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Original Article

Characterization of the oral microbiota in zygomatic versus dental implants: Implications for full-mouth rehabilitation

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Abstract *Background/purpose:* Dental implants are widely used to restore oral function and esthetics in patients with tooth loss. General zygomatic implants (GZIs) are a valuable alternative for patients with severely atrophic maxillae or post-oncologic defects. However, implant placement can alter the ecological balance of the oral microbiota, potentially influencing peri-implant and sinus-related diseases. Because GZIs often traverse or adjoin the maxillary sinus, their microbial environment may differ from that of general dental implants (GDIs). This study aimed to compare microbiota taxonomy between GZI and GDI sites.

Materials and methods: Generally healthy adults treated with either general zygomatic implants (GZI group) or general dental implants (GDI group) at a teaching hospital dental clinic were recruited. Biofilm samples were collected from implant surfaces under aseptic conditions. Microbial DNA was extracted and analyzed using 16S rRNA gene amplicon sequencing. Taxonomic classification and diversity analyses were performed through bioinformatic pipelines to identify bacterial genera and compare microbial community composition between groups.

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Results: Distinct microbial profiles were observed between the GZI and GDI groups. *Rothia*, *Thermus*, and *Sphingomonas* were significantly more abundant in the GZI group, whereas *Capnocytophaga* and *Leptotrichia* predominated in the GDI group, reflecting greater richness in the latter. Notably, *Rothia* species commonly linked to sinus infections were enriched in the GZI group, suggesting sinus-associated microbiome alterations.

Conclusion: Implant type significantly influences peri-implant microbiota composition. Zygomatic implants exhibit distinct microbial communities potentially associated with sinus involvement. Further studies are needed to clarify their role in peri-implant and sinus-related pathogenesis.

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Introduction

Rehabilitating the problem of tooth loss, oral implants are amongst the foremost treatment option. Oral implants include zygomatic implants in addition to dental implants. Dental implants are short implants (mean length: 6–18 mm) embedded on the alveolar bone for functional substitution of edentulous lost tooth.^{1,2} Nevertheless, if the alveolar ridge with severe atrophy as well as hard rehabilitation by bone graft surgery, then zygomatic implant will be considered. Zygomatic implants are longer implants (mean length: 30–60 mm) applied in cases that short implants couldn't be placed, like patients edentulous totally with high sinus pneumatization along with extensive posterior alveolar ridge resorption.³ In comparison with conventional dentures, oral implants have greater advantages while overcoming tooth loss problems like tooth preparation avoidance with its sequels potential, lessen of the mechanical perils of typical bridges, as well as achievement of favorable retention additionally superior vertical support of the above denture.²

However, while such treatment effectiveness is high, the environment around implants is prone to getting infected by microorganisms from surgical wounds, improperly placed implants, biomechanical causes, remaining bone cement, implant properties, or poor biological sealing.^{2,4,5} As for treatments, microorganisms may play a crucial role in treatment outcomes. Some evidence shows that microbial dysbiosis is connected with implant infections and may play a role in such infections' severity.^{6,7} Other study results could support such indications. Periodontal disease and peri-implantitis are similar oral infectious disease complications that are characterized by inflammation of oral implant connective tissue, destruction of underlying bone, an increased probing depth, and eventual failure of the tooth/implant.^{8–10} However, peri-implantitis's development proceeds at a faster rate than that of periodontal disease's development.⁸ Further, the relative composition of microorganisms differs between peri-implantitis and periodontal disease.¹¹

The disease of sinusitis is widespread in zygomatic implants patients. Prevalence is close to 3.9 cases per 100 zygomatic implants placed that could be connected with implants often traveling through the maxillary sinus.¹²

Likewise, zygomatic implant peri-implantitis could involve sinus microorganisms/connected sinusitis. Thus, oral implants' type could also impact microbiome taxonomy. But the relationship is yet unknown.

Apart from that, 16S rRNA gene amplicon sequencing method was used to study the taxonomy of the body's microbiome, just by getting the DNA of the specimen, amplifying, and sequencing the specified portion of the 16S rRNA gene and comparing the similarity of the public database's 16S rRNA gene sequences. In contrast to the traditional culture method, 16S rRNA gene amplicon sequencing method does not need culturing of the specimen, and is able to identify all the bacteria of the specimen.^{13,14} The method has proved very popular for investigating the microbiome at anybody location in researches.¹⁵ In response to our concerns, the method has frequently been used for detecting bacteria at the surfaces of the teeth or oral implants, neighboring tissues, and oral pockets,^{15–17} but extremely few for zygomatic implants.

The focus of current work was oral implant types (dental implant vs. zygomatic implant), microbiome taxonomy, and corresponding outcome measurement by adopting 16S rRNA gene amplicon sequencing technologies. Further, since very few studies have adopted the use of 16S rRNA gene amplicon sequencing technologies for zygomatic implants, current study also expands its range of application towards zygomatic implants and downwards the zygomatic implant infection.

Materials and methods

Study design, patient recruitment, and sample collection

This observational paired-sample study comparing zygomatic implants with conventional dental implants peri-implant microbiota within the same patients was conducted. In order to minimize oral microbiome interindividual variation as far as possible, only openly healthy adults aged between 18 and 80 years ($n = 17$) who received both zygomatic implants and dental implants were selected. Patients were enrolled from July 1, 2022, until July 1, 2024, at routine follow-up appointments of patients at a

teaching-hospital-affiliated dental clinic. Peri-implant microbiological samples were collected separately from each of the two types of implant surfaces of each subject, providing two groups: general dental implants (GDI, $n = 17$) and general zygomatic implants (GZI, $n = 17$), with a total of 34 samples.

The study protocol received approval from the Institutional Review Board of Taipei Tzu Chi Hospital (IRB No.: 11-XD-087). All participants provided written informed consent after an explanation of the purpose of the study, its procedure, its potential hazards, as well as their rights.

Genomic DNA was extracted from harvested samples, while the V3–V4 hypervariable subregion of the 16S rRNA gene was PCR-amplified for library construction. Sequencing was performed on the Illumina MiSeq platform (2×300 bp). Quality filtering of the raw reads was carried out by the DADA2 pipeline for read merging and effective read generation. Taxonomic characterization against the SILVA reference database (release 138) was used for amplicon sequence variant (ASV) identification. Further analyses included taxonomic profiling, alpha and beta diversity rarefactions, comparison of groups, statistical analysis of metagenomic profiles (STAMP), and functional prediction.

DNA extraction and PCR amplification

Genomic DNA of the implant surface samples was isolated by QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The primer pair of 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') was used for amplifying the V3–V4 hypervariable regions of the bacterial 16S rRNA gene. Each of the PCR reaction mixtures of 25 μ L volume consisted of 2 ng of template DNA, 5 \times KAPA HiFi Buffer, KAPA dNTP Mix of 10 mM concentration, KAPA HiFi DNA Polymerase of 1 U/ μ L concentration (KAPA Biosystems, Boston, MA, USA), and each primer of 0.3 μ M concentration (Tri-I, New Taipei City, Taiwan). PCR amplification was conducted by the following thermal cycling program: initial denaturation of 95 °C for 3 min; denaturation at 98 °C for 20 s; annealing at 57.5 °C for 20 s; extension at 72 °C for 20 s; repeated for 25 cycles; followed by the final extension at 72 °C for 3 min.

PCR products were verified by gel electrophoresis on 2 % agarose gels (SeaKem® LE Agarose, Lonza, ME, USA), purified by the MinElute Gel Extraction Kit (Qiagen), and quantified by the QuantiFluor® dsDNA System (Promega, Madison, WI, USA) by means of a Qubit® 2.0 Fluorometer (Invitrogen, Grand Island, NY, USA). Index barcoding was carried out by secondary 5-cycle PCR under the same thermal treatment and another round of purification.

Library preparation and sequencing

Barcoded amplicons were pooled in equimolar concentrations and prepared for sequencing using the Celero DNA-Seq System (Tecan Genomics, San Carlos, CA, USA) following the manufacturer's guidelines. Library concentration and quality were assessed using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) with a DNA 1000

LabChip. Sequencing was carried out on an Illumina MiSeq platform with the MiSeq Reagent Kit v3 (600 cycles), producing paired-end reads (2×301 bp, dual indexing).

Bioinformatics and statistical analysis

Raw read sequencing was denoised by subjecting the read sequences to the DADA2 plugin of the QIIME 2 pipeline for denoising sequences, chimeras removal, and ASVs inference. Taxonomic assignment was done against the SILVA 138 reference database, while taxa summary tables were generated. Alpha diversity analysis (Chao1 richness, Shannon index, Simpson index) was measured by QIIME/QIIME 2, with rarefaction analysis done for normalization of sequencing depth. Beta diversity was measured by principal coordinate analysis (PCoA) of unweighted as well as weighted UniFrac distances.

Further sophisticated techniques of ordination were then employed for defining microbial community structure. Non-metric multidimensional scaling was done by vegan R package, while t-distributed stochastic neighbor embedding analysis was done by Rtsne package. Gplots R package was used for creating heatmaps of taxonomic distributions.

Differential abundance of implant types was also examined by STAMP and linear discriminant analysis effect Size (LEfSe). Additional comparisons at the ASV-level were made by the DESeq2 R package that applies a negative binomial generalized linear model to adjust for variation across sequencing data.

Statistical significance of variation of microbial community structure was determined by permutational multivariate analysis of variance (PERMANOVA/ADONIS) software of QIIME. Results were deemed statistically significant at $P < 0.05$ after appropriate multiple comparison corrections.

Statistical analysis

Descriptive statistics summarized participant characteristics. For microbiota analysis, microbial diversity was evaluated based on ASV counts and the Chao1 richness index. Group differences were assessed with Wilcoxon rank-sum tests. Comparative taxonomic analyses between GDI and GZI groups were conducted using STAMP, with false discovery rate (FDR) correction applied where necessary.

Results

Microbial diversity and community composition differ between GDI and GZI groups

The patient recruitment process is outlined in Fig. 1. A total of 17 patients consented to participate in the study. For each participant, microbial samples were collected separately from the surfaces of their dental implants and zygomatic implants. The samples were subsequently analyzed using 16S rRNA gene sequencing to characterize and compare the microbiota profiles associated with each implant type. Participant demographics and clinical characteristics are summarized in Table 1. A total of 78 GDIs and 40 GZIs were included in the analysis. The majority of

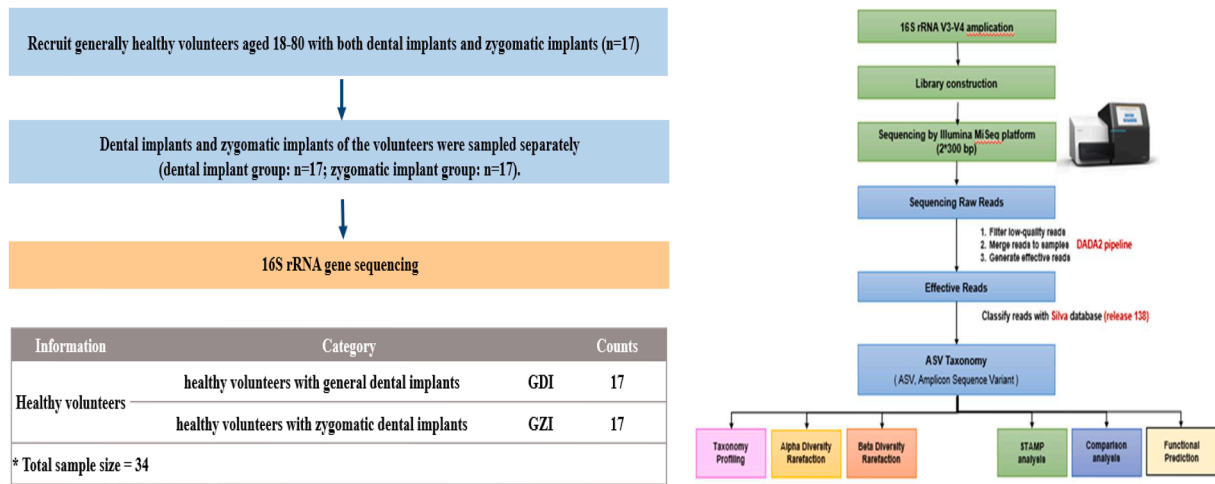


Figure 1 Study design and workflow for 16S rRNA gene sequencing of peri-implant microbiota. Healthy volunteers with general dental implants (GDI) and healthy volunteers with general zygomatic implants (GZI) were recruited (n = 17). Generally healthy volunteers aged 18–80 years (n = 17) with both GDI and GZI were recruited. Samples were collected separately from each implant type, yielding two groups (GDI, n = 17; GZI, n = 17; total = 34). Genomic DNA was extracted, and the V3–V4 region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform (2 × 300 bp). Raw reads were processed using the DADA2 pipeline, followed by taxonomic assignment with the SILVA 138 database to generate amplicon sequence variants (ASVs). Downstream analyses included taxonomy profiling, alpha and beta diversity rarefaction, group comparison, statistical analysis of metagenomic profiles (STAMP), and functional prediction.

Table 1 Background of patients.

Variables	General healthy volunteers/ implants included N (%)	
	Dental implants	Zygomatic implants
Total implants ^a	78	40
Age (years)	57.18 ± 9.63	
Sex ^b		
Female	6 (35.29 %)	
Male	11 (64.70 %)	
History of alcohol consumption ^b		
No	15 (88.23 %)	
Yes	2 (11.76 %)	
History of betel nut consumption ^b		
No	14 (82.35 %)	
Yes	3 (17.65 %)	
Bruxism/clenching ^b		
No	13 (76.47 %)	
Yes	4 (23.53 %)	
History of sinusitis ^b		
No	14 (82.35 %)	
Yes	3 (17.65 %)	
History of periodontitis ^b		
No	7 (41.18 %)	
Yes	10 (58.82 %)	
Complications of prostheses ^b		
No	13 (76.47 %)	
Yes	4 (23.53 %)	

^a The N was the number of implants included in this study.

^b The N was the number of participants.

participants were male (64.7 %) and reported no history of alcohol consumption (88.2 %). Additionally, most participants had no prior history of bruxism (76.5 %), sinusitis (82.4 %), or prosthetic complications (76.5 %). Notably, 41.2 % of participants reported no history of periodontitis.

Analysis of microbial diversity revealed significant differences between the GDI and GZI groups. Adonis analysis confirmed that overall microbiome distributions differed significantly between the two groups ($P < 0.05$) (Fig. 2A). Alpha diversity, assessed by amplicon sequence variants (ASVs) and the Chao1 richness index, was significantly higher in the GZI group compared with GDI ($P < 0.001$) (Fig. 2B). Beta diversity analyses demonstrated distinct community structures. Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity showed clear clustering between groups (Fig. 2C), while non-metric multidimensional scaling (NMDS) further confirmed the separation of microbial profiles (Fig. 2D). Together, these findings indicate that microbial communities associated with GDI and GZI differ significantly in both diversity and composition, despite being sampled from the same individuals.

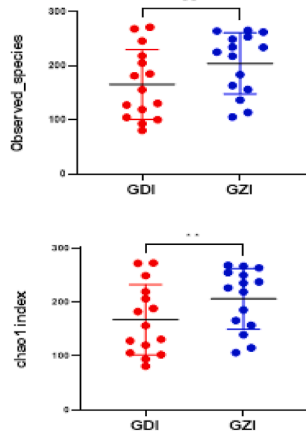
Phylum-level microbial composition

The relative abundance of the top 10 bacterial phyla was compared between GDI and GZI samples (n = 17 per group). Both groups were dominated by Actinobacteriota, Firmicutes, Fusobacteriota, Proteobacteria, and Bacteroidota (Fig. 3A). In the GDI group, Actinobacteriota represented the largest proportion of the microbiota, followed by Firmicutes, Fusobacteriota, Proteobacteria, and Bacteroidota. In contrast, the GZI group exhibited higher relative abundances of Actinobacteriota and

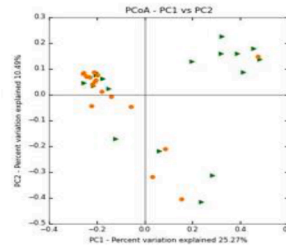
A.

Comparison Type	P-value	F value	Df - Category	Sum Of Sqs - Category	MeanSqs - Category	R2 - Category	Df - Residuals	SumOfSqs - Residuals	MeanSqs - Residuals	R2 - Residuals
GZI vs GDI	0.02	2.0654	1	0.5349	0.53487	0.06063	32	8.2868	0.25896	0.93937

B.



C.



D.

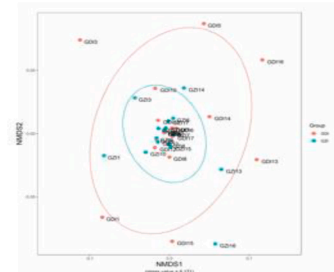


Figure 2 Comparative analysis of microbial diversity and community composition between general dental implants (GDI) and general zygomatic implants (GZI). (A) Adonis analysis of differences in microbiome composition between implant groups. (B) Alpha diversity analysis based on amplicon sequence variants (ASVs, upper panel) and the Chao1 richness index (lower panel). Group differences were assessed using Wilcoxon tests (** $P < 0.01$). (C) Beta diversity analysis by principal coordinates analysis (PCoA) showing clustering of microbial communities in GDI (brown) and GZI (green) groups. (D) Non-metric multidimensional scaling (NMDS) illustrating dissimilarity in microbial profiles between groups. Each point represents the microbiota composition of one sample; the degree of separation indicates beta diversity differences. Statistical significance was evaluated using permutational multivariate analysis of variance (PERMANOVA).

A.

Phylum top10	GDI mean	GZI mean
<u>Actinobacteriota</u>	27.60%	30.54%
<u>Firmicutes</u>	18.72%	18.35%
<u>Fusobacteriota</u>	16.99%	19.76%
<u>Proteobacteria</u>	14.12%	12.44%
<u>Bacteroidota</u>	13.60%	7.25%
Chloroflexi	2.25%	0.88%
Patescibacteria	1.73%	1.27%
Spirochaetota	1.51%	3.86%
Campilobacterota	0.99%	0.63%
Synergistota	0.70%	0.18%
Gemmatimonadota	0.32%	1.48%
Desulfobacterota	0.20%	0.68%
Others	1.07%	1.77%
Unclassified	0.19%	0.91%

B.

Taxonomy profiling at phylum level_top10

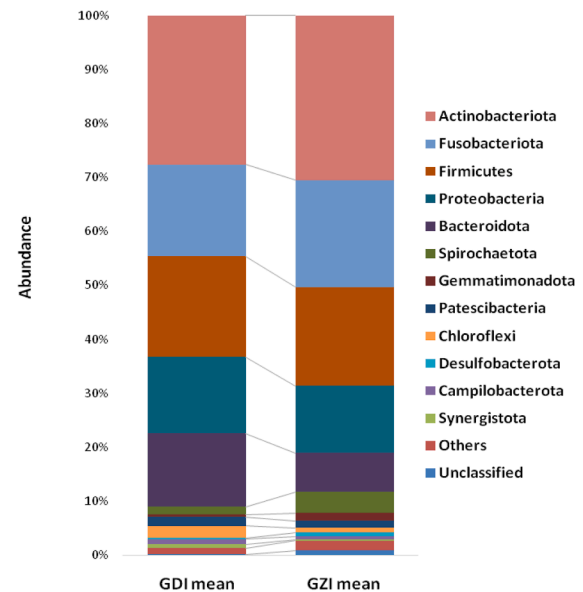


Figure 3 Comparative analysis of phylum-level microbial composition in general dental implants (GDI) and general zygomatic implants (GZI) groups. (A) Relative abundance of the top 10 bacterial phyla identified by 16S rRNA sequencing ($n = 17$ per group). Less abundant phyla were classified as "Others." (B) Bar plots showing phylum-level taxonomic composition, highlighting differences in dominant phyla between GDI and GZI groups.

Fusobacteriota, with Bacteroidota appearing slightly lower compared with GDI. These shifts suggest potential ecological differences in peri-implant microbial communities associated with zygomatic implants. Bar plot visualization further highlighted distinct phylum-level profiles between groups, with less abundant phyla grouped as “Others” (Fig. 3B). These variations reflect differential colonization patterns and microbial structures at GDI and GZI sites, even in generally healthy individuals.

Family-level microbial composition

Taxonomic profiling at the family level revealed further compositional differences between the groups (Fig. 4). In the GDI group, Actinomycetaceae (13.95 %), Fusobacteriaceae (12.55 %), Streptococcaceae (7.71 %), and Prevotellaceae (6.52 %) were most abundant. In contrast, the GZI group showed higher relative abundances of Fusobacteriaceae (18.99 %) and Streptococcaceae (10.61 %), while Actinomycetaceae (10.75 %) and Prevotellaceae (4.21 %) were reduced. Families with lower abundance were grouped as “Others.” Bar plot analysis illustrated clear differences in family-level taxonomic profiles (Fig. 4B). These findings suggest distinct ecological adaptations between implant types, with Fusobacteriaceae and Streptococcaceae emerging as potential microbial signatures of zygomatic implants. Importantly, these family-level shifts were further reflected at the genus level.

Genus-level microbial composition

The top 20 bacterial genera were compared between groups ($n = 17$ per group). Stacked bar charts revealed distinct dominant genera, with clear compositional differences between GDI and GZI (Fig. 5A–C). STAMP analysis identified several genera with significantly different relative abundances between groups ($P < 0.05$), with enrichment observed in either GDI or GZI samples (Fig. 5D). Statistical significance was determined using Welch’s t-test with Benjamini–Hochberg false discovery rate (FDR) correction. These results demonstrate distinct microbial community structures at the genus level between conventional dental and zygomatic implants.

Species-level microbial composition and sinusitis-related taxa

Species-level comparisons revealed further microbial differences between GDI and GZI groups. STAMP analysis identified species with significantly different relative abundances ($P < 0.05$, Welch’s t-test with Benjamini–Hochberg FDR correction), with several enriched in either implant type (Fig. 6A). Bar chart profiling of taxa commonly associated with sinusitis showed variations in their mean relative abundance between groups (Fig. 6B), suggesting implant type–specific associations with sinus-pathogenic bacteria. Notably, specific sinusitis-related taxa exhibited significantly

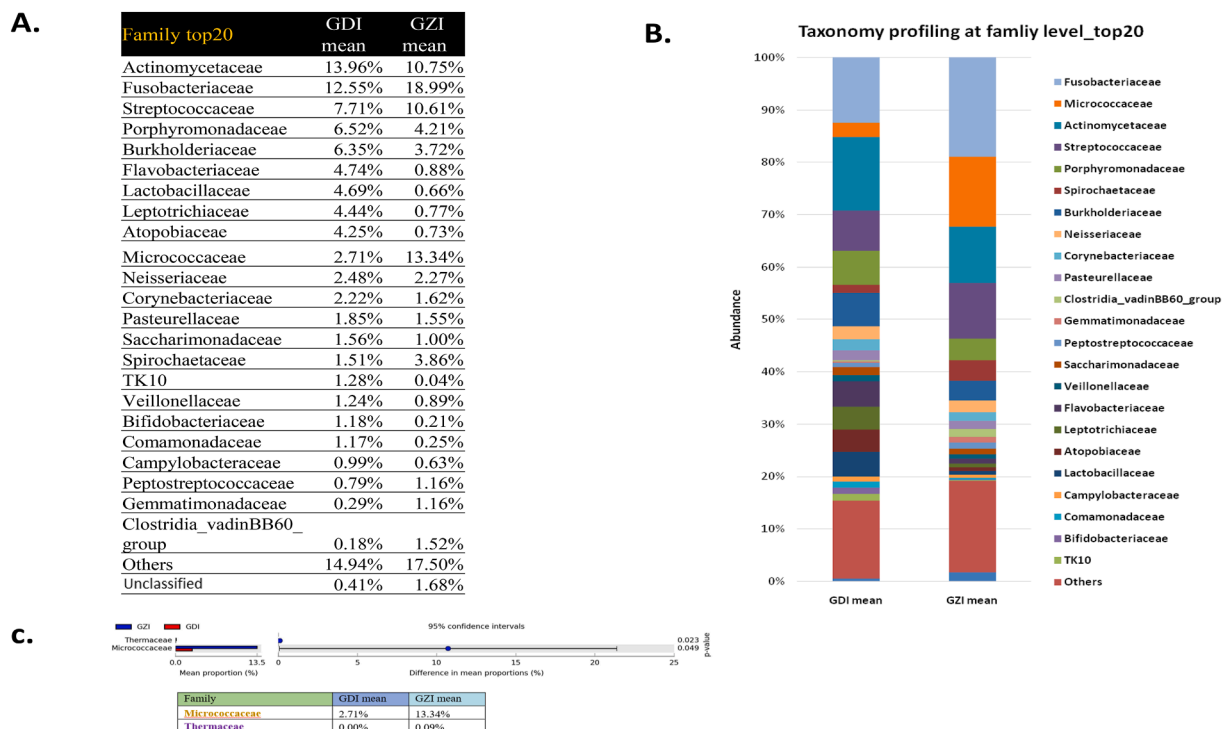


Figure 4 Comparison of the top 20 bacterial families in general dental implants (GDI) and general zygomatic implants (GZI) groups. (A) Relative abundance of the top 20 most prevalent bacterial families identified by 16S rRNA sequencing in GDI and GZI groups ($n = 17$ each). Each data point represents the mean phylum-level composition across all samples in the group; less abundant families are grouped under “Others.” (B) Bar plot visualization of families-level taxonomic composition, highlighting differences in dominant bacterial phyla between GDI and GZI. GDI: healthy volunteers with general dental implants. GZI: healthy volunteers with general zygomatic implants.

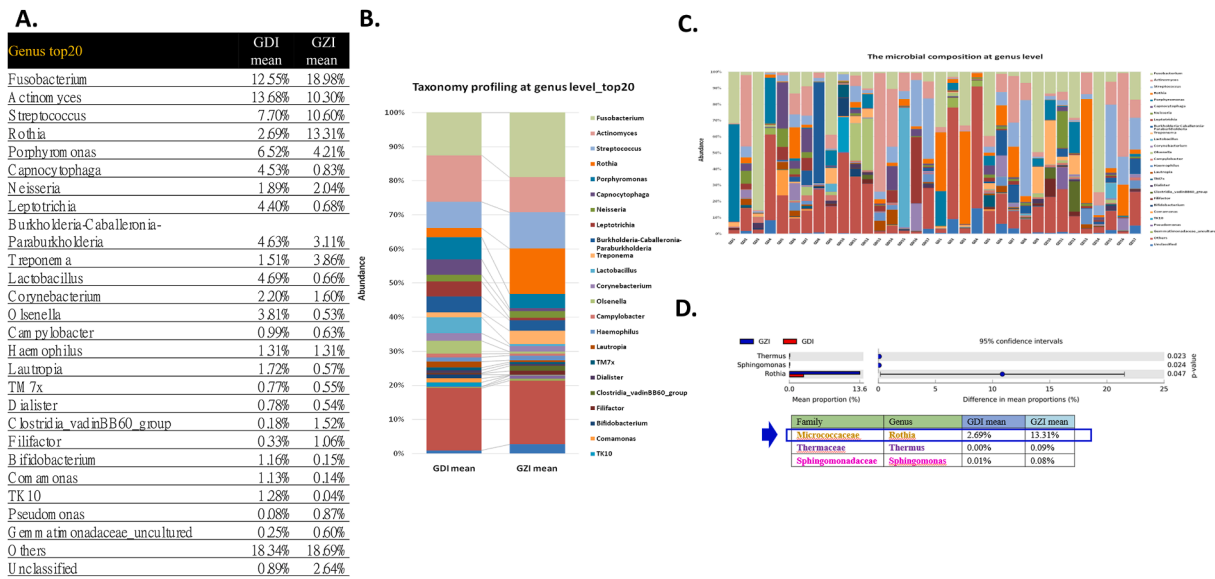


Figure 5 Comparative analysis of bacterial genera in general dental implants (GDI) and general zygomatic implants (GZI) groups. (A–C) Genus-level taxonomic composition. Stacked bar charts show the relative abundance of the top 20 bacterial genera identified by 16S rRNA gene sequencing in GDI and GZI groups ($n = 17$ each). Each bar represents the average genus-level composition per group, with less abundant genera classified as “Others.” (D) Statistical analysis of metagenomic profiles (STAMP) of differential bacterial genera between GDI and GZI. Genera with significantly different relative abundances ($P < 0.05$) are shown with 95 % confidence intervals. Enriched genera in each group are indicated. Statistical significance was determined using Welch’s t-test with Benjamini–Hochberg FDR correction.

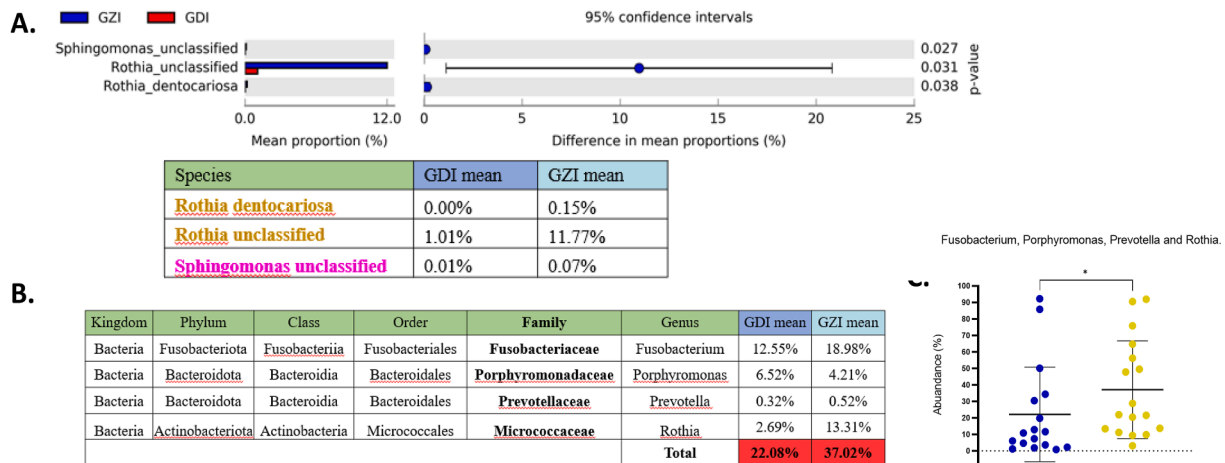


Figure 6 Comparative analysis of bacterial species and sinusitis-related microbiota in general dental implants (GDI) and general zygomatic implants (GZI) groups. (A) STAMP analysis of differential bacterial species based on 16S rRNA gene sequencing ($n = 17$ per group). Species with significantly different relative abundances ($P < 0.05$, Welch’s t-test with Benjamini–Hochberg FDR correction) are shown with 95 % confidence intervals, with enriched species indicated for each group. (B) Distribution of sinusitis-related bacterial taxa. Bar plots depict the relative abundance of genera and species commonly associated with sinusitis in GDI and GZI groups, with each bar representing the mean abundance across samples. (C) Differential abundance of key sinusitis-associated taxa between GDI and GZI. Taxa showing significant differences in relative abundance are highlighted, providing insight into the potential influence of implant type on the peri-implant sinonasal microbiome.

different abundances between GDI and GZI (Fig. 6C). Collectively, these results indicate that the peri-implant sinonasal microbiome may be influenced by implant type, with zygomatic and conventional implants harboring distinct microbial profiles.

Discussion

This study provides the first comparative analysis of peri-implant microbiota between GDI and GZI within the same individuals. By minimizing interindividual variability, our

paired-sample design allowed us to directly assess microbial differences attributable to implant type. The results demonstrated significant divergence in microbial diversity and taxonomic composition across phylum, family, genus, and species levels, highlighting distinct ecological adaptations associated with zygomatic implant sites.

Alpha diversity was significantly higher in the GZI group, suggesting that zygomatic implants may harbor more complex microbial communities than conventional implants. This contrasts with findings from previous peri-implant microbiome studies, where reduced diversity has often been associated with dysbiosis and disease progression.^{18,19} However, higher diversity in the GZI group may reflect colonization of the maxillary sinus environment, which is anatomically distinct from alveolar bone-anchored sites. Beta diversity analyses further confirmed that GZI samples clustered separately from GDI, supporting the hypothesis that anatomical location and implant design shape microbial community assembly.²⁰

At the phylum level, both groups were dominated by Actinobacteriota, Firmicutes, Fusobacteriota, Proteobacteria, and Bacteroidota, consistent with the core oral microbiome.²¹ However, the greater abundance of Actinobacteriota and Fusobacteriota in GZI suggests a shift toward taxa commonly linked to mucosal and sinus-associated niches.²² At the family level, enrichment of Fusobacteriaceae and Streptococcaceae in GZI is noteworthy, as both families are associated with biofilm formation and peri-implant inflammation.^{18,23,24} These findings suggest that zygomatic implants may provide a unique ecological niche favoring colonization by these families.

Genus-level analyses identified distinct dominant taxa between implant types, with STAMP analysis confirming several statistically significant differences. These compositional shifts mirror previously reported variations in microbial signatures between peri-implant health and peri-implantitis,^{25,26} raising the possibility that zygomatic implants may be predisposed to unique microbial colonization patterns that influence long-term clinical outcomes.

At the species level, several sinusitis-related taxa were differentially abundant between GDI and GZI. This is clinically significant because zygomatic implants often extend into or near the maxillary sinus, raising concern about potential sinonasal complications. Previous studies have linked peri-implant microbiota with maxillary sinus health,^{27,28} and our findings suggest that implant type may influence the peri-implant sinonasal microbiome. The enrichment of sinus-associated taxa in GZI underscores the need for careful monitoring of sinus health in patients with zygomatic implants.

A major strength of this study is the paired-sample design, which reduces interindividual variability and provides direct comparisons of microbial composition across implant types. The use of 16S rRNA gene sequencing and multiple statistical approaches enhances the robustness of our findings. However, limitations should be acknowledged. First, the sample size was relatively small, reflecting the limited availability of patients with both implant types. Second, 16S rRNA sequencing provides limited resolution at the species level and does not capture functional activity. Future studies using shotgun metagenomics or metatranscriptomics could provide deeper insights into functional

differences between microbial communities.²⁹ Third, while our study included generally healthy individuals, longitudinal studies are needed to assess whether these microbial differences translate into distinct clinical outcomes such as peri-implantitis or sinusitis.³⁰

In summary, this study demonstrates that peri-implant microbiota differ significantly between general and zygomatic implants across multiple taxonomic levels. Zygomatic implants were associated with greater microbial diversity, enrichment of Fusobacteriaceae and Streptococcaceae, and the presence of sinusitis-related taxa, suggesting distinct ecological and clinical implications. These findings provide a foundation for future research exploring the role of microbiota in the long-term success and complication risks of zygomatic implants.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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