International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 6, 2025
https://theaspd.com/index.php

Microbial Air Analysis in Implant Dentistry: Open Flap vs. Flapless Approach

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Abstract
Aerosol-generating procedures in dentistry pose a significant risk of airborne microbial contamination, yet evidence quantifying bioaerosol generation during implant placement—particularly comparing open flap and flapless techniques—has been lacking. In this observational study of 20 healthy patients receiving a single mandibular molar implant (10 via open flap, 10 via flapless), passive air sampling was performed with blood agar settle plates positioned centrally, near the operator, and near the assistant; CFU/dm²/hr was calculated post-incubation, and isolates were identified using API Staph and API 20 Strep biochemical tests. Statistical analysis (Wilcoxon rank-sum, Mann-Whitney U, Friedman, and Spearman's correlation; $p < 0.05$) demonstrated a significant increase in airborne microbial load during procedures (operator and assistant zones) compared to baseline ($p < 0.001$), but no difference between those zones ($p = 0.845$) or between surgical techniques at any location. Both groups, however, exhibited significant intra-procedural increases in CFU counts ($p < 0.01$), with predominant isolates including Micrococcus sp., Staphylococcus capitis, Streptococcus sp., and Staphylococcus epidermidis. These findings indicate that dental implant placement moderately but significantly elevates airborne microbial contamination regardless of flap design, underscoring the importance of stringent infection control measures and supporting passive air sampling with the IMA standard as reliable methods for evaluating and managing operatory biosafety in implantology.
Keywords: *Aerosols, Air Sampling, Bacterial Load, Biosafety, Dental Implantation, Minimally Invasive Surgical Procedures, Surgical Flaps*

INTRODUCTION

Aerosol-generating procedures (AGPs) are integral to contemporary dental practice, employing instruments such as high-speed handpieces, ultrasonic scalers, and air polishers. These devices produce aerosols comprising a mixture of air, water, saliva, blood, and microbial content, posing potential risks for airborne transmission of infectious agents within the dental operatory. Notably, aerosols generated during dental procedures predominantly consist of particles less than 5 μm in diameter, allowing them to remain suspended in the air for extended periods and settle on surfaces at considerable distances from the source (Allison et al. 2021; Harrel & Molinari 2004).

Numerous studies have identified a diverse array of microorganisms dispersed during various dental procedures. For instance, *Streptococcus viridans*, *Staphylococcus aureus*, *Actinomyces* spp., and *Fusobacterium* spp. have been detected during restorative, endodontic, and periodontal therapies (Szymanska 2007). An increased airborne microbial density during endodontic access procedures—particularly in proximity to the patient's head—has been reported (Manarte Monteiro et al. 2013). Similarly, the use of rotary instruments has been shown to significantly elevate aerosol contamination levels in the operatory environment (Rautemaa et al. 2006).

Despite extensive research on aerosol contamination associated with routine dental procedures, there is a notable paucity of evidence regarding bioaerosol generation during dental implant placement (Zemouri et al. 2017). Implant surgery typically involves the use of surgical handpieces with irrigation systems that have the potential to aerosolize microorganisms. However, no studies till date have quantified the microbial load generated specifically during implant placement or assessed the spatial distribution of bioaerosols in such settings (Bentancor Fort et al. 2023). Moreover, the impact of surgical variables—such as open flap versus flapless implant techniques—on airborne microbial contamination has not been previously evaluated.

This gap in the literature is particularly relevant in the context of infection control and occupational safety in dental implantology. Understanding the extent of airborne microbial contamination during implant placement is essential for refining operatory disinfection protocols, enhancing personal protective strategies, and ensuring the long-term success of implant outcomes by mitigating perioperative contamination (Allison et al. 2021).

The present study was designed to evaluate microbial contamination of ambient air in a controlled clinical operatory during dental implant placement. Utilizing passive air sampling techniques, we aimed to quantify and compare the colony-forming units (CFUs) isolated from blood agar plates placed before and during the procedure. Additionally, we investigated whether different surgical approaches—specifically open flap versus flapless implant placement—had any influence on airborne microbial dispersion. This investigation seeks to provide novel insights into the microbial burden associated with implant placement, thereby addressing a critical gap in aerosol-related research within implant dentistry.

METHODS

This study was designed as an observational clinical investigation. A sequential sampling technique was used to recruit patients scheduled for single implant placement in the mandibular first molar region. Inclusion criteria consisted of systemically healthy individuals (ASA I classification) aged between 25 and 60 years, presenting with a healed edentulous site and maintaining good oral hygiene with no active oral or systemic infections. Exclusion criteria included a history of smoking or tobacco use, recent antibiotic therapy within the past 30 days, immunocompromised conditions (e.g., diabetes, HIV), untreated periodontal disease, or the need for bone augmentation or simultaneous surgical interventions. These criteria ensured that microbial load variations were attributable to the surgical procedure rather than patient-related confounders (Kearney et al. 2022; Smith et al. 2021).

The study was conducted in a fully enclosed clinical operatory measuring 18.5 m^2, with ethical approval obtained from the Institutional Ethics Committee. The ventilation system remained unchanged throughout the study and was regularly inspected (Jones et al. 2020). A total of 20 patients were enrolled, with ten undergoing open flap implant placement and ten receiving flapless implants alternatively, enabling a direct comparison of surgical technique influence on microbial dispersion. Informed consent was obtained from all patients.

To control for baseline contamination, the operatory was fumigated before and after each procedure. All surfaces, including the physio dispenser, were disinfected using an aldehyde-free disinfectant. Implant handpieces and surgical kits were sterilized by autoclaving, and dental unit waterlines were disinfected daily using a 0.1% sodium hypochlorite flush followed by sterile water (Miller et al. 2019).

All procedures were performed in an air-conditioned environment, with windows kept closed throughout the clinical session. Patients performed a preprocedural oral rinse with 0.2% chlorhexidine for 60 seconds prior to entering the operatory (Marui et al. 2019). Implant osteotomy was standardized at a drilling speed of 1200 rpm with continuous irrigation at a flow rate of 75 mL/min. A single experienced right-handed implantologist performed all surgeries to eliminate operator variability in aerosol generation (Nguyen et al. 2021).

Airborne microbial contamination was assessed using a passive air sampling technique (Pasquarella et al. 2000). Three sterile 90-mm blood agar plates were used for each patient to assess microbial load. Plate 1 (Figure 1) was placed centrally in the operatory, at operatory chair armrest height, one hour prior to the commencement of the implant procedure. After the exposure period, the plate was closed with its lid. Plates 2 and 3 were positioned at 0.5 meters from the patient's oral cavity on the right and left sides, respectively, at operatory chair armrest height. These plates were exposed for the entire duration of the implant placement, which lasted approximately one hour. They were labelled accordingly (Plate 2: operator side; Plate 3: assistant side) to evaluate spatial microbial distribution near operator and assistant zones. Post-exposure, all blood agar plates were incubated aerobically at 37°C for 48 hours (Figures 2, 3, and 4). Colony-forming units (CFUs) were manually counted, and CFU/cm^2/hr was calculated (Table 1).

The qualitative analysis of airborne microbial contamination was conducted using classical microbiological identification techniques. Representative colonies were initially selected based on their

macroscopic features on blood agar, including colony colour, shape, size, texture, and haemolytic patterns. Subsequent identification involved Gram staining and evaluation of cellular morphology. Enzymatic tests such as catalase, oxidase, coagulase, and DNase assays were also performed. Based on these preliminary characteristics, isolates were further identified using commercially available biochemical test kits: API Staph and API 20 Strep (bioMérieux, Linda-a-Velha, Portugal), as appropriate.

Statistical analysis for this project involved both descriptive and inferential statistics. Initially, data were assessed for normality using the Shapiro-Wilk test, which revealed that none of the datasets were normally distributed ($p < 0.05$). Consequently, non-parametric tests were selected for subsequent analyses. Pairwise comparisons between the three plates (Plate 1, Plate 2, and Plate 3) were conducted using the Wilcoxon rank-sum test. Additionally, Spearman's rank correlation was performed to assess the strength and direction of relationships among the plates. To assess the influence of flap design on microbial dispersion, Mann-Whitney U tests were used to compare CFU values between open flap and flapless groups. Intragroup comparisons across plate locations were analysed using the Friedman test. All statistical tests were conducted with a significance level set at $p < 0.05$, using SPSS software (version X.X, IBM Corp., Armonk, NY, USA).

RESULTS

Initial testing for data normality using the Shapiro-Wilk test revealed non-normal distribution across all three groups (Plate 1, $p < 0.001$; Plate 2, $p = 0.032$; Plate 3, $p = 0.008$). Consequently, non-parametric statistical analyses were utilized. Pairwise comparisons using the Wilcoxon rank-sum test demonstrated highly significant differences between Plate 1 and Plate 2 ($U = 29.0$, $p < 0.001$), as well as between Plate 1 and Plate 3 ($U = 30.0$, $p < 0.001$). However, there was no statistically significant difference between Plate 2 and Plate 3 ($U = 192.5$, $p = 0.845$).

Correlation analysis using Spearman's rank correlation test showed weak and non-significant correlations between Plate 1 and Plate 2 ($\rho = 0.179$, $p = 0.450$), and Plate 1 and Plate 3 ($\rho = 0.118$, $p = 0.621$). However, a moderate positive correlation approaching borderline significance was observed between Plate 2 and Plate 3 ($\rho = 0.432$, $p = 0.057$).

When stratified by surgical approach, Mann-Whitney U test comparisons revealed no statistically significant differences in CFU/dm²/hr values between the open flap and flapless groups at any of the three plate locations (Plate 1: $p = 0.696$; Plate 2: $p = 0.782$; Plate 3: $p = 0.757$). However, within-group comparisons using the Friedman test indicated significant differences across plates in both open flap ($\chi^2 = 9.50$, $p = 0.009$) and flapless ($\chi^2 = 14.26$, $p < 0.001$) groups, confirming an overall rise in airborne microbial load during surgical activity in both protocols.

When patients were grouped based on surgical approach—with the first ten undergoing open flap and the next ten undergoing flapless implant placement—no statistically significant differences were found in CFU/dm²/hr values at any plate location between the two groups. Mann–Whitney U test comparisons yielded non-significant p-values for Plate 1 ($p = 0.696$), Plate 2 ($p = 0.782$), and Plate 3 ($p = 0.757$), indicating that flap design did not significantly influence airborne microbial counts during implant placement. However, within-group analysis using the Friedman test revealed statistically significant differences across the three plate positions in both the open flap ($\chi^2 = 9.50$, $p = 0.009$) and flapless ($\chi^2 = 14.26$, $p < 0.001$) groups. Spearman's rank correlation showed no significant relationship between preprocedural and intraoperative contamination levels in the open flap group, whereas the flapless group demonstrated a moderate positive correlation between Plate 1 and Plate 2 values ($\rho = 0.619$, $p = 0.057$). Gram-positive cocci were the predominant microorganisms identified in the samples. The isolates included Micrococcus sp. (99.9%), Staphylococcus capitis (99.8%), Streptococcus sp. (99.9%), Staphylococcus epidermidis (84.8%), and Staphylococcus sp. (99.9%).

DISCUSSION

The present study is the first to specifically quantify airborne microbial contamination during dental implant placement using standardized passive air sampling. Our findings revealed mean CFU/dm²/hr values of 2.4 ± 0.7 for Plate 1 (pre-procedural), 5.6 ± 1.9 for Plate 2 (operator zone), and 6.1 ± 2.4 for Plate 3 (assistant zone). Statistically significant increases were noted from baseline (Plate 1) to intra-

procedural values (Plates 2 and 3) ($p < 0.001$), while no significant difference was observed between Plates 2 and 3 ($p = 0.845$). This uniform aerosol dispersion suggests symmetrical microbial spread across operative zones during implant osteotomy.

In a meta-analysis (Pasquarella et al. 2025), IMA values (equivalent to CFU/dm²/hr) during procedures ranged from 19 to 53.3, with a pooled mean of 33 CFU/dm²/hr, significantly higher than the implant-related values reported in this study. For instance, restorative dentistry recorded values up to 39.6 CFU/dm²/hr, periodontics up to 53.3, and endodontics around 35–40 CFU/dm²/hr. Other studies (Manarte Monteiro et al. 2013; Hosseini et al. 2014) demonstrated elevated microbial air contamination particularly during ultrasonic scaling, periodontal instrumentation, and extractions, likely due to higher aerosolization potential and tissue manipulation. In contrast, the lower values in implantology, as observed in our study, may be attributed to minimal soft tissue trauma, enclosed surgical drilling systems, preoperative mouth rinses, and strict sterilization protocols.

Moreover, our CFU values remain well below the proposed IMA threshold of 33 CFU/dm²/hr for intra-procedural dental settings, indicating that implant placement in a controlled operatory qualifies as a low-risk aerosol-generating procedure. The percent increase in CFU from Plate 1 to Plate 3 was approximately 154%, markedly lower than the 230% spike reported in general dental treatments (Pasquarella et al. 2025).

The Spearman correlation analysis in our study showed weak non-significant associations between Plate 1 and intraoperative plates, indicating that ambient contamination played a minimal role in the observed microbial load. A moderate correlation between Plates 2 and 3 ($\rho = 0.432$, $p = 0.057$) suggests simultaneous aerosol exposure at both operator and assistant zones, supporting ergonomic and ventilation planning.

These results confirm that implant surgery—regardless of flap elevation—leads to a significant rise in aerosolized microbial contamination throughout the intraoperative phase. The lack of between-group differences suggests that both open flap and flapless techniques generate similar levels of airborne dispersion under standardized conditions. The moderate correlation observed in the flapless group implies that baseline ambient contamination may exert a greater influence when soft-tissue disruption is minimized. Clinically, this underscores the importance of stringent aerosol control measures across all surgical protocols, as the choice of flap design alone does not mitigate microbial spread.

Microbial species isolated were predominantly Gram-positive cocci, including *Micrococcus sp.*, *Staphylococcus capitis*, *Streptococcus sp.*, *Staphylococcus epidermidis*, and *Staphylococcus sp.*, as determined by API Staph and API 20 Strep systems. These organisms are consistent with those reported in previous air microbiota studies in dental clinics (Petti et al. 2003; Manarte Monteiro et al. 2013), where staphylococci and streptococci were frequent due to their origin in human skin and oral cavity. While *Streptococcus* species suggest oral origin of aerosols, *Staphylococcus* and *Micrococcus* species are more indicative of human presence and environmental shedding.

Clinically, our findings support enhanced precautions during implant surgery. Although CFU levels are lower than in other dental specialties, microbial dispersion remains evident. Strategies such as high-volume suction, preprocedural rinses, barrier protection, and air filtration should be integrated into implant operatory protocols. Our data also reinforce the utility of settle plate-based microbial monitoring using the IMA standard, as a practical infection control tool. Furthermore, the use of settle plates provides a cumulative representation of microbial fallout, which is especially relevant for procedures requiring aseptic field maintenance, like implant placement.

Future research should aim to compare implant-related bioaerosol levels under variable ventilation, with and without adjunctive mitigation devices, and expand into species-specific pathogen analysis using molecular techniques.

LIMITATIONS

The main limitations of this study include its relatively small sample size and focus on a single implant site and patient cohort, which may limit generalizability across different anatomical regions and patient populations. Passive air sampling captures only settling particles and may underestimate total aerosolized load, and the exclusive use of blood agar restricts detection to cultivable bacteria, omitting fungi and

viruses. Future research should employ larger, multisite cohorts, integrate active air-sampling and molecular detection methods to quantify a broader spectrum of pathogens, and evaluate the efficacy of mitigation strategies—such as high-volume evacuation, air purification systems, and procedural modifications—on reducing bioaerosol dispersion during implant surgery.

CONCLUSION

This study is the first to comprehensively quantify and compare airborne microbial contamination during dental implant placement, with additional evaluation of the influence of surgical approach on aerosol dispersion. The findings revealed that although implant osteotomy generates a measurable increase in airborne CFUs, the microbial load remains significantly lower than that observed in other aerosol-generating dental procedures. Importantly, no statistically significant difference was found between open flap and flapless techniques, suggesting that implant-related bioaerosol contamination is more influenced by procedural factors common to both approaches—such as drilling and irrigation—rather than surgical access design. These results underscore the importance of implementing universal infection control protocols in all implant surgeries, regardless of technique.

RECOMMENDATIONS

To enhance biosafety in implantology, practitioners should adopt and rigorously enforce standardized infection control measures—such as high-volume evacuation, surgical draping, and preprocedural mouth rinses—for every implant procedure and ensure that clinical policies and training programs emphasize that flap design (open versus flapless) does not substantially influence bioaerosol risk. Implant surgery suites should also integrate environmental biosafety improvements, including optimized operatory ventilation and HEPA-filtered air purification systems. Finally, the scientific community is encouraged to pursue further research comparing bioaerosol generation during implant placement with other aerosol-generating procedures, to refine risk-management protocols across all dental disciplines.

ACKNOWLEDGEMENTS

None

FUNDING

None

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL

Ethical approval was obtained from the Institute Ethics Committee, in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.

DISCLOSURE STATEMENT

There are no relevant financial or non-financial competing interests to report.

DISCLAIMER

None

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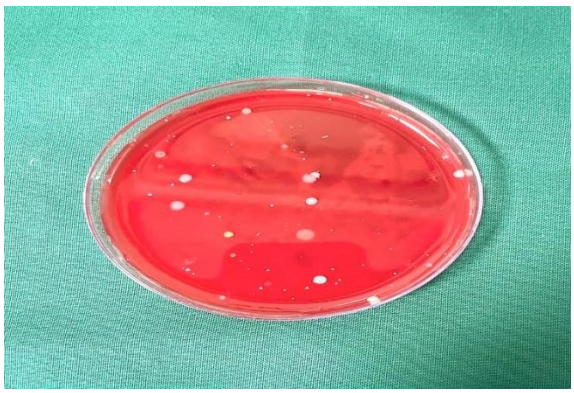
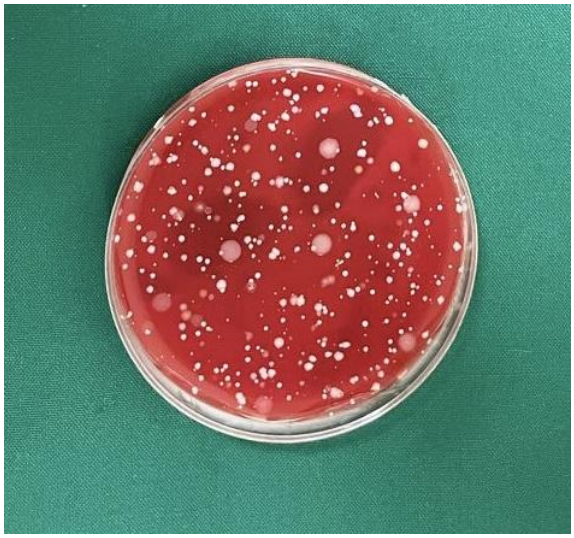
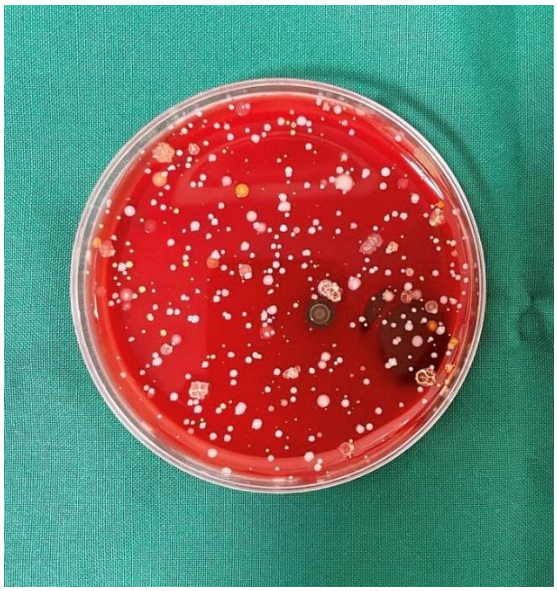
1.Plate 1 prior to the start of the procedure



2.Plate 1 after incubation



3.Plate 2 after incubation



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Patient	Plate 1	Plate 2	Plate 3
1	1.6	4.7	6.3
2	3.1	4.7	3.1
3	4.7	3.1	3.1
4	1.6	6.3	6.3
5	1.6	6.3	4.7
6	1.6	3.1	6.3
7	4.7	6.3	14.1

Table 1. Mean CFU/dm²/hr Recorded From 20 Patients Across Sampling Plates

8	1.6	7.9	7.9
9	1.6	4.7	3.1
10	1.6	6.3	4.7
11	3.1	7.9	9.4
12	3.1	6.3	4.7
13	1.6	3.1	4.7
14	1.6	6.3	3.1
15	1.6	3.1	6.3
16	1.6	6.3	4.7
17	3.1	9.4	9.4
18	1.6	3.1	6.3
19	3.1	4.7	3.1
20	3.1	6.3	7.9

Table 2: Wilcoxon Rank-Sum Test Results for Pairwise Comparisons Between Sampling Plates

Comparison	U Statistic	p-value
IMA Plate 1 vs IMA Plate 2	29	2.00E-06
IMA Plate 1 vs IMA Plate 3	30	2.41E-06
IMA Plate 2 vs IMA Plate 3	192.5	0.844979078

Table 3: Spearman's Rank Correlation Between Sampling Plate CFU Values

Comparison	Spearman Correlation	p-value
Plate 1 vs Plate 2	0.179	0.45
Plate 1 vs Plate 3	0.118	0.621
Plate 2 vs Plate 3	0.432	0.057

Table 4: Mann-Whitney U Test Comparing Open Flap and Flapless Groups at Each Sampling Plate

Plate	U-statistic	p-value
Plate 1	45	0.696
Plate 2	46	0.782
Plate 3	45.5	0.757

Table 5: Friedman Test Results for Intra-Group CFU Differences Across Plates

Group	Chi-square	p-value
Open Flap	9.5	0.009
Flapless	14.26	0.001

Table 6: Spearman Correlation Between Baseline and Intra-Procedural CFU Levels in Each Group

Group	Comparison	Spearman's rho	p-value
Open Flap	Plate 1 vs Plate 2	-0.267	0.456
Open Flap	Plate 1 vs Plate 3	-0.131	0.718
Flapless	Plate 1 vs Plate 2	0.619	0.057
Flapless	Plate 1 vs Plate 3	0.356	0.313