

Identification of bioaerosol composition in preclinical dental laboratories using MALDI-TOF MS

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ABSTRACT

Aims: The grinding and polishing procedures carried out during dental training result in the release of high levels of particulate matter and microorganisms into the environment. This study aimed to assess the microbial air quality in the preclinical laboratory environment and to identify the predominant species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Methods: Sampling was performed before and during the preclinical session using the passive deposition method. The isolated microorganisms were identified using MALDI-TOF MS.

Results: The total microbial load was 22.67 ± 1.15 CFU/plate prior to the preclinical session, whereas during the session it rose to 169.00 ± 13.86 colony forming units (CFU)/plate, representing an approximately 7.5-fold increase. During the study, fungal pathogens such as *Aspergillus* spp. and *Candida tropicalis*, as well as *Staphylococcus* and *Micrococcus* species, were found to be predominant.

Conclusion: Laboratory activities increase the microbial load by approximately 7.5-fold. This highlights the importance of adequate ventilation and the use of personal protective equipment.

Keywords: Bioaerosol, air pollution, preclinical training, MALDI-TOF MS

INTRODUCTION

Dental training involves intensive practical sessions in preclinical laboratories. The high-speed rotary instruments used during this process lead to the formation of bioaerosols, which consist of liquid and solid particles containing microbial agents. Bioaerosols are microscopic particles that can remain suspended in the air for long periods and reach the depths of the lungs (alveoli) via the respiratory tract.¹

Preclinical laboratories are areas where the risk of cross-contamination is high due to the large number of students working simultaneously. The literature reports that particulate matter released into the environment during dental procedures is not merely a physical contaminant but also acts as a carrier (vector) for pathogenic microorganisms. In particular, dust-generating procedures such as acrylic restoration and tooth cutting significantly increase the microbial load (CFU/plate) in the environment.² The aim of this study is to characterize the microbial diversity in a dental faculty preclinical laboratory and to assess the microbial load in the ambient air using the passive deposition method. It was hypothesized that active acrylic trimming procedures would increase both the airborne microbial load and microbial diversity in the preclinical laboratory compared with the baseline period.

METHODS

Ethics

This study was conducted as an in vitro environmental microbiological assessment in a preclinical dental laboratory. No human participants, patient data, human biological samples, or animal subjects were involved. Therefore, according to institutional practice, ethics committee approval was not required for this study.

Study Design

The study was conducted at the Preclinical Laboratory of Dicle University. The preclinical laboratory had a capacity of approximately 150 students, and 70 students were present during the experimental session. During the active sampling period, the students performed acrylic trimming procedures using micromotors and rotary burs. The exact operating speed (rpm) of the micromotors was not recorded. Local suction systems located beneath each workstation were in operation during the procedures. The air-conditioning system was operating, and the doors and windows were open throughout the sampling period. Room temperature and relative humidity were not recorded.

Passive air sampling was performed at three designated locations on the same day. Baseline sampling was conducted

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in the morning, following routine cleaning of the laboratory and before the students entered and laboratory activities commenced. Petri dishes were placed on work surfaces approximately 1 m above floor level and exposed for 1 h. Sampling was subsequently repeated for 1 h at the same designated locations while 70 students were performing acrylic trimming procedures. One sampling point was located on the academic staff desk, approximately 1.5 m from the student working area, whereas the other two sampling points were located on the student workstations closest to the academic staff desk. The sampling points were approximately 1.5 m apart.

The passive settling method was used to determine the microbial load in the ambient air. This method is based on the principle that microorganisms settle onto the surface of the culture medium under the influence of gravity.³

As part of the passive settling method used to assess the load of microorganisms in the air, microorganisms that settled onto culture media left exposed for specific periods were isolated following incubation. Pure cultures obtained from the developing colonies were identified in the microbiology laboratory using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Zybio Inc., China), a method based on protein fingerprint analysis.^{4,5} The results obtained enabled the qualitative and species-level assessment of microbial contamination in ambient air.

For sampling purposes, blood agar was used to assess total bacterial count and haemolytic properties; EMB agar was used for the selective isolation of gram-negative bacteria; and Sabouraud dextrose agar was used to detect fungal (yeast and mould) contamination.

Following sampling, the Petri dishes were transferred to the microbiology laboratory; bacteria were incubated at 37°C for 24–48 hours, whilst fungi were incubated at 25°C for 5–7 days. At the end of incubation, the developed colonies were counted, and the results were recorded as colony forming units (CFU/plate). Although EMB agar was used for the selective isolation of gram-negative bacteria, as these microorganisms also grew on blood agar, species-level identification and colony counting were carried out using isolates obtained from colonies that developed on blood agar.

Statistical Analysis

Descriptive statistics were expressed as mean±standard deviation (SD). Since measurements before and during acrylic trimming were obtained from the same three sampling locations, the observations were treated as paired. The normality of the paired differences was assessed using the Shapiro-Wilk test. No statistically significant deviation from normality was detected ($p=0.132$); therefore, microbial loads before and during acrylic trimming were compared using a paired-samples t-test. Statistical significance was set at $p<0.05$. Statistical analyses were performed using Python with the SciPy statistical package.

RESULTS

Measurements carried out in a preclinical laboratory setting revealed significant differences in microbial load and diversity before and during laboratory activities.

It was determined that during preclinical procedures, the total microbial load increased from 22.67 ± 1.15 CFU/plate to 169.00 ± 13.86 CFU/plate, representing an approximate 7.5-

fold increase. Statistical analysis between the groups revealed that the difference was statistically significant ($p=0.003$).

Prior to the preclinical session, a limited number of bacterial species were isolated, while no fungal growth was detected. The main microorganisms isolated during this period were *Staphylococcus hominis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Bacillus* species (Table 1).

Table 1. CFU/plate distribution of microorganisms isolated prior to preclinical laboratory testing

Microorganism	Plate 1 (CFU/plate)	Plate 2 (CFU/plate)	Plate 3 (CFU/plate)
<i>Staphylococcus hominis</i>	7	4	7
<i>Micrococcus luteus</i>	7	5	10
<i>Staphylococcus epidermidis</i>	4	8	3
<i>Bacillus</i> spp.	2	4	2
<i>Mesobacillus thioparans</i>	1	0	0
<i>Pseudarthrobacter oxydans</i>	2	1	0
<i>Oerskovia turbata</i>	1	0	0

CFU: Colony forming units

In contrast, a marked increase in both microbial diversity and colony density was observed during preclinical trials (Figure). During this period, in addition to species such as *Micrococcus luteus*, *Staphylococcus pasteurii*, *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Streptococcus mitis/oralis*, environmental and potentially pathogenic microorganisms (*Escherichia coli*, *Pseudomonas oryzihabitans*, and *Trueperella pyogenes*) were also isolated (Table 2).



Before Preclinical Training

During Preclinical Training

Figure. Appearance of colonies obtained using the passive agar plate method in a preclinical laboratory setting

Table 2. Distribution of microorganisms isolated during preclinical laboratory testing, expressed as CFU per plate

Microorganism	Plate 1 (CFU/plate)	Plate 2 (CFU/plate)	Plate 3 (CFU/plate)
<i>Micrococcus luteus</i>	45	80	50
<i>Staphylococcus pasteurii</i>	15	10	18
<i>Staphylococcus xylosus</i>	10	7	8
<i>Staphylococcus epidermidis</i>	15	20	28
<i>Staphylococcus haemolyticus</i>	31	15	10
<i>Streptococcus mitis/oralis</i>	15	5	7
<i>Bacillus subtilis</i>	3	8	11
<i>Corynebacterium callunae</i>	3	2	4
<i>Moraxella osloensis</i>	9	18	7
<i>Pseudomonas oryzihabitans</i>	1	3	1
<i>Staphylococcus warneri</i>	8	7	13
<i>Escherichia coli</i>	2	1	0
<i>Trueperella pyogenes</i>	1	2	0
<i>Aspergillus</i> spp.	2	5	4
<i>Candida tropicalis</i>	1	2	0

CFU: Colony forming units



Furthermore, fungal microorganisms not detected prior to the preclinical phase emerged during the procedures; in particular, species of *Aspergillus* spp. and *Candida tropicalis* were isolated.

The data obtained indicate that during laboratory activities, not only did the microbial load increase, but the diversity of microorganisms present in the environment also expanded significantly (Table 2).

DISCUSSION

In this study, the effect of procedures carried out in a preclinical dental laboratory setting on microbial air contamination via bioaerosol formation was evaluated. The passive deposition method was used to determine the microbial load and bioaerosol diversity in the ambient air.

The findings revealed a significant increase in the microbial load during preclinical procedures. The average colony count prior to preclinical procedures was approximately 22.67 ± 1.15 CFU/plate; this value rose to approximately 169.00 ± 13.86 CFU/plate during preclinical procedures, demonstrating that laboratory activities significantly increased the biological load in the ambient air. This increase is consistent with studies reporting that aerosol and particle formation during dental procedures enhance microbial spread.^{6,7}

Upon examining the distribution of microorganisms, it was observed that *Staphylococcus*, *Micrococcus*, and *Bacillus* species were predominant. These species are frequently reported in dental aerosol studies and are among the microorganisms that may be of human origin. However, the fact that species such as *Escherichia coli*, *Pseudomonas oryzihabitans*, and *Trueperella pyogenes* were detected only during preclinical procedures suggests that environmental contamination, as well as human sources, may be a factor in the environment.^{7,8}

A particularly striking finding in this context is that, although certain microorganisms were not detected prior to the preclinical phase, they appeared in high concentrations only during the procedure. This suggests that these microorganisms were introduced into the environment via bioaerosols generated during laboratory activities, rather than originating from the ambient flora. Such a 'absence-to-presence' transition can be interpreted as a direct indicator of bioaerosol formation.⁷

The findings regarding fungal contamination are similarly noteworthy. While no fungal growth was detected prior to preclinical procedures, the isolation of *Aspergillus* spp. and *Candida tropicalis* during procedures suggests that fungal spores can remain airborne and become mobilized in the environment due to particle formation. This indicates that bioaerosols in dental laboratory environments contain not only bacterial but also fungal components.⁹

Increased microbial load and diversity pose a potential exposure risk, particularly for students working for extended periods in preclinical laboratories. Bioaerosols generated during procedures performed at close range are significant in terms of cross-contamination and respiratory exposure.⁶ Furthermore, previous studies assessing airborne particles and microbial components have highlighted the potential occupational health risks associated with bioaerosol exposure in such environments.^{7,10}

In light of these findings, it is important to enhance the effectiveness of ventilation systems in preclinical laboratory settings, utilize high-vacuum aspiration systems, and standardize the use of personal protective equipment. The implementation of engineering controls, such as air purification systems fitted with HEPA filters, may also contribute to improving ambient air quality.^{2,6}

Recent studies have further emphasized the importance of monitoring and controlling aerosols in dental environments. Dental procedures may generate particles of varying sizes with a microbiological component, highlighting their potential relevance to cross-contamination in clinical environments.¹¹ In preclinical dental training settings, ventilation, air-conditioning, and evacuation strategies have also been investigated as important factors influencing indoor air quality during aerosol-generating procedures.¹² Furthermore, recent systematic evidence indicates that aerosol-control measures, particularly suction devices, can reduce microbial contamination generated during dental procedures.¹³ These findings support the importance of appropriate ventilation and aerosol-control strategies in dental training environments where multiple individuals work simultaneously.

Limitations

The limitations of this study include the use of passive sampling methods and the limited duration of sampling. The relatively short sampling period may not fully capture temporal variations in microbial load and diversity throughout different laboratory sessions or time periods. In future studies, longer and repeated sampling periods, the use of active air sampling methods, and data obtained from larger sample groups would contribute to a more comprehensive assessment of microbial dynamics in dental laboratory environments.

CONCLUSION

This study demonstrates that procedures carried out in a preclinical dental laboratory setting significantly increase microbial air contamination via bioaerosol formation. A statistically significant increase was observed in both total colony count and microbial diversity during preclinical procedures; in particular, the fact that certain microorganisms appeared only during the procedures suggests that these species were introduced into the environment via bioaerosols. Furthermore, the isolation of fungal microorganisms that were not detected prior to the preclinical procedures indicates that particle formation may contribute to the suspension of fungal spores in the air during these procedures.

The findings indicate that preclinical laboratories are not merely educational environments but also high-risk work areas where potential exposure to bioaerosols is a concern. Consequently, the use of effective ventilation systems, the widespread adoption of high-vacuum aspiration systems, and the routine use of personal protective equipment are recommended.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study did not require ethical approval as it did not involve any human subjects or animal experiments.



Informed Consent

Since the study was conducted without the participation of any living being, no written consent form was obtained.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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Author Contributions

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