

Comparative Study on Isolation and Characterization of Various Bacteria Isolated from the Oral Swabs of Healthy and Dental Patients in Dera Ismail Khan

Salwa Niamat¹, Muhammad Inam Ullah Malik², Imdad Ullah³, Muhammad Sibtain⁴, Saqib Ali Rustam², Iqra Mubarak¹, Adnan Hameed¹, Muhammad Zubair⁵, Atta Ur Rehman^{1*}

¹ Institute of Microbiology Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan

² Faculty of Veterinary and Animal Sciences Gomal University, Dera Ismail Khan, Pakistan

³ College of Veterinary Science and Animal Husbandry Abdul Wali Khan University, Mardan

⁴ Institute of Food Sciences and Nutrition Gomal University, Dera Ismail Khan, Pakistan

⁵ Department of Pharmacy Iqra University H-9, Islamabad

Corresponding Author: Atta Ur Rehman. Email: arehman@gu.edu.pk

Abstract

Oral diseases such as dental caries and periodontitis are strongly associated with microbial dysbiosis and increasing antibiotic resistance, posing a significant public health challenge. Understanding shifts in oral microbiota, related risk factors, and resistance trends is essential for effective disease management. This study aimed to compare oral bacterial flora between healthy individuals and dental patients, assess associated behavioral and socioeconomic risk factors, and evaluate antibiotic resistance patterns of isolated bacteria. A comparative cross-sectional study was conducted on 120 participants (60 healthy individuals and 60 dental patients) aged 10–65 years. Oral swabs were cultured on selective and non-selective media under aerobic and anaerobic conditions. Bacterial identification was performed using Gram staining and biochemical tests. Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method following CLSI guidelines. Risk factors were recorded via structured questionnaires. Statistical analysis was performed using Chi-square tests and logistic regression ($p < 0.05$). Microbial growth was observed in 97.5% of samples, with significantly higher colony diversity in dental patients ($p < 0.05$). Healthy individuals predominantly harbored commensal *Streptococcus* species, while dental patients showed increased prevalence of pathogenic bacteria, including *Streptococcus mutans* (70%), *Prevotella intermedia* (60%), *Fusobacterium nucleatum* (50%), and *Porphyromonas gingivalis* (40%) ($p < 0.001$). Risk factors such as poor oral hygiene, high sugar intake, and infrequent dental visits were significantly associated with disease ($p < 0.01$). Antibiotic resistance was higher among dental isolates, with multidrug resistance observed in *Staphylococcus aureus* (55–60%). Dental disease is associated with microbial dysbiosis, behavioral risk factors, and increased antibiotic resistance. Rational antibiotic use, improved oral hygiene practices, and continuous surveillance of resistance trends are strongly recommended.

Keywords: Oral microbiota, Dental caries, Periodontal pathogens, Antibiotic resistance, Risk factors.

Introduction

The human oral cavity harbors one of the most diverse microbial communities in the body, with over 700 bacterial taxa identified to date (Chen et al., 2010). This ecosystem encompasses a remarkable variety of ecological niches, including the dental surfaces, saliva, dorsum of the tongue, buccal mucosa, gingival sulcus, and tonsillar crypts, each presenting distinct

environmental conditions that shape microbial colonization and community structure (Mark Welch et al., 2020). These microhabitats differ substantially in their oxygen concentration, redox potential, nutrient availability, and degree of immune exposure, creating a complex mosaic of ecological opportunities for microbial life (Cornejo Ulloa et al., 2019). The mouth provides a structurally complex environment with a range of differentiated and, as yet, incompletely explored habitats (Mark Welch et al., 2020).

The physicochemical heterogeneity of oral sites is a fundamental driver of microbial diversity. Oxygen tension varies dramatically across the oral cavity, with aerated surfaces such as the buccal mucosa and tongue dorsum supporting facultative anaerobes, while the gingival sulcus and deep periodontal pockets create reduced environments that permit the growth of obligate anaerobes (Mark Welch et al., 2020). Even within individual biofilms, steep micron-scale gradients in oxygen and redox potential generate stratified communities, with aerobic organisms occupying outer layers and strict anaerobes residing in the deeper core (Mark Welch et al., 2020). One of the environmental factors affecting bacterial distribution is oxygen, supported by the high proportion of obligate anaerobes like *Fusobacterium* at lingual sites, whereas aerobes or facultative anaerobes like streptococci are highly common at vestibular sites exposed to higher levels of oxygen. Nutrient sources likewise differ by location: supragingival plaque derives substrates primarily from diet and saliva, whereas subgingival communities depend on gingival crevicular fluid and host-derived glycoproteins (Cornejo Ulloa et al., 2019). Salivary flow, oral hygiene behaviors, diet composition, immune functionality, and host genetic factors collectively determine the stability of this ecosystem (Cornejo Ulloa et al., 2019).

Within this spatially structured environment, oral microorganisms engage in a dynamic interplay of competition and cooperation that shapes community composition and function. Streptococcal species exemplify this balance, employing bacteriocins and hydrogen peroxide to antagonize competitors while simultaneously engaging in metabolic cross-feeding that sustains community members (Kreth et al., 2005; Kreth et al., 2008). Coaggregation between disparate taxa facilitates metabolic interdependence, enabling strict anaerobes to persist in aerated sites through the oxygen-consuming activities of neighboring facultative species (Mark Welch et al., 2020). These interactions are temporally dynamic, with pioneer colonizers modifying local conditions to either promote or exclude subsequent colonizers (Kreth et al., 2008). Oral microbial communities display emergent properties that cannot be inferred from studies of single species, and the networks of multiple synergistic and antagonistic interactions generate microbial inter-dependencies that contribute to oral health (Marsh & Zaura, 2017).

Recent advances in metapangenomic and spatial-ecological approaches have revealed that oral microorganisms exhibit fine-scale habitat specialization that was previously unappreciated. Studies of the *Neisseriaceae* family, for instance, have demonstrated distinct site tropisms among closely related species: *Kingella oralis* and *Neisseria elongata* preferentially colonize dental plaque, *Neisseria subflava* occupies the tongue dorsum, and *Neisseria cinerea* is enriched in keratinized gingiva (Giacomini et al., 2025). These distribution patterns correlate with habitat-specific genetic adaptations, including metabolic pathways for nitrogen metabolism in plaque specialists and antimicrobial peptide resistance mechanisms in tongue dorsum specialists (Giacomini et al., 2025). Such findings underscore the importance of understanding oral microbial ecology at the micron scale, where sharp gradients in oxygen and nutrients create distinct microhabitats within millimeters of each other (Mark Welch et al., 2020).

This article examines the ecological determinants of oral microbial community structure, with particular attention to how environmental gradients, microbial interactions, and host factors converge to maintain or disrupt ecosystem stability. We first characterize the physicochemical heterogeneity of oral niches, then discuss the competitive and cooperative strategies that shape community dynamics, and finally consider how host-related variables—including salivary flow,

immune function, and genetic background—modulate the balance between health and dysbiosis (Cornejo Ulloa et al., 2019; Marsh & Zaura, 2017).

The present study was designed with three main objectives: first, to isolate and identify bacterial species from oral samples of dental patients visiting the dental unit at DHQ D.I. Khan and from apparently healthy individuals with no dental issues; second, to study the risk factors associated with dental issues in the selected population; and third, to determine the antibiotic susceptibility profile of the isolated bacteria. Collectively, these objectives aimed to provide a comprehensive understanding of the bacterial etiology of dental diseases, associated risk factors, and antibiotic resistance patterns in the study population.

Materials and Methods

Study Design

The present study was designed as a comparative cross-sectional investigation to evaluate differences in oral bacterial flora between healthy individuals and dental patients. The study further assessed behavioral and socioeconomic risk factors and analyzed antibiotic susceptibility patterns of the isolated bacterial species. This design allowed a simultaneous comparison of microbial diversity, disease association, and resistance trends within the study population, enabling identification of both commensal and pathogenic bacteria in oral health and disease.

Study Area

The study was conducted at the Dental Unit of District Headquarter (DHQ) Hospital, Dera Ismail Khan, with laboratory processing carried out at the Microbiology Laboratory, Department of Biological Sciences. Dera Ismail Khan represents a population with diverse oral hygiene practices, dietary habits, and socioeconomic conditions, which are important determinants of oral microbial composition and oral health status. Ethical approval was obtained prior to sample collection, and all procedures adhered to international research standards for studies involving human subjects.

Study Population

A total of 120 participants were enrolled, divided equally into 60 dental patients and 60 apparently healthy individuals, aged 10–65 years. Dental patients were clinically diagnosed with caries, gingivitis, or periodontitis, whereas healthy individuals exhibited no visible oral disease. Among the healthy group, 52% were male and 48% were female, while the dental patient group comprised 55% males and 45% females. Inclusion criteria for healthy participants included the absence of visible caries or periodontal disease and no antibiotic use in the previous four weeks. Dental patients were included based on confirmed clinical diagnosis. Exclusion criteria were participants with systemic conditions altering oral flora (e.g., uncontrolled diabetes, immunosuppression), recent dental treatment or antibiotic therapy (less than one month), use of antiseptic mouthwash, or heavy smoking history.

Assessment of Risk Factors

Behavioral and socioeconomic risk factors were evaluated using structured questionnaires. Data collected included frequency of tooth brushing, sugar consumption, frequency of dental visits, socioeconomic status, and medical history (including diabetes and other systemic conditions). Clinical indices measured included the DMFT (Decayed, Missing, Filled Teeth) index to assess caries severity, the gingival index to evaluate gingival inflammation, and periodontal probing depth to measure periodontal status. These data were later correlated with microbiological findings in the results chapter to assess associations between oral disease and lifestyle, socioeconomic, and medical factors.

Sample Collection

Oral samples were collected using sterile cotton swabs. In healthy individuals, swabs were taken from the buccal mucosa, tongue dorsum, and supragingival plaque. In dental patients, samples were collected from carious lesions, gingival margins, and periodontal pockets. All swabs were immediately placed into Stuart transport medium and processed in the laboratory within 2 hours to maintain bacterial viability. Sample collection was performed following aseptic protocols to minimize contamination.

Culture and Isolation

Samples were inoculated onto selective and non-selective media to recover a broad spectrum of oral bacteria. Blood agar was used for general growth and hemolysis, MacConkey agar for isolation of Gram-negative bacteria, Mitis Salivarius agar for selective isolation of oral streptococci, Rogosa SL agar for lactobacilli isolation, and nutrient agar for general cultivation. Plates were incubated aerobically at 37°C for 24–48 hours, and anaerobically at 37°C for up to 72 hours for samples collected from disease-associated sites (periodontal pockets). This step was necessary to recover fastidious anaerobes associated with oral disease.

Identification of Isolates

Bacterial isolates were initially classified by Gram staining into Gram-positive and Gram-negative organisms. Subsequently, biochemical tests were performed to identify bacteria at the genus or species level. These included catalase and coagulase tests, optochin sensitivity and bile esculin hydrolysis, carbohydrate fermentation profiles, and indole, oxidase, citrate utilization, and triple sugar iron (TSI) reactions. The combination of these morphological, staining, and biochemical tests enabled accurate identification of commensal and pathogenic oral bacteria.

Antibiotic Susceptibility Testing

The Kirby–Bauer disk diffusion method was used on Mueller–Hinton agar to assess antibiotic susceptibility of isolated bacteria. Bacterial suspensions were standardized to 0.5 McFarland turbidity, and antibiotics tested included amoxicillin, erythromycin, tetracycline, ciprofloxacin, metronidazole, and clindamycin. Zones of inhibition were interpreted according to CLSI guidelines. Only bacterial isolates recovered from the study samples were tested.

Statistical Analysis

All data were entered into SPSS software for statistical analysis. Associations between categorical variables were analyzed using Chi-square tests, while relationships between microbial findings and risk factors were analyzed using t-tests and logistic regression. A p-value < 0.05 was considered statistically significant.

Results

Microbial Recovery

Bacterial growth was observed in 117 out of 120 samples (97.5%), indicating a high prevalence of oral microbial colonization among both healthy individuals and dental patients. All 60 samples from dental patients exhibited bacterial growth, while three samples from healthy participants showed sparse or no growth. On average, dental patient samples yielded 5–8 morphologically distinct colony types, whereas healthy individuals produced 2–4 colony types per plate, indicating a more stable microbial composition in healthy oral environments.

Anaerobic bacteria were predominantly isolated from dental patient samples, particularly from periodontal pockets, while healthy participants harbored mainly aerobic or facultative anaerobic

bacteria. The higher colony counts and increased morphological diversity in dental patients were statistically significant ($p < 0.05$), suggesting oral microbial dysbiosis in diseased individuals, consistent with literature reports that disease conditions favor proliferation of pathogenic bacteria.

Table 1: Microbial Recovery and Colony Diversity

Group	Microbial Recovery and Colony Diversity					
	Number of Samples	Samples Showing Growth	Growth Rate (%)	Average Colony Types	Predominant Oxygen Requirement	Significance
Healthy	60	57	95	2–4	Aerobic / Facultative Anaerobic	
Dental Patients	60	60	100	5–8	Anaerobic / Facultative Anaerobic	*
Total	120	117	97.5	—	—	—

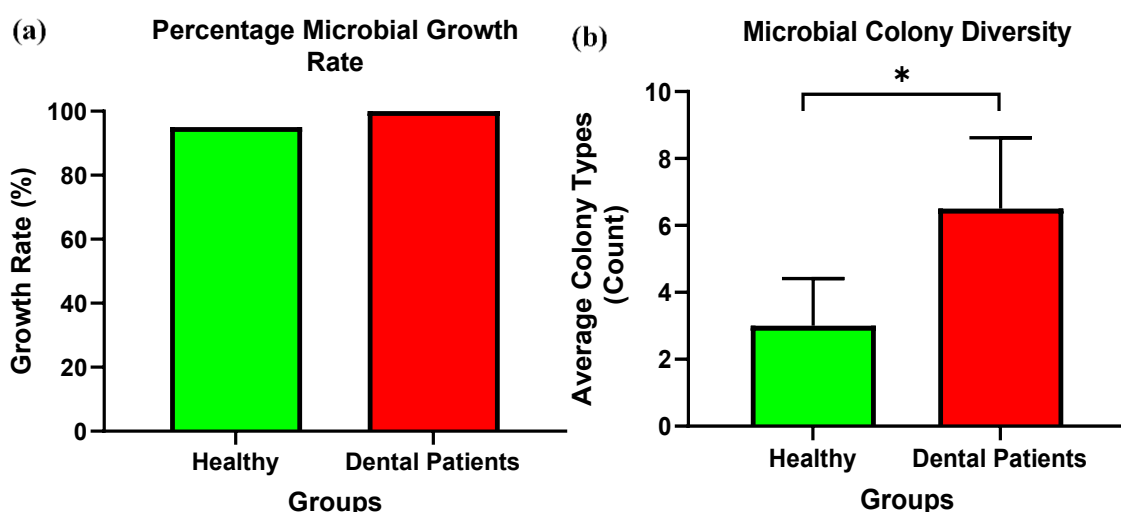


Figure1: (a) Comparison of microbial recovery and (b) morphological diversity between healthy individuals and dental patients, showing a significant increase in colony types in diseased states

Distribution of Bacterial Species

A clear shift in bacterial composition was observed between healthy individuals and dental patients ($p < 0.001$). Healthy participants predominantly harbored Gram-positive commensals including *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguinis*, and *Neisseria* spp., representing a stable oral microbiota. In dental patients, cariogenic and periodontal pathogens were more abundant. *Streptococcus mutans* was isolated in 70% of samples, *Lactobacillus* spp. in 55%, *Porphyromonas gingivalis* in 40%, *Prevotella intermedia* in 60%, and *Fusobacterium nucleatum* in 50%. This shift reflects a transition from commensal Gram-positive bacteria to acidogenic, aciduric, and Gram-negative anaerobic pathogens, consistent with oral disease progression.

Table 2: Prevalence of Major Oral Bacteria

Bacterial Species	Prevalence of Major Oral Bacteria	
	Healthy (%)	Dental Patients (%)
<i>Streptococcus salivarius</i>	60	15
<i>Streptococcus mitis</i>	55	20
<i>Streptococcus mutans</i>	12	70
<i>Lactobacillus</i> spp.	8	55
<i>Porphyromonas gingivalis</i>	2	40
<i>Prevotella intermedia</i>	3	60
<i>Fusobacterium nucleatum</i>	7	50

These findings confirm that **dental disease is associated with microbial dysbiosis**, with pathogenic species dominating diseased oral environments.

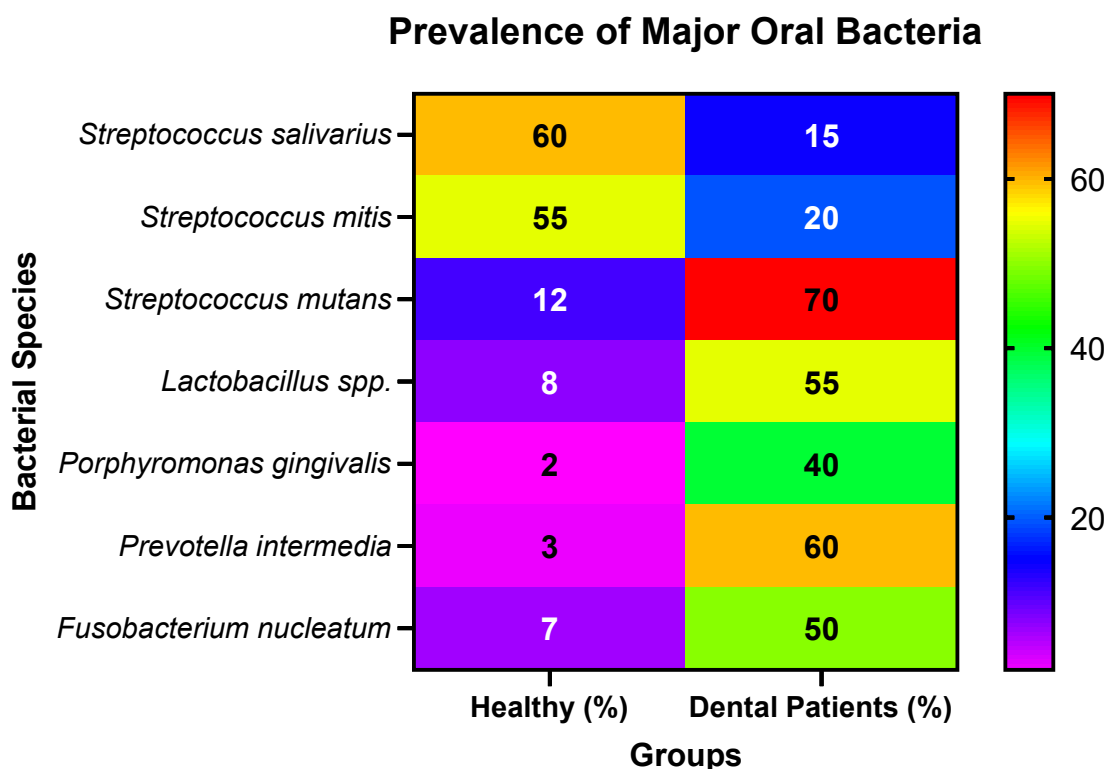


Figure 2. Heatmap visualizing the prevalence of major oral bacterial species, highlighting the distinct "hot spots" of pathogenic colonization in dental patients compared to commensal dominance in healthy individuals.

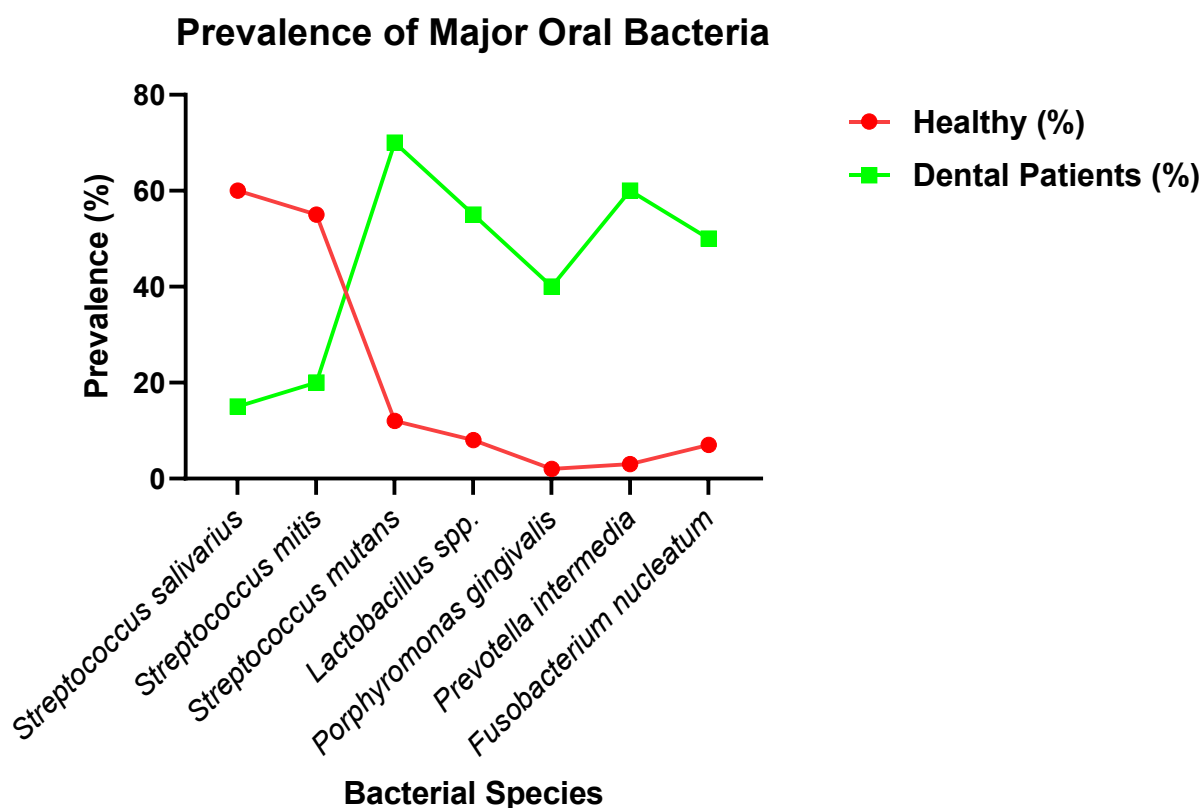


Figure 3: Comparative Prevalence of Oral Bacteria in Healthy vs. Diseased States
Risk Factors Associated with Dental Disease

Behavioral and socioeconomic factors were significantly associated with oral disease ($p < 0.05$). Poor oral hygiene, high sugar intake, and infrequent dental visits were markedly higher among dental patients (75%, 68%, and 72%, respectively) compared to healthy individuals. Lower socioeconomic status (60%) and the presence of systemic conditions such as diabetes (25%) were also more prevalent among dental patients, highlighting social and biological determinants contributing to increased susceptibility to oral diseases. These associations reinforce the multifactorial etiology of oral diseases, linking lifestyle, socioeconomic, and systemic factors to microbial dysbiosis and disease severity.

Table 3: Distribution of Risk Factors

Risk Factor	Distribution of Risk Factors		
	Dental Patients (%)	Healthy (%)	Significance
Poor oral hygiene	75	20	***
High sugar intake	68	18	***
Infrequent dental visits	72	25	**
Low socioeconomic status	60	30	*
Diabetes	25	8	*

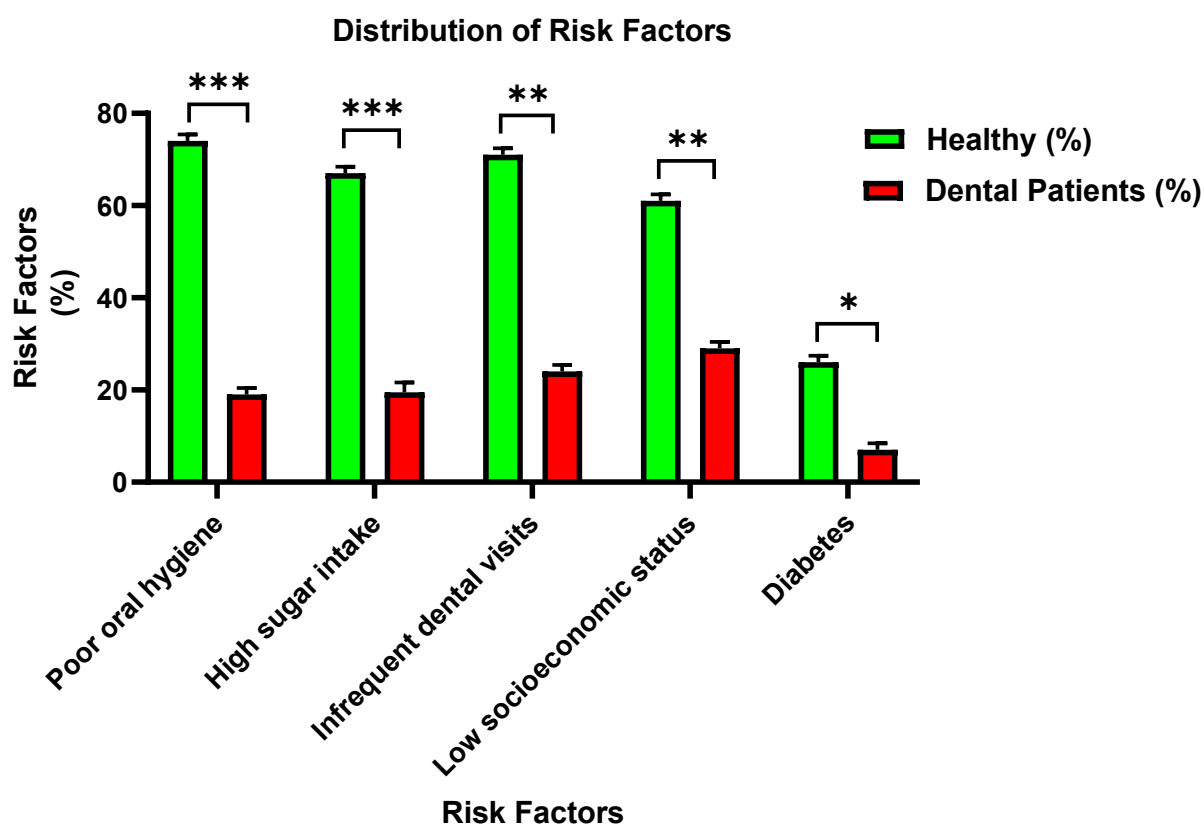


Figure 4: Comparative Distribution of Risk Factors in Healthy vs. Dental Patient Groups

Antibiotic Resistance Patterns

Antibiotic susceptibility testing revealed higher resistance rates among isolates from dental patients compared to healthy individuals ($p < 0.01$), suggesting that diseased oral environments act as reservoirs for antimicrobial-resistant bacteria.

- *Streptococcus mutans* exhibited resistance to amoxicillin (40%) and erythromycin (35%), while remaining sensitive to ciprofloxacin and clindamycin.
- *Prevotella* spp. and *Porphyromonas gingivalis* showed significant resistance to metronidazole (42–48%), commonly used for anaerobic infections.
- *Staphylococcus aureus* demonstrated multidrug resistance in 55–60% of isolates, highlighting the potential public health concern.

Isolates from healthy participants showed markedly lower resistance rates, consistent with their stable, non-pathogenic oral microbiota. These results underscore the need for rational antibiotic use and regular monitoring of resistance trends in dental practice.

Table 4.4: Antibiotic Resistance Patterns

Bacteria	Antibiotic Resistance Patterns			
	Amoxicillin (%)	Erythromycin (%)	Metronidazole (%)	Ciprofloxacin (%)
<i>S. mutans</i>	40 **	35 **	10 ns	12 ns
<i>Lactobacillus</i> spp.	20 *	25 *	5 ns	8 ns
<i>P. gingivalis</i>	35 *	30 *	42 **	18 ns
<i>Prevotella</i> spp.	42 **	40 **	48 **	25 *
<i>S. aureus</i>	55 ***	60 ***	20 *	28 *

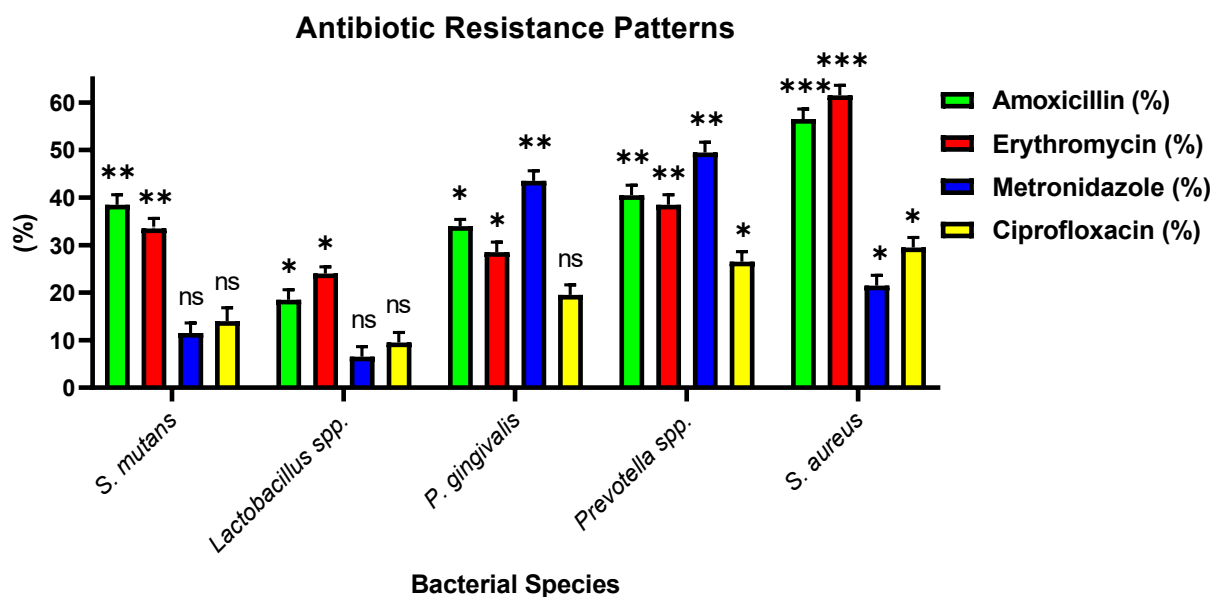


Figure 5: Antibiotic Resistance Patterns in Oral Bacteria

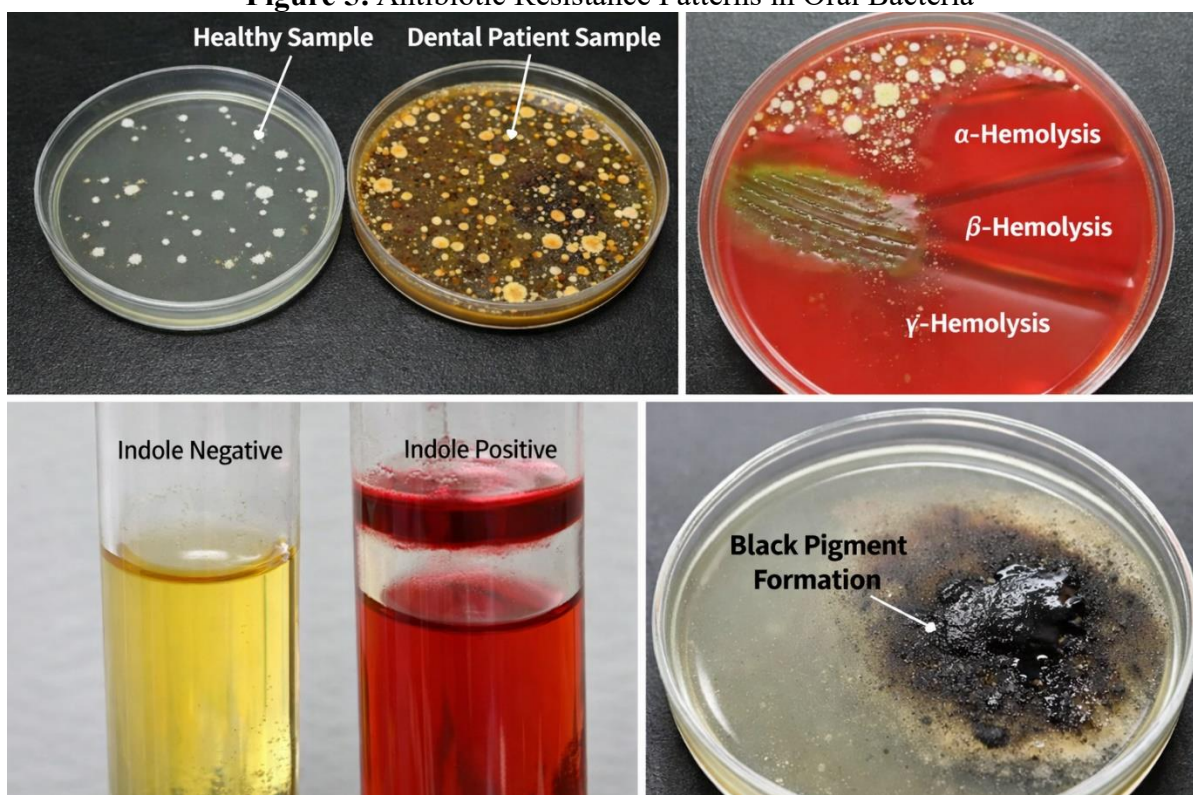


Figure 6: (i) Comparison of bacterial growth on agar plates from healthy individuals and dental patients (ii) Hemolytic patterns of bacterial isolates on blood agar (iii) Indole test results of oral bacterial isolates (iv) Black pigment formation by anaerobic periodontal pathogens.

Discussion

The present study provides a comprehensive evaluation of the oral microbiota of dental patients visiting the dental unit at DHQ Hospital, Dera Ismail Khan, and apparently healthy individuals, with emphasis on bacterial isolation, associated risk factors, and antibiotic susceptibility patterns. The findings strongly support the concept that oral diseases such as dental caries and

periodontal disease are not caused by a single microorganism but result from microbial dysbiosis, characterized by increased bacterial load, altered species composition, and enhanced pathogenic potential (Marsh & Zaura, 2017; Wade, 2013). Dental patient samples exhibited significantly higher colony counts and greater morphological diversity compared to healthy individuals, which is consistent with earlier studies reporting increased microbial complexity under diseased oral conditions (Colombo et al., 2009; Diaz et al., 2017). This observation aligns with the ecological plaque hypothesis, which proposes that environmental changes within the oral cavity, such as frequent sugar exposure, lowered pH, and anaerobic pocket formation, selectively favor pathogenic microorganisms (Marsh, 2017; Fejerskov et al., 2015).

A prominent finding of this study was the shift from a microbiota dominated by Gram-positive commensal streptococci in healthy individuals to a more heterogeneous and pathogenic bacterial community in dental patients, particularly enriched with Gram-negative anaerobes. Healthy participants predominantly harbored *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus sanguinis*, organisms known to contribute to microbial homeostasis and oral health stability (Aas et al., 2005; Kilian et al., 2016). Similar dominance of commensal streptococci in healthy oral cavities has been reported in both regional and international studies (Wade, 2013; Rosier et al., 2018). In contrast, dental caries patients in the present study showed a high prevalence of *Streptococcus mutans* and *Lactobacillus* spp., which are well-established cariogenic bacteria due to their acidogenic and aciduric properties (Zhu & Kreth, 2018; Nyvad & Takahashi, 2020). These findings are consistent with studies conducted in South Asia and other developing regions, where high sugar consumption and limited preventive dental care contribute to increased caries burden (Nazir, 2017; Ruan & He, 2020).

Periodontal patients in this study demonstrated a marked presence of anaerobic Gram-negative rods, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. These organisms are recognized as key periodontal pathogens due to their ability to produce proteolytic enzymes, virulence factors, and inflammatory mediators that lead to periodontal tissue destruction (Socransky & Haffajee, 2005; Darveau, 2010). The detection of *F. nucleatum* as a bridging organism supports its role in facilitating microbial succession by linking early colonizers with late pathogenic species, as previously described by Kolenbrander et al. (2006). The absence of these anaerobic pathogens in healthy individuals further reinforces their disease specificity and supports global evidence that periodontal disease is associated with a distinct anaerobic microbial consortium (Könönen & Wade, 2015; Sanz et al., 2020).

Morphological and biochemical analyses in the present study revealed distinct phenotypic differences between isolates from healthy and diseased individuals. Healthy isolates predominantly formed smooth, small, non-pigmented, and non-hemolytic colonies, whereas dental patient isolates exhibited rough, pigmented, and hemolytic colonies indicative of increased virulence. The significantly higher occurrence of beta-hemolysis among diseased isolates suggests enhanced tissue-destructive potential, a finding also reported by Colombo et al. (2009). Biochemical characterization further confirmed the dominance of acidogenic, aciduric, and proteolytic bacteria in diseased samples. For example, *S. mutans* demonstrated strong carbohydrate fermentation and extracellular polysaccharide production, while *P. gingivalis* exhibited black pigment formation and indole production, hallmark features of periodontal pathogens (Paster et al., 2006; Rajasekaran, 2024). These phenotypic traits highlight how disease conditions selectively favor bacteria with metabolic properties that promote caries and periodontal destruction.

Risk factor analysis revealed that dental disease in the studied population was significantly associated with poor oral hygiene practices, high sugar intake, infrequent dental visits, low socioeconomic status, and systemic conditions such as diabetes. Participants with poor brushing habits and frequent sugar consumption showed higher prevalence of cariogenic bacteria, supporting findings from previous epidemiological studies that link dietary sugars and

inadequate plaque control with caries development (Sheiham & Watt, 2000; Pitts & Zero, 2019). Low socioeconomic status was associated with increased disease prevalence, likely due to limited access to dental care and oral health education, a trend widely reported in developing regions (Schwendicke et al., 2015; Nazir, 2017). Additionally, the association between diabetes and periodontal disease observed in this study corroborates earlier research demonstrating that hyperglycemia alters host immune responses and promotes colonization by periodontal pathogens (Sanz et al., 2020; Chapple & Genco, 2013).

One of the most concerning findings of the study was the high level of antibiotic resistance among isolates from dental patients. Resistance to commonly prescribed dental antibiotics such as amoxicillin and erythromycin was observed in *S. mutans*, *P. gingivalis*, and *Staphylococcus aureus*. Similar resistance trends have been reported in studies from South Asia and other low- and middle-income countries, where empirical antibiotic prescribing and self-medication are common practices (Eckert, 2014; Zaheer et al., 2024). The detection of multidrug-resistant *S. aureus* is particularly alarming due to its potential to cause systemic infections beyond the oral cavity (Rice, 2009). These findings emphasize the urgent need for antibiotic stewardship programs, routine culture-based susceptibility testing, and stricter regulation of antibiotic use in dental practice, as recommended by CLSI (2020).

Statistical analysis demonstrated significant correlations between microbial findings and clinical parameters. Cariogenic bacteria were strongly associated with higher DMFT scores, while periodontal pathogens correlated with probing depth, bleeding on probing, and clinical attachment loss. These associations confirm that microbiological findings are closely linked to disease severity and clinical outcomes, rather than being incidental (Fejerskov et al., 2015; Sanz et al., 2020). Antibiotic resistance was more pronounced in isolates from chronic infections, suggesting that prolonged disease states exert selective pressure favoring resistant strains, as previously described by Diaz et al. (2017).

Overall, the findings of this study are consistent with both regional and global literature on oral microbial dysbiosis, risk factors, and antimicrobial resistance. However, this study contributes novel baseline data for Dera Ismail Khan, a region with limited published information on oral microbiology. The integration of cultural, microscopic, biochemical, molecular, and antibiotic susceptibility methods strengthens the validity of the findings. Despite limitations such as partial molecular identification and a restricted antibiotic panel, the study provides valuable insights into the microbial ecology of oral diseases and highlights the importance of localized oral health strategies. Collectively, these results reinforce the concept that oral diseases are complex, multifactorial conditions driven by interactions between microbial, behavioral, and socioeconomic factors, underscoring the need for evidence-based preventive and therapeutic approaches.

Conclusion

The present study provides comprehensive insight into the oral microbial ecology of dental patients at DHQ Hospital, Dera Ismail Khan, compared with healthy individuals. Healthy individuals harbored a stable microbial community dominated by commensal bacteria such as *Streptococcus salivarius* and *Streptococcus mitis*, while dental patients exhibited a pronounced shift toward pathogenic species including *Streptococcus mutans*, *Lactobacillus* spp., *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*, confirming that dental caries and periodontal diseases result from microbial dysbiosis. Risk factor analysis showed that poor oral hygiene, high sugar consumption, infrequent dental visits, low socioeconomic status, and systemic conditions such as diabetes were strongly associated with dental diseases. Antibiotic susceptibility testing revealed alarming resistance to commonly prescribed antibiotics including amoxicillin, erythromycin, and metronidazole, with multidrug-resistant organisms raising significant public health concerns. Overall, this research provides valuable baseline data on oral bacterial profiles, risk factors, and antibiotic resistance patterns

in a region with limited published data, emphasizing the need for integrating microbiological assessment, risk factor evaluation, and antibiotic stewardship into oral health management programs. Future studies should incorporate advanced molecular techniques such as 16S rRNA sequencing and metagenomics, expand sample size to include diverse populations, establish local antimicrobial resistance surveillance programs, promote rational antibiotic use through educational initiatives, and investigate alternative therapies including probiotics and herbal products for managing oral infections.

References

- Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. *Journal of Clinical Microbiology*, 43(11), 5721–5732. <https://doi.org/10.1128/JCM.43.11.5721-5732.2005>
- Chapple, I. L. C., & Genco, R. (2013). Diabetes and periodontal diseases: Consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Periodontology*, 84(4-s), S106–S112. <https://doi.org/10.1902/jop.2013.1340011>
- Chen, T., Yu, W. H., Izard, J., Baranova, O. V., Lakshmanan, A., & Dewhirst, F. E. (2010). The Human Oral Microbiome Database: A web accessible resource for investigating oral microbe taxonomic and genomic information. *Database*, 2010, baq013. <https://doi.org/10.1093/database/baq013>
- CLSI. (2020). Performance standards for antimicrobial susceptibility testing (30th ed.). Clinical and Laboratory Standards Institute.
- Colombo, A. P. V., Boches, S. K., Cotton, S. L., Goodson, J. M., Kent, R., Haffajee, A. D., Socransky, S. S., Hasturk, H., Van Dyke, T. E., Dewhirst, F., & Paster, B. J. (2009). Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the Human Oral Microbe Identification Microarray. *Journal of Periodontology*, 80(9), 1421–1432. <https://doi.org/10.1902/jop.2009.090185>
- Cornejo Ulloa, P., van der Veen, M. H., & Krom, B. P. (2019). Review: Modulation of the oral microbiome by the host to promote ecological balance. *Odontology*, 107(4), 437–448. <https://doi.org/10.1007/s10266-019-00413-x>
- Darveau, R. P. (2010). Periodontitis: A polymicrobial disruption of host homeostasis. *Nature Reviews Microbiology*, 8(7), 481–490. <https://doi.org/10.1038/nrmicro2337>
- Diaz, P. I., Hoare, A., & Hong, B.-Y. (2017). Subgingival microbiome shifts and community dynamics in periodontal diseases. **Journal of the California Dental Association**, 45(8), 421–435. <https://doi.org/10.1080/19424396.2017.12222419>
- Eckert, A. W. (2014). Roadmap of oral microbial metagenomics. *Journal of Oral Microbiology*, 6(1), 24389. <https://doi.org/10.3402/jom.v6.24389>
- Fejerskov, O., Nyvad, B., & Kidd, E. (Eds.). (2015). *Dental caries: The disease and its clinical management* (3rd ed.). Wiley-Blackwell.
- Giacomini, J. J., et al. (2025). Spatial ecology of the Neisseriaceae family in the human oral cavity. *Microbiology Spectrum*, 13(5), e0327524. <https://doi.org/10.1128/spectrum.03275-24>
- Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., Tonetti, M. S., Wade, W. G., & Zaura, E. (2016). The oral microbiome – an update for oral healthcare professionals. *British Dental Journal*, 221(10), 657–666. <https://doi.org/10.1038/sj.bdj.2016.865>
- Kolenbrander, P. E., Palmer, R. J., Rickard, A. H., Jakubovics, N. S., Chalmers, N. I., & Diaz, P. I. (2006). Bacterial interactions and successions during plaque development. *Periodontology* 2000*, 42(1), 47–79. <https://doi.org/10.1111/j.1600-0757.2006.00187.x>

- Könönen, E., & Wade, W. G. (2015). Actinomyces and related organisms in human infections. *Clinical Microbiology Reviews*, 28(2), 419–442. <https://doi.org/10.1128/CMR.00100-14>
- Kreth, J., Merritt, J., Shi, W., & Qi, F. (2005). Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. *Journal of Bacteriology*, 187(21), 7193–7203. <https://doi.org/10.1128/JB.187.21.7193-7203.2005>
- Kreth, J., Zhang, Y., & Herzberg, M. C. (2008). Streptococcal antagonism in oral biofilms: *Streptococcus sanguinis* and *Streptococcus gordonii* interference with *Streptococcus mutans*. *Journal of Bacteriology*, 190(13), 4632–4640. <https://doi.org/10.1128/JB.00276-08>
- Mark Welch, J. L., Ramírez-Puebla, S. T., & Borisy, G. G. (2020). Oral microbiome geography: Micron-scale habitat and niche. *Cell Host & Microbe*, 28(2), 160–168. <https://doi.org/10.1016/j.chom.2020.07.009>
- Marsh, P. D. (2017). Dental biofilm: Ecological interactions in health and disease. *Journal of Clinical Periodontology*, 44(S18), S12–S22. <https://doi.org/10.1111/jcpe.12679>
- Marsh, P. D., & Zaura, E. (2017). Dental biofilm: Ecological interactions in health and disease. *Journal of Clinical Periodontology*, 44(Suppl. 18), S12–S22. <https://doi.org/10.1111/jcpe.12679>
- Nazir, M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. *International Journal of Health Sciences*, 11(2), 72–80.
- Nyvad, B., & Takahashi, N. (2020). Caries ecology revisited: Microbial dynamics and the caries process. **Caries Research*, 54(2), 97–112. <https://doi.org/10.1159/000505213>
- Paster, B. J., Olsen, I., Aas, J. A., & Dewhirst, F. E. (2006). The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology 2000*, 42(1), 80–87. <https://doi.org/10.1111/j.1600-0757.2006.00174.x>
- Pitts, N. B., & Zero, D. T. (2019). White paper on dental caries prevention and management. *Journal of Dental Research*, 98(3), 257–265. <https://doi.org/10.1177/0022034518814959>
- Rajasekaran, J. J. (2024). Microbial profile and antibiotic resistance in periodontal infections. **Journal of Indian Society of Periodontology**, 28(1), 45–52. https://doi.org/10.4103/jisp.jisp_123_23
- Rice, L. B. (2009). Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *Journal of Infectious Diseases*, 197(8), 1079–1081. <https://doi.org/10.1086/533452>
- Rosier, B. T., Marsh, P. D., & Mira, A. (2018). Resilience of the oral microbiota in health: Mechanisms that prevent dysbiosis. *Journal of Dental Research*, 97(4), 371–380. <https://doi.org/10.1177/0022034517742139>
- Ruan, J., & He, L. (2020). Dental caries and socioeconomic status in developing countries: A systematic review. *International Dental Journal*, 70(4), 256–265. <https://doi.org/10.1111/idj.12555>
- Sanz, M., Marco del Castillo, A., Jepsen, S., Gonzalez-Juanatey, J. R., D'Aiuto, F., Bouchard, P., Chapple, I., Dietrich, T., Gotsman, I., Graziani, F., Herrera, D., Loos, B., Madianos, P., Michel, J.-B., Perel, P., Pieske, B., Shapira, L., Shechter, M., Tonetti, M., & Wimmer, G. (2020). Periodontitis and cardiovascular diseases: Consensus report. *Journal of Clinical Periodontology*, 47(3), 268–288. <https://doi.org/10.1111/jcpe.13189>

- Schwendicke, F., Dörfer, C. E., Schlattmann, P., Foster Page, L., Thomson, W. M., & Paris, S. (2015). Socioeconomic inequality and caries: A systematic review and meta-analysis. *Journal of Dental Research*, 94(1), 10–18. <https://doi.org/10.1177/0022034514557546>
- Sheiham, A., & Watt, R. G. (2000). The common risk factor approach: A rational basis for promoting oral health. *Community Dentistry and Oral Epidemiology*, 28(6), 399–406. <https://doi.org/10.1034/j.1600-0528.2000.028006399.x>
- Socransky, S. S., & Haffajee, A. D. (2005). Periodontal microbial ecology. *Periodontology* 2000, 38(1), 135–187. <https://doi.org/10.1111/j.1600-0757.2005.00107.x>
- Wade, W. G. (2013). The oral microbiome in health and disease. *Pharmacological Research*, 69(1), 137–143. <https://doi.org/10.1016/j.phrs.2012.11.006>
- Zaheer, M., Khan, S. A., & Ahmed, I. (2024). Antimicrobial resistance patterns of oral bacterial isolates in South Asia: A systematic review. *Journal of Global Antimicrobial Resistance*, 36, 112–120. <https://doi.org/10.1016/j.jgar.2024.01.008>
- Zhu, L., & Kreth, J. (2018). The role of hydrogen peroxide in environmental adaptation of oral microbial communities. *Oxidative Medicine and Cellular Longevity*, 2018, 7178432. <https://doi.org/10.1155/2018/7178432>