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Influence of different cleaning methods on the concentration of airborne endotoxins and microbial aerosols in the oral clinical environment

Yaru Du¹, Ran Tao², Meiling Shi³, Bing Liu⁴ and Fei Zhao^{4*}

Abstract

Aim This study aims to evaluate the effectiveness of various cleaning methods in reducing airborne endotoxin and microbial aerosols during oral cleaning procedures.

Method Forty patients undergoing oral cleaning procedures were randomly assigned to one of four groups ($n = 10$ per group). Group A received strong suction alone; Group B received strong suction combined with an air disinfection machine; Group C received strong suction combined with a dental electric suction machine; Group D received strong suction in conjunction with both an air disinfection machine and a dental electric suction machine. Airborne aerosol concentrations were assessed at four-time points: before treatment, 30 min into treatment, immediately after treatment, and 60 min after treatment ended. Samples were collected at distances of 20 cm, 60 cm, and 1 m from the patient's oral cavity using the natural sedimentation method. T-test was used to evaluate the difference among tested groups.

Results Airborne endotoxins and microbial aerosols levels increased significantly during treatment, with the highest levels observed at 20 cm from the patient's mouth. During treatment, groups with additional cleaning methods (Groups B, C, and D) exhibited higher levels of airborne endotoxins and microbial aerosols compared to Group A (strong suction alone). However, post-treatment analysis revealed that Group D demonstrated the lowest level of airborne endotoxins and microbial aerosols, while Group A exhibited the highest.

Conclusions Implementing effective aerosol management strategies can significantly reduce aerosol dispersion in the oral clinical environment. Continuous monitoring aerosol concentrations and the application of appropriate control measures are essential for minimizing infection risks for both patients and healthcare providers during oral cleaning procedures.

Keywords Airborne endotoxins, Microbial aerosols, Natural settling method, Oral clinical environment, Surface sampling method

*Correspondence:

Fei Zhao
zhaofei@hebmu.edu.cn

Full list of author information is available at the end of the article



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Background

The oral clinical environment is particularly vulnerable to bioaerosol contamination due to the extensive use of medical instruments that generate substantial quantities of aerosols [1, 2]. The oral cavity, a known reservoir for microorganisms, contributes to the production of microbial aerosols, potentially harboring pathogens such as *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, and *Legionella* [3]. These aerosols can easily enter the respiratory tract during oral procedures, posing significant health risks, including mucosal irritation, congestion, edema, bleeding, and ulceration.

Bioaerosols, particularly microbial aerosols, have a significant impact on human health. The World Health Organization reports that 14 out of 41 major infectious diseases worldwide are transmitted through microbial aerosols with respiratory infections from these aerosols contributing to nearly 20% of global cases [4]. Particles with diameters between 1 and 5 μm are of particular concern as they can penetrate deeply into the alveoli, leading to severe pulmonary infections [5].

Endotoxins, specific biohazardous substances produced by bacteria during metabolism, primarily originate from the cell walls of Gram-negative bacteria and cyanobacteria. Comprising a mixture of lipopolysaccharides (LPS) and proteins, endotoxins are highly toxic [6, 7]. These lipophilic organic substances can dissolve cell membranes, disrupt membrane proteins, and induce cell death, leading to bacterial lysis. Upon entering the bloodstream, endotoxins can disseminate throughout the circulatory system, affecting various organs and tissues, potentially leading to organ dysfunction or failure. Individuals with compromised immune systems are particularly vulnerable to severe infections and even death resulting from endotoxin exposure [7].

While various methods, including high-volume suction systems, air disinfection machines, and dental electric suction machines, are employed to manage dental aerosols [1, 2, 8], there is limited understanding of the most effective combination of techniques for reducing airborne endotoxins and microbial aerosols in dental clinical environments [9]. Given the potential risks associated with microbial aerosols and endotoxins, it is essential to explore and refine methods for reducing their concentration in dental settings to improve infection control and ensure a safer clinical environment. Therefore, this study aims to investigate methods for reducing airborne endotoxins and microbial aerosols during oral cleaning procedures to improve infection control and ensure a safer clinical environment.

Materials and methods

Study subjects

Forty patients undergoing oral cleaning procedures in the dental cleaning room from March 2022 to December 2022 were included in the present study. Patients were randomly assigned to one of the four groups: Group A ($n=10$) received oral cleaning with a strong suction device alone, Group B ($n=10$) received oral cleaning with a strong suction device (A-dec300, U.S.) plus an air disinfection machine (KeK®XD-B800, China), Group C ($n=10$) received oral cleaning with a strong suction device plus a dental electric suction machine (AeroVac Pro D, China), and Group D ($n=10$) received oral cleaning with a strong suction device plus an air disinfection machine and a dental electric suction machine.

Sampling

The study utilized both natural sedimentation and surface sampling methods. Aerosol content was assessed at distances of 20 cm, 60 cm, and 1 m from the patient's mouth at four-time points: 0 min before treatment, 30 min after treatment started, immediately after the treatment completion, and 60 min after treatment ended. The natural sedimentation method involved a 10-minute sampling duration.

Surface sampling was performed using sterile swabs moistened with phosphate-buffered saline (PBS). Swabs were taken from the right arm of the operator and the left arm of the assistant at the following time points: 5 min before treatment, immediately before treatment, 30 min after treatment started, and at the end of treatment. The swabs were transported to the laboratory within 2 h of collection.

Airborne endotoxin measurement

Airborne endotoxin was sampled using an air microbiological sampler. The sampler was placed at the center of the clinic, 1.5 m above the ground. Sampling was performed with 50 ml of pyrogen-free water as the medium, an airflow of 12.5 L/min, and a sampling duration of 20 min. Sampling was conducted at three equidistant diagonal positions, each measured three times. Samples were transported to the laboratory under refrigerated conditions (4 °C) and processed within 24 h. The pH was adjusted to between 7 and 8, and endotoxin concentration was determined using the Limulus Amebocyte Lysate (LAL) assay. The endotoxin concentration per cubic meter of air in the ward is calculated using the following formula: Concentration of airborne endotoxins per cubic meter of air = $(1000 \times \text{endotoxin concentration in the sample} \times \text{volume of the sampling medium}) / (\text{AGI airflow rate} \times \text{sampling time})$.

Table 1 Distribution of airborne endotoxins and microbial aerosols at different distances

Parameters		20 cm	60 cm	1 m
Before treatment	Airborne endotoxins / EU(m ³) ⁻¹	67.52 ± 27.45	67.85 ± 24.85	67.57 ± 22.75
	Microbial aerosols / CFU(m ³) ⁻¹	8.75 ± 2.87	8.85 ± 2.84	8.77 ± 2.65
During treatment (30 min)	Airborne endotoxins / EU(m ³) ⁻¹	68.52 ± 27.45	67.85 ± 24.85	67.57 ± 22.75
	Microbial aerosols / CFU(m ³) ⁻¹	8.45 ± 2.91	8.38 ± 1.83	8.17 ± 2.04
After treatment (0 min)	Airborne endotoxins / EU(m ³) ⁻¹	169.45 ± 26.45 ^{ab}	134.54 ± 22.77 ^b	110.88 ± 21.75
	Microbial aerosols / CFU(m ³) ⁻¹	59.75 ± 24.58 ^{ab}	45.78 ± 18.78 ^b	40.81 ± 20.75
After treatment (60 min)	Airborne endotoxins / EU(m ³) ⁻¹	99.78 ± 37.58 ^{ab}	78.78 ± 34.88 ^b	75.45 ± 21.54
	Microbial aerosols / CFU(m ³) ⁻¹	30.74 ± 16.87 ^{ab}	27.75 ± 15.75 ^b	22.75 ± 14.85

Note: ^a Compared with 60 cm, *P*-value < 0.05; ^b Compared with 1 m, *P*-value < 0.05

Table 2 Distribution of airborne endotoxins and microbial aerosols in the oral clinical environment among four groups of patients

Parameters		Group A	Group B	Group C	Group D
Before treatment	Airborne endotoxins / EU(m ³) ⁻¹	68.27 ± 27.89	67.93 ± 21.87	66.35 ± 25.43	62 ± 24.08
	Microbial aerosols / CFU(m ³) ⁻¹	8.96 ± 2.57	8.77 ± 2.39	8.78 ± 3.04	8.85 ± 3.92
During treatment	Airborne endotoxins / EU(m ³) ⁻¹	175.12 ± 69.6 ^a	114.07 ± 65.02 ^{ac}	113.33 ± 67.2 ^{ac}	70.24 ± 25.18 ^{acde}
	Microbial aerosols / CFU(m ³) ⁻¹	58.25 ± 28.9 ^a	28.06 ± 11.6 ^{ac}	26.48 ± 17.3 ^{ac}	10.79 ± 4.85 ^{acde}
After treatment	Airborne endotoxins / EU(m ³) ⁻¹	95.9 ± 41.87 ^a	78.89 ± 37.24 ^{ac}	75.48 ± 39.91 ^{ac}	68.71 ± 15.39 ^{acde}
	Microbial aerosols / CFU(m ³) ⁻¹	27.59 ± 17.95 ^a	23.28 ± 13.53 ^{ac}	21.79 ± 13.59 ^{ac}	9.85 ± 2.65 ^{acde}

Note:

^a Compared with before treatment, *P*-value < 0.05;

^b Compared with during treatment, *P*-value < 0.05;

^c Compared with the Group A, *P*-value < 0.05;

^d Compared with the Group B, *P*-value < 0.05

^e Compared with the Group C, *P*-value < 0.05;

Microbial aerosol measurement

Microbial aerosols were sampled using an impact air microbiological sampler, placed at the center of the dental clinic, 1.5 m above the ground, with an air velocity of 28.3 L/min for 5 min. Sampling was collected at three equidistant positions and repeated three repetitions per position. Samples were cultured on selective media for Gram-negative bacteria, and the sampling duration was controlled between 5 and 10 min to ensure the collection of microorganisms on all six agar plates, aiming for a colony count between 30 and 300. Following collection, blood agar plates were incubated at 37 °C for 2 to 3 days. After incubation, colony counts were adjusted using the Andersen (1958) calibration table to determine the concentration of airborne bacteria (CFU/m³). Colonies were Gram-stained, and Gram-negative bacteria were isolated and cultured. API-20E tests were conducted to identify the Gram-negative bacteria present on each plate based on staining reactions and identification results.

Statistical analysis

SPSS software (version 26.0) was used to analyse the collected data. The air environmental parameters were expressed as mean ± standard deviation. The t-test was utilized to compare the differences, with statistical significance set at *P*-value < 0.05.

Results

Distribution of airborne endotoxin and microbial aerosols at different distances

No significant differences in airborne endotoxin and microbial aerosol levels were observed across different distances before treatment (Table 1). However, during treatment, airborne endotoxins and microbial aerosols increased significantly at a distance of 20 cm (*P*-value < 0.05), with the highest production observed at this distance. Similarly, the highest airborne endotoxins and microbial aerosols production were also observed at 20 cm after treatment (*P*-value < 0.05).

Effect of different cleaning methods

There was no statistical difference among the four groups before treatment, see Table 2. During treatment, compared to before treatment, there was a significant increase in airborne endotoxins and microbial aerosols production, with the highest production observed at this time (*P*-value < 0.05). During treatment, the groups using additional methods (Group C, Group B, and Group D) had significantly higher levels of airborne endotoxins and microbial aerosols compared to the group using strong suction alone (Group A) (*P*-value < 0.05). Notably, Group D exhibited the highest levels of endotoxins and microbial aerosols among all groups during treatment but showed the lowest levels of airborne endotoxin and

microbial aerosols after treatment, while Group A had the highest post-treatment levels (P -value < 0.05).

Comparison of sampling methods

Compared to the surface sampling method, the natural settling method captured significantly lower concentrations of airborne endotoxins and microbial aerosols at 20 cm (P -value < 0.05), see Table 3, indicating its superior accuracy in reflecting airborne contamination.

Discussion

The findings of this study underscore the critical importance of effective aerosol management in dental procedures. Notably, our results revealed that while the use of strong suction combined with additional methods, such as air disinfection or dental electric suction, was associated with an increase in aerosol levels during treatment, it resulted in the lowest levels post-treatment. This counterintuitive outcome highlights the need for more precise and targeted control measures to effectively manage aerosols throughout the entirety of dental procedures.

The quality of the oral clinical environment directly affects patient outcomes, particularly in terms of survival and recovery [7, 10–12]. Eliminating airborne endotoxins and microbial aerosols is a critical issue in the prevention and control of oral environmental infections. Various dental instruments generate significant quantities of aerosols that contain a myriad of microorganisms. In our study, aerosol levels were consistent across groups prior to treatment. However, a significant increase in aerosol production was observed during treatment, particularly within 20 cm of the source. This finding aligns with previous research by Choudhary et al. [2] and Adhikari et al. [8], who reported increased aerosolized bacteria and microbial activity during dental procedures. The failure to maintain patients in a semi-open state during procedures can allow these bacteria and viruses to enter the body through the respiratory tract, posing severe health risks [10]. Furthermore, patient movements during treatment exacerbate the transmission of aerosols through blood, air, and skin contact [11]. Moreover, activities such as coughing and sneezing during dental visits contribute to the contamination of the environment with airborne endotoxins and microbial aerosols. Additionally, common patient behaviors such as coughing and sneezing

during dental visits contribute to the contamination of the environment with highly infectious airborne endotoxins and microbial aerosols, presenting significant health risks, especially in high-turnover settings with insufficient protective measures [13].

Our findings also reveal significant differences in aerosol levels depending on the suction methods employed. The highest aerosol levels were observed within 20 cm of the source at the end of treatment. Among the patient groups studied, those using high-powered suction alone generated the most aerosols, followed by those using high-powered suction in combination with a dental electric suction device, high-powered suction with an air disinfection machine, and finally, the combination of all three methods. This hierarchy suggests that the combined use of high-powered suction, air disinfection, and a dental electric suction device is the most effective strategy for reducing aerosol levels. Future research should focus on continuous monitoring of microbial aerosol concentrations in dental environments to further enhance environmental quality and safety. Consistent with our findings, Choudhary et al. [2] reported that the highest aerosol concentrations were generated during ultrasonic scaling and high-speed drilling of anterior teeth. They recommended the use of conical or ISOVAC high-volume evacuators rather than standard-tip evacuators to mitigate aerosols produced during routine clinical practice.

The study also highlighted the effectiveness of different sampling methods in assessing aerosol contamination [14]. Our results suggest that the natural sedimentation method provides a more accurate reflection of real-time air pollution levels compared to surface sampling. To our knowledge, no prior studies have directly compared these two methods within dental clinics. Surface sampling detects contaminants that have already settled, potentially underestimating the actual airborne contamination levels. In contrast, the natural sedimentation method captures suspended particles, offering a more accurate assessment of airborne contaminants. Previous studies conducted in the U.S [8], Canada [15], Italy [16], and Germany [17] have investigated airborne bacterial concentrations in dental clinics using various sampling methods. However, there remains a paucity of data on airborne bacteria in dental settings during cleaning

Table 3 Distribution of airborne endotoxins and microbial aerosols under different sampling methods

Parameters		Natural settling method	Surface sampling method	P-value
Before treatment	Airborne endotoxins / EU(m ³) ⁻¹	64.87 ± 24.87	66.57 ± 22.47	< 0.05
	Microbial aerosols / CFU(m ³) ⁻¹	8.88 ± 2.13	8.97 ± 2.97	< 0.05
During treatment	Airborne endotoxins / EU(m ³) ⁻¹	158.75 ± 24.58	167.78 ± 23.37	< 0.05
	Microbial aerosols / CFU(m ³) ⁻¹	55.78 ± 26.38	69.78 ± 17.82	< 0.05
After treatment	Airborne endotoxins / EU(m ³) ⁻¹	92.75 ± 35.78	98.78 ± 33.56	< 0.05
	Microbial aerosols / CFU(m ³) ⁻¹	26.97 ± 13.34	29.53 ± 24.61	< 0.05

procedures, particularly within the context of Chinese dental clinics.

Despite the valuable insights provided by our study, several limitations should be acknowledged. The relatively small sample size and single-center design may limit the generalizability of our findings. To address these limitations, future research should aim to include larger sample sizes and engage in multi-center collaborations to provide a broader perspective on aerosol management. Such expanded research efforts will enhance the generalizability of our findings and contribute to the development of more effective aerosol control strategies.

Conclusion

In conclusion, our study emphasizes the need for comprehensive strategies, including the use of strong suction, air disinfection, and dental electric suction, to mitigate airborne endotoxins and microbial aerosols during dental procedures. Although aerosol production increased significantly during treatment, especially at 20 cm from the patient's mouth, the combined approach effectively reduced aerosol levels over time. The natural settling method proved superior to surface sampling in capturing airborne contaminants. Implementing these findings is crucial for improving infection control and protecting both patients and healthcare providers. Further research is needed to refine aerosol management strategies in dental and other environments.

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Not applicable.

Author contributions

Du Y. is the guarantor of the entire study. Du Y. designed this study and did the literature research. Tao R., Shi M. and Liu B. acquired the data and analyzed the data. Zhao F. contributed to the project administration. Du Y. drafted the paper. ZHao F. revised the paper. All authors have read and approved the final paper.

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Data availability

The datasets used during the present study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Hebei Key Laboratory of Stomatology, Hebei Clinical Research Center for Oral Diseases, School and Hospital of Stomatology, Hebei Medical University. All subjects signed the informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Hospital Infection Management/Hebei Key Laboratory of Stomatology/Hebei Technology Innovation Center of Oral Health, School and Hospital of Stomatology, Hebei Medical University, Shijiazhuang 050017, PR China

²Department of Pharmacy/Hebei Key Laboratory of Stomatology/Hebei Technology Innovation Center of Oral Health, School and Hospital of Stomatology, Hebei Medical University, Shijiazhuang 050017, PR China

³Department of Periodontal II/Hebei Key Laboratory of Stomatology/Hebei Technology Innovation Center of Oral Health, School and Hospital of Stomatology, Hebei Medical University, Shijiazhuang 050017, PR China

⁴Department of Periodontal I/Hebei Key Laboratory of Stomatology/Hebei Technology Innovation Center of Oral Health, School and Hospital of Stomatology, Hebei Medical University, Room 408, Hospital of Stomatology Hebei Medical University 383 Zhongshan East Road, Chang'an District, Shijiazhuang 050017, PR China

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