative, particularly in vulnerable populations such as CLP patients, who may require recurrent antibiotic treatments post-surgery (Britton et al., 2014; Valenza et al., 2014). Additionally, the ability of AMPs to target multiple pathogens simultaneously makes them a versatile option for addressing the diverse microbiome composition in CLP patients (Sangar et al., 2021; Malay et al., 2020; Ribeiro et al., 2021).

The findings of this study underscore the need for further research to optimize AMP formulations for clinical application. While AMPs demonstrated promising antimicrobial activity, factors such as peptide stability, cytotoxicity, and bioavailability need to be explored to enhance their therapeutic potential (Han & Wang, 2013; Zhou et al., 2018; Jenssen et al., 2006). Future studies should also investigate the efficacy of AMPs in *in vivo* models and explore their role in promoting

wound healing and reducing post-surgical infections in CLP patients (Li & Tanner, 2015; Morris et al., 2017; Fujimoto et al., 2016).

Conclusion

This study provides valuable insights into the microbial composition of CLP patients and highlights the potential of AMPs as an effective antimicrobial strategy. The ability of AMPs to inhibit pathogenic microorganisms suggests a promising therapeutic approach for managing oral infections in CLP patients. However, further research is necessary to refine AMP-based treatments and assess their long-term clinical benefits. By addressing microbial dysbiosis and infection risks, AMPs may contribute to improved oral and systemic health outcomes in CLP patients.

References

1. Zaura E, Keijser BJF, Huse SM, Crielaard W. Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol. 2009;9:259.
2. Zhou Y, Gao H, Mihindukulasuriya KA, La Rosa PS, Wylie KM, Martin JC, et al. Biogeography of the oral microbiome in individuals with cleft lip and palate. PLoS One. 2018;13(8):e0201311.
3. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2014;326(5960):1694–7.
4. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology. 2014;146(6):1547–53.
5. Han X, Wang Y. Characteristics and functions of antimicrobial peptides. Biotechnol Adv. 2013;31(6):1136–49.
6. Li Y, Tanner ACR. Effect of antimicrobial peptides on Streptococcus mutans biofilms. Caries Res. 2015;49(6):561–9.
7. Valenza G, Tappe D, Turnwald D, Frosch M, König C, Hebestreit A, et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. Int J Med Microbiol. 2014;304(3-4):295–302.
8. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Am J Respir Crit Care Med. 2017;195(8):1043–52.
9. Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24(12):1551–7.

10. Maragathavalli G. Microbial colonization in cleft lip and palate patients: A systematic review. J Clin Diagn Res. 2021;15(2):ZE01–ZE05.
11. Brook I. The impact of antimicrobial resistance on oral and respiratory tract infections. Curr Infect Dis Rep. 2017;19(12):46.
12. Priyadarsini R, et al. Oral microbial flora in cleft lip and palate patients: A review. J Indian Orthod Soc. 2023;57(1):23–9.
13. Sivakumar A, et al. Oral health and microbial profile of cleft lip and palate patients. J Clin Pediatr Dent. 2020;44(5):301–7.
14. Malay S, et al. Gingival health status and microbial profile in cleft patients. J Oral Microbiol. 2020;12(1):1769381.
15. Fujimoto Y, et al. The prevalence of Staphylococcus aureus in cleft patients undergoing surgery. J Craniofac Surg. 2016;27(4):879–85.
16. Patturaja K, Leelavathi D. Public awareness of cleft lip and palate conditions. J Public Health Res. 2019;8(3):156–62.
17. Ng WL, et al. Candida species in cleft patients and antifungal susceptibility patterns. Mycopathologia. 2018;183(4):627–33.
18. Raaj G, Ravindran S. Oral health and microbial dysbiosis in cleft patients. Int J Paediatr Dent. 2020;30(6):730–8.
19. Giuliani A, et al. Antimicrobial peptides as potential therapeutic agents against oral pathogens. Front Microbiol. 2019;10:2727.
20. Jenssen H, et al. Host defense peptides in human health and disease. J Innate Immun. 2006;1(3):171–87.
21. Sangar V, et al. Oral mucosal lesions in cleft lip and palate patients. Int J Oral Sci. 2021;13(1):21.
22. Ribeiro RM, et al. Microbiome diversity in cleft lip and palate patients. J Dent Res. 2021;100(8):879–86.
23. Mookherjee N, et al. Antimicrobial host defense peptides: Mechanisms and therapeutic potential. Nat Rev Drug Discov. 2020;19(5):311–32.
24. Hancock REW, et al. Antimicrobial peptides and their potential clinical applications. Exp Biol Med. 2016;241(8):1014–31.
25. Wang G, et al. Development of novel antimicrobial peptides: Mechanisms and future applications. Acta Pharm Sin B. 2019;9(5):857–72.