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AAO Foundation Final Report Form
(a/o 6/30/2020)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

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Please prepare a report that addresses the following:

Type of Award, Biomedical Research Award

Name(s) of Principal Investigator(s): Carlos Flores-Mir, Khaled Altabtbaei

Institution: University of Alberta

Title of Project: Characterization of Aerosols Generated by Various Dental Procedures and Their Relevance to COVID19: Clinical study

Period of AAOF Support: 07-01-21 to 06-30-22

Amount of Funding: 30,000 USD

Summary/Abstract:

Abstract (as per accepted publication J Dent Res 2022)
Many dental procedures are considered aerosol-generating procedures that may put the dental operator and patients at risk for cross-infection due to contamination from nasal secretions and saliva. This aerosol, depending on the size of the particles, may stay suspended in the air for hours. The primary objective of the study was to characterize the size and concentrations of particles emitted from 7 different dental procedures, as well as estimate the contribution of the nasal and salivary fluids of the patient to the microbiota in the emitted bioaerosol. This cross-

sectional study was conducted in an open-concept dental clinic with multiple operators at the same time. Particle size characterization and mass and particle concentrations were done by using 2 direct reading instruments: Dust-Trak DRX (Model 8534) and optical particle sizer (Model 3330). Active bioaerosol sampling was done before and during procedures. Bayesian modeling (SourceTracker2) of long-reads of the 16S ribosomal DNA was used to estimate the contribution of the patients' nasal and salivary fluids to the bioaerosol. Aerosols in most dental procedures were sub-PM_1dominant. Orthodontic debonding and denture adjustment consistently demonstrated more particles in the PM_1, $PM_{2.5}$, PM_4, and PM_{10} ranges. The microbiota in bioaerosol samples were significantly different from saliva and nasal samples in both membership and abundance ($P < 0.05$) but not different from preoperative ambient air samples. A median of 80.15% of operator exposure was attributable to sources other than the patients' salivary or nasal fluids. Median operator's exposure from patients' fluids ranged from 1.45% to 2.75%. Corridor microbiota showed more patients' nasal bioaerosols than oral bioaerosols. High-volume saliva ejector and saliva ejector were effective in reducing bioaerosol escape. Patient nasal and salivary fluids are minor contributors to the operator's bioaerosol exposure, which has important implications for COVID-19. Control of bioaerosolization of nasal fluids warrants further investigation.

Respond to the following questions:

1. Were the original, specific aims of the proposal realized?

   Yes. We were successful in answering the stated research questions.

2. Were the results published?
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   Rafiee A, Carvalho RS, Lunardon D, Flores-Mir C, Major PW, Quemerais B, Altabtbaei K. Particle size, mass concentration and microbiota in dental aerosols. J Dent Res 2022; 101: 785-92. Doi: 10.1177/00220345221087880.

   It has to be noted that this publication is part of the journal's editor collection (20 most influential JDR articles since 2019): https://journals.sagepub.com/topic/collections-jdr/jdr-1-featured_articles/jdrb?pageSize=20

   b. Was AAOF support acknowledged?

   Acknowledged noted.

   c. If not, are there plans to publish? If not, why not?

   Not applicable

3. Have the results of this proposal been presented?

a. If so, list titles, author or co-authors of these presentation/s, year and locations.
   Dental Discovery Seminar – May 4th 2022
   ACFD conference – June 18th 2022.

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Journal of Dental Research
2022, Vol. 101(7) 785–792

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Particle Size, Mass Concentration, and Microbiota in Dental Aerosols

A. Rafiee[1], R. Carvalho[2], D. Lunardon[2], C. Flores-Mir[2], P. Major[2], B. Quemerais[1*], and K. Altabtbaei[2*]

Abstract

Many dental procedures are considered aerosol-generating procedures that may put the dental operator and patients at risk for cross-infection due to contamination from nasal secretions and saliva. This aerosol, depending on the size of the particles, may stay suspended in the air for hours. The primary objective of the study was to characterize the size and concentrations of particles emitted from 7 different dental procedures, as well as estimate the contribution of the nasal and salivary fluids of the patient to the microbiota in the emitted bioaerosol. This cross-sectional study was conducted in an open-concept dental clinic with multiple operators at the same time. Particle size characterization and mass and particle concentrations were done by using 2 direct reading instruments: DustTrak DRX (Model 8534) and optical particle sizer (Model 3330). Active bioaerosol sampling was done before and during procedures. Bayesian modeling (SourceTracker2) of long-reads of the 16S ribosomal DNA was used to estimate the contribution of the patients' nasal and salivary fluids to the bioaerosol. Aerosols in most dental procedures were sub-PM_1 dominant. Orthodontic debonding and denture adjustment consistently demonstrated more particles in the PM_1, $PM_{2.5}$, PM_4, and PM_{10} ranges. The microbiota in bioaerosol samples were significantly different from saliva and nasal samples in both membership and abundance ($P < 0.05$) but not different from preoperative ambient air samples. A median of 80.15% of operator exposure was attributable to sources other than the patients' salivary or nasal fluids. Median operator's exposure from patients' fluids ranged from 1.45% to 2.75%. Corridor microbiota showed more patients' nasal bioaerosols than oral bioaerosols. High-volume saliva ejector and saliva ejector were effective in reducing bioaerosol escape. Patient nasal and salivary fluids are minor contributors to the operator's bioaerosol exposure, which has important implications for COVID-19. Control of bioaerosolization of nasal fluids warrants further investigation.

Keywords: dentistry, aerosol, bioinformatics, nose, saliva, COVID-19

Introduction

Dental professionals are potentially exposed to a variety of occupational health hazards in dental offices, among which aerosols are of the utmost importance because of their adverse impacts on health (Ayatollahi et al. 2012; Polednik 2021). Dental instruments, such as air–water syringes, ultrasonic scalers, and handpieces used during dental procedures, have been recognized as major sources of aerosols in dentistry (Day et al. 2008; Nulty et al. 2020). Using these types of instruments can lead to the air dispersion of particles of various sizes, including spatter, droplets with a diameter >50 µm, and ultrafine particles, with the latter having the potential to remain suspended in air for extended periods of time and the potential to deposit within the respiratory system (Adhikari et al. 2017; Abramovitz et al. 2020). The location of this deposition is size dependent, with the smaller particles having higher potential to cause respiratory sequelae (Peters et al. 1997; Oberdörster 2001; Chen et al. 2017). Particulate matter (PM) <10 µm and <4 µm in diameter (PM_{10}, PM_4) can deposit beyond the larynx and past the nonciliated airways, respectively. Particles <2.5 µm in diameter ($PM_{2.5}$) can deposit in the alveoli of the lung (Yang et al. 2020). Studies have also suggested that a significant part of the adverse health effects is induced by particles with a diameter <1 µm (PM_1) or <0.1 µm ($PM_{0.1}$), with the latter being small enough to enter the bloodstream (Peters et al. 1997; Oberdörster 2001; Ohlwein et al. 2019).

The spread of particles is well established in dental literature. Pierre-Bez et al. (2021) reported that at 0 to 1.2 m from the patient's head, there is an increase in mass concentrations of particles compared to 1.2 to 2.4 m, indicating that these larger particles can settle and contaminate surfaces adjacent to the emission source. The same study found a significantly higher mass concentration of PM_1 compared to all other studied PM fractions at these distances (Pierre-Bez et al. 2021). The increase in submicron-particle concentrations is corroborated in another study that used various aerosol-generating

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A supplemental appendix to this article is available online.

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dental procedures (AGPs), with the highest increase observed during drilling/grinding (Polednik 2014).

The spread of bioaerosols, a subcategory of particles containing biologics such as bacteria and viruses, has been demonstrated during various dental procedures (Nóbrega et al. 2021). The microbial content of bioaerosols in dentistry is mainly attributed to patients' nasopharyngeal secretions, saliva, blood, and the dental-unit water line (Harrel and Molinari 2004; Zemouri et al. 2020). As the human body's second-most complex microbiota (Dewhirst et al. 2010), the oral cavity serves as a colonization habitat for various microorganisms, including the coronavirus disease 2019 (COVID-19)–causing severe acute respiratory virus coronavirus 2 (SARS-CoV-2). High expression of angiotensin-converting enzyme 2 (ACE2), the primary host-cell receptor for coronaviruses, was identified in the mucosa of the oral cavity, epithelial cells of the tongue, and respiratory cells in the nasal cavity, nasopharynx, and oropharynx (Sungnak et al. 2020; Xu et al. 2020; Zou et al. 2020). With this in mind, and because of the aerosol-generating nature of dental procedures, the US Occupational Safety and Health Administration (OSHA 2020) listed dentistry among the highest-risk occupations regarding the transmission of SARS-CoV-2.

Knowing the origin of the bioaerosols helps infer contagion risk to the exposed individuals. Meethil et al. (2021) attributed the majority of bioaerosols to the dental-unit water line (DUWL), with a median of 0% attributed to saliva. The nature of dentistry necessitates that the operator works in close contact with 2 biological fluids laden with potential pathogens: the nasal and salivary fluids. To our knowledge, no other study has simultaneously examined the operator's exposure to both patients' fluids.

The present study attempts to 1) characterize aerosols generated during common dental procedures and 2) identify patterns of bioaerosol spread across dental clinics from patients' nasal and oral fluids.

Materials and Methods

Study Area and Sampling Strategy

The study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria. The cross-sectional study was conducted at the KAYE Edmonton clinic (details are provided in the Appendix), affiliated with the University of Alberta, Canada (ethics #Pro00103510). Inclusion criteria included participants who could provide saliva/nasal samples and were undergoing AGPs. All participating patients signed a consent form indicating their voluntary participation.

The study design used multiple dental operatories simultaneously performing a combination of 7 different procedures: 1) ultrasonics with high-volume evacuator (HVE) and saliva ejector (henceforth *dual suction*), 2) ultrasonics with saliva ejector only, tooth preparation 3) with and 4) without rubber dam (HVE only), 5) orthodontic bonding and 6) debonding (HVE only), and 7) denture adjustment (removable cone attached to HVE). The operator was instructed to perform the denture adjustment as close to the cone as possible. Dentures were soaked in diluted bleach for 10 s before any adjustments. Patients did not use mouthwash before the procedures to minimize disturbance to the salivary microbiota.

Each of the following procedures used the following instruments as needed: ultrasonics used Cavitron tips, tooth preparation with/without rubber dam used high-speed handpieces with water coolant and air/water syringe (Ti-Max Z95L High Speed 1:5, maximum motor speed = 40,000 rpm, water flow rate >37 mL/min), orthodontic bonding/debonding used air/water syringe, and orthodontic debonding and denture adjustment used a handpiece without water coolant (Ti-Max Z25L Slow Speed 1:1, maximum motor speed = 40,000 rpm) for debonding and HA-43A Contra Nose Cone 1:1 (maximum motor speed = 40,000 rpm for denture adjustment).

Aerosol Measurements

A pilot study was done to determine which direct-reading instruments to use and their position (details are provided in the Appendix). The mass concentration of particles was measured using the Dust-Trak DRX (Model 8534; TSI), which simultaneously measures the size-segregated mass concentrations corresponding to particles with a diameter PM_1, $PM_{2.5}$, and PM_{10}; respirable; and total particulate matter. An optical particle sizer (OPS; 3330; TSI) was used to measure the particle concentration across 13 sizes: 0.3 µm, 0.4 µm, 0.5 µm, 0.7 µm, 1 µm, 1.3 µm, 1.7 µm, 2.2 µm, 3 µm, 4 µm, 5.5 µm, 7 µm, and 10 µm. Air changes/h and room temperature information are provided in Appendix Table 2.

Sampling started with a 20-min preprocedural background measurement in the middle of all operatories, which was approximately 1 m away from the operatory chairs' heads. This was followed by a 40-min collection while the dentist worked on the patient (instruments located approximately 50 cm from the breathing zone of both the patient and the dental personnel; Appendix Fig. 2). After 40 min, AGPs were stopped, and patients/operators were asked to move out of the sampling area. Twenty minutes after, background measurement was done in the same position used during the preprocedural sampling.

Bioaerosol Sampling and Identification

Bioaerosol samples were collected using GilAir Plus Personal Air Sampling pumps calibrated at a 2-L/min suction rate (henceforth termed *pumps*). Air intercepted through these pumps was collected on sterile 25-mm diameter gelatin filters (catalogue ID: 12602--25----alk; Sartorius Corporation). Prior to patient arrival, ambient air was sampled for 30 min (same position as direct-reading instruments) and the gelatin filter removed and stored for analysis. After patients were seated, they were asked to collect 5 mL of saliva by drooling into sterile tubes. No mouthwashes were used to minimize disturbance to the salivary microbiota. Next, patients inserted a sterile nasal swab 2 cm into their nostrils, twisted it in place, then placed it in a sterile tube with

Table 1. Particle Characterization: Breakdown of Number of Repetitions Done per Procedure, Sampling Duration, and Number of Collected Samples for Dust-Trak, OPS, and Bioaerosol Sampling.

	Air Characterization			Bioaerosol Testing
Dental Procedures	No. of Repeats[a]	No. of Data Points (OPS)	No. of Data Points (Dust-Trak)	No. of Dental Procedures That Yielded Samples
Ultrasonic—HVE and saliva ejector	3	120	720	7
Ultrasonic—saliva ejector	3	120	678	7
Denture adjustment	3	120	720	5
Orthodontic bonding	3	120	720	10
Orthodontic debonding	4	145	852	14
Tooth prep with rubber dam	4	151	890	8
Tooth prep without rubber dam	3	120	672	Excluded

HVE, high-volume evacuator; OPS, optical particle sizer.
[a]Number of repeats for aerosol testing (OPS and Dust-Trak).

RNA*later* (Qiagen). This validated method is commonly used to diagnose nasal *Staphylococcus aureus* (van Cleef et al. 2012). Each operator wore a pump attached to their face shield (henceforth *personal samplers;* Appendix Fig. 3), which they activated immediately before starting AGP for 40 min. In the lab, the gelatin filters were cut in half using sterile scissors, dissolved in sterile phosphate-buffered saline, and then processed using QIAmp DNA Mini Kits (Qiagen) according to the manufacturer's instructions. Technical errors in the lab resulted in exclusion of all personal samplers used during tooth preparation without a rubber dam. Out of 64 personal samplers, 13 had nonlaboratory technical issues (e.g., no amplifications found, <100 sequences) and as such were excluded from analysis. This hindered analysis of the operator's patient versus others stratified per procedure. A total 184 samples were available for microbial analysis (51 personal samplers, 51 nasal swabs, 51 saliva samples, 11 preprocedural air samples, 20 corridor samplers).

Samples were sequenced in LoopGenomics facilities for the entire 16S ribosomal DNA (rDNA) region, recently reported with per-nucleotide accuracy of 99.995% (Callahan et al. 2021). Low-quality sequences, singletons, and partial sequences not covering all 9 rDNA hypervariable regions were removed. Bias correction was done using DADA2 to amplicon sequence variant (ASV). Species were assigned using trained classifier SILVA138.

Statistical Analysis

STATA 15.0 (StataCorp LLC) and GraphPad Prism 6.0 (GraphPad Software) were used to conduct the statistical analysis. Data normality of the particle and mass concentrations was assessed with the Shapiro–Wilk normality test. The Kruskal–Wallis test was performed to compare the mass/particle concentrations during AGPs with pre- and postbackground levels. The correlation between mass and particle concentrations was assessed using Pearson correlation. In all statistical tests, $P < 0.05$ was considered statistically significant.

Percentage contribution of the sources was assessed using Bayesian modeling (SourceTracker2; Knights et al. 2011) using default settings (source rarefaction depth = 1,000, burn-in = 100, restart = 10, $\alpha = 0.001$, $\beta = 0.01$; Appendix Fig. 1). Analysis was done for patient to own operator and patient to other operators (a total of 179 one-to-many comparisons, excluding own patient–operator pair). Since distribution was positively skewed, $\log_{10}$ transformation was done before analysis. Analysis was done on RStudio v1.4.1717, and raw data were graphed using ggplot2. Sequences are available in NCBI-SRA (PRJNA776892). Alpha rarefaction and SourceTracker2 with standard deviations are provided in the Appendix. Number of samples per procedure is found in Table 1.

Results

Particle and Mass Concentrations in Dental Procedures

In total, 896 particle concentration data points, as measured with OPS, and 5,252 particle mass concentrations were collected with Dust-Trak for a total of 6,148 data points (Table 1). Overall, mass and particle concentrations correlated strongly across the different PM sizes in all procedures (Pearson's r range = 0.92–1; Table 2). All procedures produced significantly more particles when compared to the preprocedural ambient air ($P < 0.05$, Kruskal–Wallis test, mass, particle concentrations, or both). Mass concentration in orthodontic bonding was not significantly different from preprocedural ambient air. Particle concentration following tooth preparation with rubber dam was not different from the background; however, mass concentration was significantly higher. When comparing the different procedures against each other, ultrasonics with dual suction had the lowest particle concentration.

In all procedures, there was an inverse relationship between the 13 different particle sizes and their abundance, with particles ≤0.7 µm being significantly more abundant compared to those ≥1 µm ($P < 0.05$, Wilcoxon rank-sum test; Appendix Table 2). Although aerosols in dental procedures were sub-PM_1 dominant, concentrations of particle sizes varied significantly by procedure, especially particles ≥1 µm in diameter. Orthodontic debonding and denture adjustment consistently had more particles in the PM_1, $PM_{2.5}$, PM_4, and PM_{10} ranges compared to other procedures (Appendix Table 3).

Table 2. Comparison of Mass and Number Concentrations of Particles Generated during and Postbackground of Various Dental Procedures with Preprocedural Background (Kruskal–Wallis Nonparametric Test).

Dental Procedure	Particle Characteristics	Background, Geometric Mean (95% CI)	Increase during the Procedure Compared to Background (*P* Value)	During, Geometric Mean (95% CI)	Increase during the Procedure Compared to Postbackground (*P* Value)	Postbackground, Geometric Mean (95% CI)	Increase in Postbackground Compared to Background (*P* Value)
Ultrasonic scaling—HVE and saliva ejector	Number concentration	474 (461–487)	Yes (<0.001)	632 (604–662)	No[a] (<0.001)	1,218 (1,185–1,252)	Yes (<0.001)
	Mass concentration	0.0069 (0.006–0.007)	No (0.973)	0.0073 (0.071–0.074)	No[a] (0.001)	0.008 (0.0076–0.0084)	Yes (<0.001)
Ultrasonic scaling—saliva ejector only	Number concentration	1,789 (1,529–2,093)	No (0.985)	1835 (1525–2207)	No[a] (0.0017)	2794 (2151–3629)	Yes (0.009)
	Mass concentration	0.0077 (0.0074–0.0079)	Yes (<0.001)	0.0108 (0.0104–0.011)	No (0.335)	0.01 (0.0096–0.0104)	Yes (<0.001)
Orthodontic bonding	Number concentration	6,036 (5,956–6,117)	Yes (<0.001)	6,996 (6,952–7,042)	No[a] (<0.001)	8,633 (8,555–8,712)	Yes (<0.001)
	Mass concentration	0.0105 (0.010–0.011)	No (0.4)	0.011 (0.0107–0.0112)	No[a] (<0.001)	0.012 (0.0113–0.0123)	Yes (0.003)
Orthodontic debonding	Number concentration	2021 (1846–2213)	Yes (<0.001)	5912 (5208–6812)	Yes (<0.001)	2,723 (2,518–2,944)	Yes (<0.001)
	Mass concentration	0.007 (0.0069–0.0071)	Yes (<0.001)	0.016 (0.0158–0.0173)	Yes (<0.001)	0.009 (0.0088–0.0093)	Yes (<0.001)
Denture adjustment	Number concentration	5,388 (5,336–5,441)	Yes (<0.001)	8646 (8296–9012)	No[a] (<0.001)	9,201 (9,109–9,295)	Yes (<0.001)
	Mass concentration	0.009 (0.009–0.093)	Yes (<0.001)	0.014 (0.0139–0.0148)	Yes (<0.001)	0.01 (0.01–0.011)	Yes (<0.001)
Tooth prep with rubber dam	Number concentration	3,364 (3,025–3,742)	No (0.89)	2966 (2667–3299)	No[a] (<0.001)	5214 (4634–5866)	Yes (<0.001)
	Mass concentration	0.0072 (0.007–0.0074)	Yes (0.001)	0.0109 (0.01–0.011)	Yes (<0.001)	0.009 (0.0087–0.0092)	Yes (<0.001)
Tooth prep without rubber dam	Number concentration	1792 (1693–1898)	Yes (0.007)	2145 (2000–2301)	No (0.054)	1930 (1842–2022)	No (0.13)
	Mass concentration	0.006 (0.0062–0.0067)	Yes (<0.001)	0.0095 (0.0091–0.01)	Yes (<0.001)	0.007 (0.0067–0.0071)	Yes (<0.001)

CI, confidence interval; HVE, high-volume evacuator.
[a]The mass/number concentration of particles measured during the dental procedure was higher than the postbackground levels.

Bioaerosol Analysis

LoopSeq sequencing of 184 samples from 51 dental procedures (Table 1) resulted in 1,481,238 high-quality sequences, with a median (interquartile range [IQR]) of 9,379 (3,281) sequences with a median (IQR) of 1,489 (17) base pairs per sequence. Personal samplers and corridor samples were significantly different from saliva and nose samples in microbial membership and abundance ($P<0.05$, ANOSIM of Jaccard and Bray–Curtis indices, respectively) but not different from preoperative ambient air samples ($P>0.05$). Bayesian modeling estimated the potential sources of operator exposure (Fig. 1). The greatest percentage (median = 80.15%) of the exposure was not traceable to any of the collected samples. Ambient air microbiota contributed the second largest percentage (median = 10.5%), and patient fluids contributed the remaining exposure (median = 8.7%). It was interesting to note that the microbiota on each operator's personal sampler could be traced almost equally to their own patient's salivary (median = 1.5%) and nasal (median = 2.4%) microbiota as well as to salivary (median = 2.1%) and nasal (median = 2.7%) microbiota of adjacent patients ($P>0.05$, analysis of variance [ANOVA] with Tukey post-hoc Honest Significant Difference Test [HSD]; Fig. 1A). Corridor microbiota was traceable more to the nasal than to the salivary microbiota ($P<0.05$, ANOVA with Tukey post-hoc HSD; Fig. 1B).

The escape of bioaerosols from the patient's area to adjacent operatories was examined (Fig. 2). Bioaerosols in operatories adjacent to the patient area also demonstrated microbiota from patient fluids, irrespective of the procedure type ($P>0.05$, ANOVA with Tukey post-hoc HSD). However, the median contribution of patient fluid to this bioaerosol was ≤8%. For ultrasonic procedures, when dual suction was used, there was ~10-fold reduction in the biological material from a patient to the other areas (median = 0.2%, $P<0.05$, ANOVA with Tukey post hoc HSD).

To investigate the potential sources of microbiota that were not traceable to any of the collected samples (i.e., patient saliva, patient's nasal swabs, preprocedural ambient air), we compared the taxonomy of the "unknown" ASVs to the Human Oral Microbiome Database (HOMD) (Chen et al. 2010). The underlying rationale was that if these bacteria were of oral or nasal origin, they would be found in this database and therefore could potentially be attributed to the operator themselves as a source. In total, 3,902 ASVs were condensed to 545 phylotypes, 219 of which were identifiable through HOMD. Their mean relative abundance was 27.54% and 17.73% in all samplers and corridor samplers, respectively, suggesting that the operator themselves may be sources of microbiota in environmental bioaerosols (Appendix Fig. 4).

Discussion

Dentistry, when performed in large teaching institutions or health centers, is typically practiced in open-bay areas. While ventilation and personal protection devices play a vital role in preventing contamination, the COVID-19 pandemic has raised the possibility that AGPs can carry a payload of respiratory and other airborne pathogens across distances and therefore contribute to widespread disease transmission. As a teaching

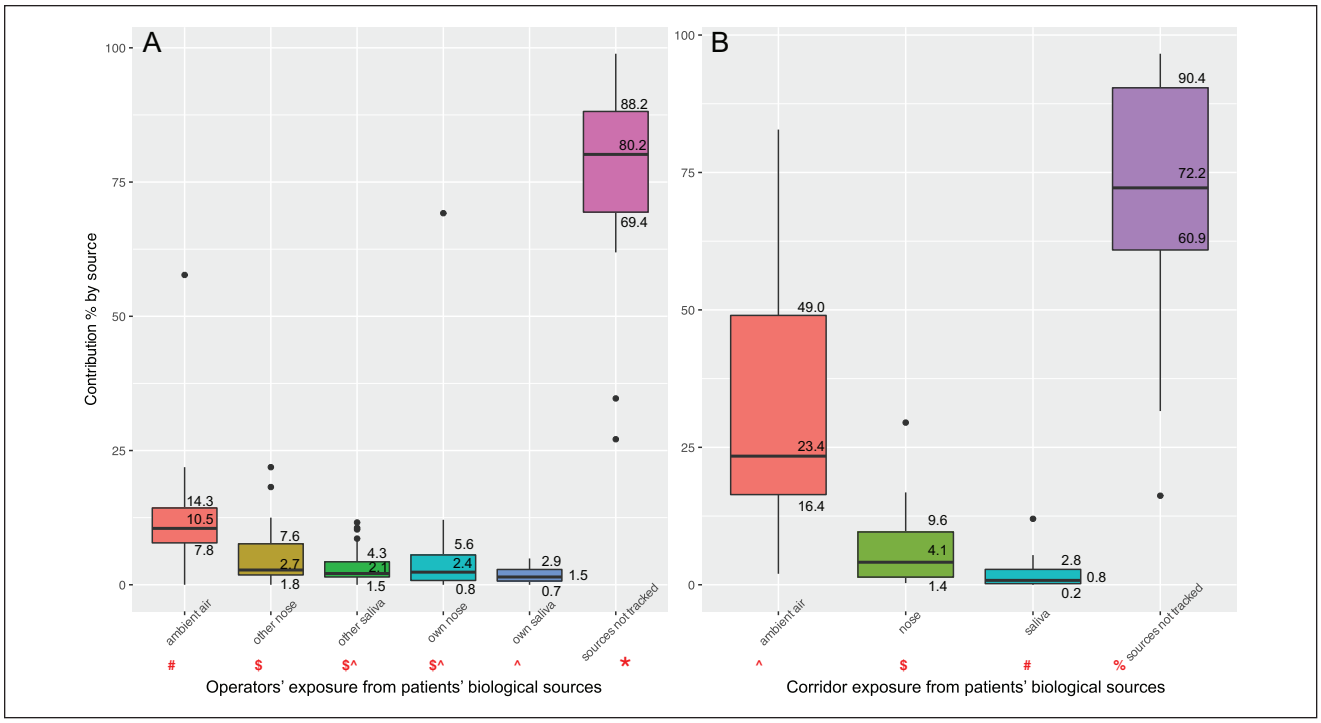

Figure 1. Percent contribution of bioaerosols from identified sources. (**A**) Contribution of operator's own patient biological sources compared to those from other patients in the area, aggregated from all procedures. (**B**) Contribution from patients in the area on the corridor. Categories sharing a symbol are not significantly different from each other (ANOVA with Tukey Post-hoc HSD). HSD, Honest Significant Difference.

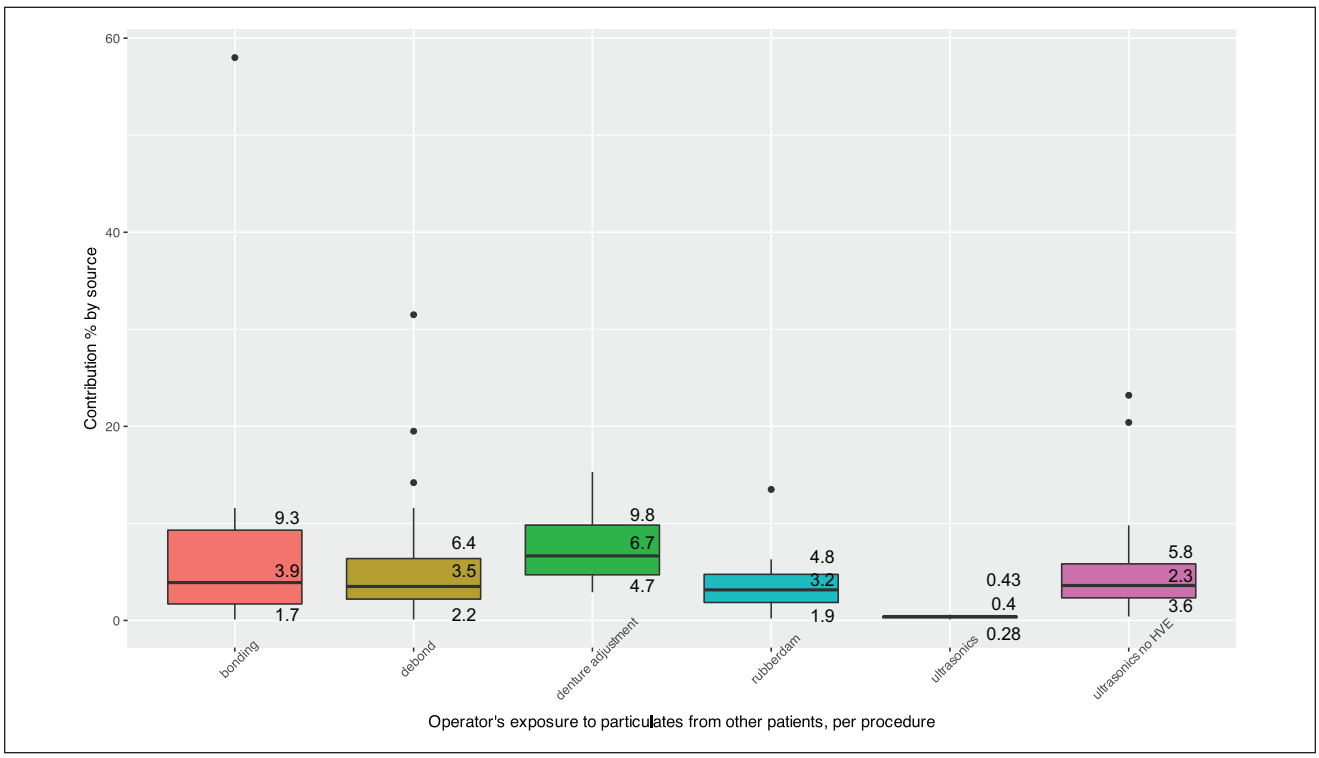

Figure 2. Percent contribution of bioaerosols from identified sources that has traveled to the other operatories, stratified per procedure.

institution with open-bay cubicles, we were ideally positioned to examine the spread of dental aerosols.

Our data corroborate previous evidence in the literature that particles can spread from one operatory to other clinic areas in

a multioperator open-bay setting (Timmerman et al. 2004; Rautemaa et al. 2006; Dutil et al. 2009; Manarte-Monteiro et al. 2013). There are several potential sources of particles: aerosols generated during activities such as talking and coughing, the AGP itself, spread from adjacent cubicles/bays, and activities such as movement of the patient or dental personnel. To test this, we collected 3 particle data sets: pre-, intra-, and postprocedural. We hypothesized that since aerosol bloom dissipates rapidly away from the source, if the intraprocedural particles are less than the postprocedural ones, one may infer that the measurements are heavily influenced by changes in the environment (i.e., inside or outside clinic) and not the procedure itself. In the present investigation, intraprocedure particle and mass concentrations were significantly greater than preoperative measures, suggesting that the AGP was the primary source. However, the postprocedural concentrations were higher than or similar to the intraoperative levels, even though the postprocedural sampling was done after the area was cleared of procedure, patient, and personnel. It is possible that aerosols from AGP take more than 20 min to settle or that the increased mass concentration is due to changes in the environment. It was not within the scope of this investigation to test that, and further studies are warranted to measure dental aerosol settling times.

This research also confirms the results that dental procedures, regardless of aerosol-generating instrument used, are dominated by sub-PM_1 particles (Ehtezazi et al. 2021). The probability of a particle containing at least 1 virion decreases with the decrease in particle size. Thus, a 3-μm particle from saliva has an associated probability of 0.01% (Stadnytskyi et al. 2020). Since these probabilities are based on saliva without water coolant dilution, the probability is further reduced in dental clinical scenarios. Moreover, not all emitted particles come in contact with the patient's microbiota (Allison et al. 2021), further reducing odds of transmission.

We estimated that the median biological exposure to be 8.7%. While this percentage seems concerning, one must consider that dentistry has aerosol capture, DUWL dilution, and high air exchange capabilities and then weigh this against interactions in the real world at the same distance, which undoubtably have a higher percentage. Moreover, since the rise of human immunodeficiency virus in the 1980s, dentistry has implemented high standards of occupational hygiene, the principles of which also translate to airborne diseases. All of these may contribute to the observed low COVID-19 infection rates in dentistry.

Ultrasonic instrumentation has been a subject of much discussion as a major source of dental aerosols and, therefore, a potential source of infection transmission (Timmerman et al. 2004; Kumar and Subramanian 2020; Pierre-Bez et al. 2021). Therefore, we compared particle size, mass concentration, aerosol particle abundance, and the sources of microbiota during ultrasonic use with and without HVE. We found that dual suction resulted in ~4.8-fold reduction in particle concentration. Likewise, microbial tracking demonstrated a ~10-fold reduction. Since ultrasonics mass concentration was not significantly different from the postprocedural measurement, we suspect this incongruency is due to the aforementioned environmental changes. This underscores the importance of using concurrent sampling methods with different technologies in multioperatory environmental analysis. In agreement with Allison et al, we suspect that the efficiency of dual suctioning is due to the low momentum of particles generated from ultrasonics (Allison et al. 2021). Therefore, it is likely, from an occupational risk perspective, that ultrasonics with dual suction does not produce a substantial number of particles and therefore does not present a high risk of biological exposure. Since the same profile was not found in scaling with saliva ejector only, the most likely reason for such reduction is the addition of HVE.

A similar profile of intra- and postprocedural mass concentrations was found in orthodontic bonding, which is not surprising since its use of air–water syringe is limited to short spurts. However, HVE usage is also sporadic compared to its constant usage in ultrasonics, as such orthodontic patients consistently emit uncaptured particles when they are in the chair. This may explain the increased biological contribution from orthodontic bonding patients compared to ultrasonics with dual-suction usage. All other investigated procedures did not show a similar intra/postprocedural concentration pattern. Debonding had one of the highest emissions, which was surprising, especially when compared to the more extensive tooth preparation without a rubber dam. We suspected that this is due to the lack of water coolant usage in debonding. Indeed, Cokic et al. (2020) found that water acts as a composite "dust collector." As such, rotary instrumentation without water coolant should be discouraged.

Overall, our findings agree with Meethil et al. (2021) that the microbiological exposure from patient saliva is low (Meethil et al.: 0% vs. current: <3%), but the 2 studies were conducted in different clinical settings (open bay vs. closed operatory) and differed in the types of aerosol-producing equipment, air exchanges, and suction efficiencies. Moreover, our study employed an active sampling method instead of passive sampling. Although both techniques are acceptable in environmental hygiene, we used active air sampling as we expected the air to have a low microbial biomass. While it is possible that active sampling may overestimate bioaerosol contribution, our pumps were calibrated at 2 L/min, well below the breathing capacity of the average adult (4–6 L/min), and therefore, we believe that this provides a realistic estimate of exposure, especially when attached to the operator.

Although our primary objective was to test biological exposure to operators, our study design enabled us to also investigate the opposite scenario (personnel to patients). We found that 17.73% to 27.54% of the environmental bioaerosols could be attributed to the operator as a source. Although all operators in the present study wore ASTM-certified level 3 medical masks, which have a filtration capability of ≥95% for particles 0.1 μm in size, the masks are not designed to seal on the nose and mouth, which might be a plausible reason for the possible detection of the operator's microbes, posing a potential risk to people in the same space. Our data therefore point to the increased advantage offered by N95 instead of medical masks.

In conclusion, aerosol-generating dental procedures do not uniformly produce the same amount and types of particles. This should support the implementation of more granular infection prevention and control policies that are especially formulated to the instrument being used. Furthermore, patients' saliva and nasal fluids should not be considered the major source of bioaerosols in dentistry but rather a minor contributor to the aerosol microbial content.

Author Contributions

A. Rafiee, contributed to data sample collection, analysis, and interpretation, drafted and critically revised the manuscript; R. Carvalho, contributed to data sample collection and analysis, critically revised the manuscript; D. Lunardon, contributed to the conception and design, critically revised the manuscript; C. Flores-Mir, contributed to conception and design, critically revised the manuscript; P. Major, contributed to data interpretation, critically revised the manuscript; B. Quemerais, contributed to the conception, design, data analysis, and interpretation, drafted and critically revised the manuscript; K. Altabtbaei, contributed to the conception and design, data analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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