


## ORIGINAL ARTICLE OPEN ACCESS

# Aerosols Contamination in the Dental Practice Following Everyday Procedures: An Observational Study

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## ABSTRACT

**Objective:** The purpose of the present observational study was to evaluate the bacterial load in the air following various dental procedures.

**Methods:** Air contamination following seven aerosol-generating dental procedures was assessed. The air volume was sampled by a wet cyclone collector for 10 min during 10 sessions of the following procedures: air-polishing, ultrasonic instrumentation, manual instrumentation, rubber cup polishing, cavity preparation with a 1:5 red contra-angle, cavity preparation with turbine and Low Volume Evacuator (LVE), and cavity preparation with turbine and High Volume Evacuator (HVE). Contamination of the sampled solution was determined using ATP (Adenosine TriPhosphate) quantification of the viable bacterial count, and compared to baseline measurements.

**Results:** The baseline air contamination was 1.45 (0.85–2.04) CFUs/L of air. The highest increase in air contamination was observed after the use of a turbine with LVE, with an average of 7.38 (95% CI 3.87–10.89) CFUs/L of air ( $p < 0.01$ ). The use of the turbine with HVE and the use of the red hand-piece resulted in non-significant increases in bacterial counts compared to baseline (2.98 [1.34–4.63] and 2.70 [0.18–4.22] CFUs/L of air respectively). The application of air polishing, ultrasonic instrumentation, hand instrumentation and rubber cups did not result in a higher bacterial count than the baseline.

**Conclusion:** Routine professional oral hygiene procedures do not increase air contamination. However, cavity excavation with LVE creates a significantly higher bacterial count in the air.

## 1 | Introduction

The COVID-19 pandemic has boosted the interest in airborne diseases and appropriate infection control measures [1]. Different transmission routes in the dental setting have to be considered. Among those, the most important aspect resulting in contamination in the dental office may be the direct or indirect splatter of aerosols produced during dental routine procedures [1, 2]. As present-day dental medicine practices apply a

number of techniques that utilise drilling instruments, ultrasonics and air with abundant water spray, it is obvious that contamination of saliva and its spread in the generated aerosols provides a vehicle for pathogens [3].

Splatter is described as a mixture of water and/or solid substances in the form of droplets with a diameter higher than 50 µm. Given these dimensions, splatter does not spread far away from the area of its production and precipitates quickly on the surrounding

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surfaces [4]. On the other hand, aerosol particles are smaller than 50 µm and may remain suspended in the air for a long time [5]. Bio-aerosols may have a highly heterogeneous composition depending on their source of origin. Hence, they pose a serious risk of inhalation and infection with contained viable micro-organisms [6, 7].

Guidelines for the management of the first SARS-CoV virus strongly recommended performing dental procedures that produced lower amounts of aerosols if possible [8]. Moreover, personal protection through proper Personal Protective Equipment (PPE) was strongly recommended [8].

It has been demonstrated that aerosols produced by ultrasonic scalers, air polishing devices or even rotary drilling with air coolant can be reduced by, for example, High Volume Evacuator (HVE) [9]. The splatter of aerosols into the immediate environment around the dental chair will also be affected by applying HVE [9]. Supplementary methods to minimise the impact of aerosols include pre-treatment rinses with disinfectant mouthwashes and the use of rubber dam [1, 9–11]. However, the efficacy of mouthwashes seems to be proven only for bacteria, and limited evidence exists around viruses [12]. Airborne contamination may be evaluated in experimental and clinical settings applying various techniques:

- Aerosol sampling by passively allowing microorganisms to settle on surfaces like agar plates. This may be useful to evaluate an aerosol settling rate;
- active sampling of defined air for a set period of time [13].

Active sampling applying wet-cyclones to allow air-to-liquid transfer of the particles contained in the sampled air volume [13]. The liquid content may then be analysed.

Quantification of Adenosine TriPhosphate (ATP) content of the collected liquid is a well-known analytical method for viable microorganisms. ATP is an energy-carrying molecule found in all living prokaryotic cells. A special bioluminescence assay using the luciferin-luciferase reactions is able to determine the quantity of ATP in a solution, that is, the number of viable microorganisms. ATP assays are considered to be simple, rapid and sensitive methods for monitoring surface contamination in hospital settings, waterlines and dental settings [14, 15]. Cyclone systems are proven to have an excellent sampling performance and—together with ATP enzymatic reactions—represent an excellent solution for monitoring indoor and outdoor environments [16].

The aim of the present study was to evaluate the microbial load in the aerosol generated during various everyday dental procedures, using a wet-cyclone sampling system and ATP quantification.

## 2 | Materials and Methods

The present study represents an observational study of an environmental parameter in a private practice in Brescia (Italy). The dental treatments were provided to patients referred for professional oral hygiene or treatment of tooth decay during February and March 2021. All participants were informed about the environmental measurements and analysis. No sensitive patient data

or information has been collected for this study. Ten different sessions for each of the treatment modalities described below were sampled, for a total of 70 samples from 70 different patients.

### 2.1 | Clinical Procedures

Baseline measurements were obtained by sampling the air in the clinical room of the dental office for 10 min during a conversation between the two operators, both wearing an FFP2 facial mask. Prior to the baseline measurement the room had been disinfected and exposed to the fresh air for 15 min.

As was customary in the dental office, a pre-treatment rinse with 0.1% chlorhexidine digluconate and cetylpyridinium chloride (0.05%) was administered (BacterX Pro, EMS, Nyon, Switzerland). Either Low Volume Evacuator (LVE) only or a combination of LVE and HVE were applied. The operators were wearing full PPE equipment during each treatment.

Aerosol collection was performed during the following procedures performed by two professionals:

- Air-polishing (Airflow Prophylaxis Master, EMS) with Erythritol powder (PLUS, EMS). The power was set at 5/10 and the water supply at 10/10. HVE and LVE were both applied.
- Ultrasonic Instrumentation with a perio-slim tip (Piezon PS, EMS). The power was set at 3/10 and the water supply at 10/10. LVE was applied.
- Polishing with rubber cup (1241RA, Edenta, Switzerland) and prophylaxis paste (NUPRO, Dentsply Sirona, York, Pennsylvania, USA). A regular 1:1 contra-angle handpiece (blue band) was used. LVE was applied.
- Manual instrumentation with Gracey curettes 7/8, 11/12 and 13/14 (LM-Dental, Finland). LVE was applied.
- Cavity preparation with a Turbine with water cooling (KaVo Kerr Dental). LVE was applied.
- Cavity preparation with a Turbine with water cooling (KaVo Kerr Dental). HVE and LVE were both applied.
- Cavity preparation with 1:5 red contra-angle with water cooling (Sirona Multiplier, Dentsply Sirona). HVE and LVE were both applied.

Ten different sessions were sampled for each procedure, totaling 70 measurements. The sessions were randomly selected from the clinic's appointment books via computer randomizer.

In order to minimise external water contamination, the combined air-polishing and ultrasonic device was used with an independent bi-distilled and demineralized water supply. The dental chair was also supplied with the same water through a bottle system. Bi-distilled demineralized water was produced through a water distiller (Mophorn, USA). The clinical room was cleaned and disinfected following standard infection-control procedures, and the window was opened for 15 min between each treatment.

## 2.2 | Aerosol Sample Collection

Sampling was performed using a wet-cyclone sampler system (PRELECT, Medentex, Germany). The cyclone system consisted of two components: the upper part containing the cyclonic structure and the lower part acting as a water collector and container. The air containing the aerosol was sucked into the cyclone container. Aerosol droplets were then accelerated and centrifuged in the vortex created so that they were pushed into contact with the moving pre-filled water in the collector. The aerosol bacterial and viral debris were transferred to the collecting water, preventing desiccation. The system accumulated the living material in the collector. The collector was filled with 120 mL of 0.45- $\mu$ m syringe-filtrated water. To maximise the air collection, a suction funnel with 100 mm external diameter was added to guide the aerosol into the system. All the suction power was generated by an independent suction system (H-POWER 700, Hoover, USA) connected to the cyclone exit tube with a 30 mm tube to avoid pressure loss due to the tubing.

To obtain the best estimation of the air volume collected, the suction flow rate was measured as follows: a standard plastic bag (110 L trash bag) was fixed to the suction system with a tape. The exact air-filled bag volume in this setup was measured by filling it previously with water and resulted in 115 L. Time was then recorded to fill up the bag with air until the pressure in the bag started increasing. A flow of  $957 \pm 43$  L/min was confirmed.

To account for possible natural aerosol variations, regular measurements of the room background were taken before and after the tests. As the measurement results were consistently similar, we assumed non-relevant variations.

To control and limit system contamination during the setup preparation the following protocol was applied before each procedure:

- The upper part of the system was rinsed twice with 30 mL of 0.45  $\mu$ m syringe-filtrated water.
- The collector was also rinsed twice with 60 mL of 0.45  $\mu$ m syringe filtrated water.
- Both parts were dried with compressed medical-grade air.
- A second rinse was performed for both parts.
- The cyclone was then set up, and 120 mL of 0.45  $\mu$ m syringe filtrated water was inserted into the system through the exit channel.

In order to prevent water projections into the exit channel, the vortex movement of the liquid was progressively created. The aspiration flow rate was increased from 70 mbar to 210 mbar in 10 s. Treatments began at this stage. At the end of the treatment, the same protocol was applied in reverse to obtain a smooth switch.

For each procedure, air collection was performed for 10 min, with the cyclone system placed at about 20 cm from the patient's mouth. A total of 9 m<sup>3</sup> of air was sampled per treatment.

The samples were then labelled with a combination of letters and random numbers linking them to the type of procedure but not to the specific patient, to ensure anonymity.

## 2.3 | Sample Preparation for ATP Analysis

The bio-contamination of the aerosol was assessed by means of ATP (Adenosine TriPhosphate) quantification. Measurements were performed using an ATP bioluminescence assay based on luciferin-luciferase reactions (Institut Clinident, France). The enzymatic reaction of bioluminescence between luciferin (2 MIN REAGENT, Institut Clinident) and firefly luciferase (Test Tube, Institut Clinident) causes ATP to release energy in the form of light. The emitted light was measured with a luminometer (Lumitester Smart, Kikkoman Biochemifa Company, Japan). This measurement is strongly dependent on environmental temperature. Therefore, after every sample measurement, a referenced ATP amount (Standard 1000, Institut Clinident) was added to the same sample and the emitted light was measured again to obtain system calibration.

In order to concentrate bacteria floating in the solution and to increase the sensitivity of the system, all solutions collected from the cyclone were filtrated on a sterile syringe filter (0.45  $\mu$ m PES, Merk Millipore, Germany). Once the solutions were filtrated, 160  $\mu$ L of enzymatic luciferin agent was sucked into the filter with the syringe, forming the luciferin-ATP complex in the bacteria concentrated on the filter. The solution was then expelled through the filter into the test tube containing the luciferase, followed immediately by the light measurements (result = R1). Immediately after this first measurement, the calibration ATP STANDARD 1000 was added into the tube (40  $\mu$ L) and the light measurement was taken again (results = R2).

The amount of light measured in RLU (Relative Light Unit) can be directly transformed into ATP amount using the two equations below:

$$\text{Correction factor} = \frac{R2 - R1}{1000}$$

With R1 being the result obtained in (RLU) for the sample, and R2 the result obtained in (RLU) for the sample + STANDARD 1000

$$[\text{ATP}] = \frac{R1}{\text{Correction factor} \times V}$$

With [ATP] the concentration of ATP in (pgATP/mL), and V the volume of solution filtered in (mL).

The conversion of ATP to the total bacteria amount was made according to the equation below:

$$1 \text{ pg ATP} \approx 1000 \text{ bacteria}$$

Therefore, the ATP measurement provided directly results in CFU/mL (Colony Forming Unit):

$$\text{Bacteria} = 1000 \times [\text{ATP}]$$

With 'Bacteria' the concentration of bacteria in the collected water in (CFU/mL).

To ensure maximal sensitivity for these tests, the entire water volume ( $V$ ) was used to collect the aerosol (approximately 100 mL).

## 2.4 | Microbial Load Calculation

To obtain the microbial aerosol load, the total amount of collected CFU/mL was divided by the total amount of collected air in order to get a bacteria concentration per unit of air volume:

$$\text{Bacteria air load} = \frac{\text{Bacteria} \times V_{\text{water}}}{Q_{\text{air}} \times \text{Time}}$$

With 'Bacteria air load' being the number of bacteria suspended in the air in (CFU/L<sub>air</sub>), 'Bacteria' the concentration of bacteria in the collected water in (CFU/mL),  $V_{\text{water}}$  the total amount of water collected,  $Q_{\text{air}}$  the air suction capacity in (NL/min) (= 900 NL/min), and 'Time' the collection time in (min), which is 10 for all the tests applied.

## 2.5 | Statistical Analysis

Bacterial air load was modelled using a generalised linear model with Gamma family and identity link. This allows us to account for a substantial skewness in the data distribution. Results were reported as estimated averages and 95% confidence intervals. All tests were two-sided and assumed a 5% significance level. All analyses were performed using R (version 4.1.0).

## 3 | Results

A total of 70 air samples were collected during various dental procedures, representing seven different treatment modalities. Each of the modalities was sampled for 10 min during 10 different procedures. The mean bacterial load in the air adjacent to the dental chair is summarised in Table 1. Pre-treatment baseline assessment of the room contamination showed an average of 1.45 (95% CI 0.85–2.04) CFUs/L of air. Very similar results were seen after the use of air-polishing with HVE, manual instrumentation with LVE, ultrasonic instrumentation with LVE and the rubber cup application with LVE. On the other hand, following the use of the 1:5 red contra-angle and the turbine with HVE the air contamination was clearly above the baseline. The highest count was observed following the application of the turbine with LVE with an average of 7.38 (95% CI 3.87–10.89) CFUs/L of air, which is the only procedure that showed a statistically significant difference with baseline ( $p < 0.01$ ) (Table 2). As per Table 2, none of the air samples following all the other procedures yielded a statistically higher concentration of bacteria when compared to the baseline.

As various methods are used in a clinical setting, a combination of procedures was analysed with regard to aerosol contamination (Table 3). No statistically significant differences were noted between the contamination of the air adjacent to the chair

**TABLE 1** | Mean bacteria air load (CFU/L<sub>air</sub>) and confidence interval per each procedure performed.

Group	Estimated concentration (95% CI)
Baseline	1.45 (0.85–2.04)
Air-polishing HVE	1.44 (0.57–2.32)
Ultrasonic inst.	1.44 (0.49–2.40)
Rubber cup	1.10 (0.30–1.89)
Manual inst.	1.13 (0.32–1.95)
Turbine	7.38 (3.87–10.89)
Turbine HVE	2.98 (1.34–4.63)
1:5 contra-angle HVE	2.70 (0.18–4.22)

Abbreviations: CI—confidence interval, HVE—high volume evacuator.

**TABLE 2** | Difference in bacteria air load (CFU/L<sub>air</sub>) between baseline sample and aerosol sample of 10 min of treatment (CI—confidence interval).

Contrast	Estimate (95% CI)	<i>p</i>
1:5 contra-angle HVE vs. Baseline	1.25 (−0.38; 2.88)	0.133
Air-polishing HVE vs. Baseline	−0.00 (−1.06; 1.05)	0.995
Manual inst. vs. Baseline	−0.31 (−1.32; 0.69)	0.543
Ultrasonic inst. vs. Baseline	−0.00 (−1.13; 1.13)	0.997
Rubber cup vs. Baseline	−0.35 (−1.34; 0.64)	0.490
Turbine HVE vs. Baseline	1.54 (−0.21; 3.28)	0.085
Turbine vs. Baseline	5.93 (2.37; 9.50)	<0.01

Abbreviation: HVE—high volume evacuator.

following the application of air polishing combined with ultrasonic instrumentation and the usage of ultrasonic instrumentation and rubber cup polishing. However, the use of the turbine even with HVE led to a highly statistically significant contamination compared to the combination of air-polishing with HVE and ultrasonic instrumentation ( $p < 0.01$ ).

## 4 | Discussion

The aim of the present study was to quantify the level of environmental contamination with aerosol produced during common dental procedures. Importantly, this study wants to reflect the real everyday practice of a standard European dental clinic, therefore applied some commonly used instruments and the combination of a HVE device for those which generally require it, such as air-polishing and the use of high-speed and low-speed drilling handpieces. Sampling through a wet-cyclone system was performed to measure the microbial contamination in the air adjacent to the dental chair. Bacterial load was determined by means of an ATP bioluminescence assay.



**TABLE 3** | Comparison of bacteria air load (CFU/L<sub>air</sub>) produced by different dental procedures and combinations of instruments.

Contrast	Estimate (95% CI)	<i>p</i>
Air-polishing HVE + Ultrasonic inst. vs. Rubber cup + Ultrasonic inst.	−0.00 (−1.30; 1.30)	0.999
Air-polishing HVE + Ultrasonic inst. vs. Rubber cup + Manual inst.	0.33 (−0.71; 1.37)	0.538
Turbine vs. Turbine HVE	4.40 (0.52; 8.28)	0.026
1:5 contra-angle HVE vs. Turbine HVE	−0.29 (−2.52; 1.95)	0.802
1:5 contra-angle HVE vs. Turbine	−4.68 (−8.51; −0.85)	0.017
Air-polishing HVE + Ultrasonic inst. vs. Turbine HVE	−5.94 (−9.56; −2.32)	<0.01

Abbreviation: HVE—high volume evacuator.

To determine the environmental air contamination, baseline assessments of the room air after disinfection and air refreshment were made. This assessment resulted in a baseline value of 1.45 CFU/L air. This value was used for comparison with the contamination resulting from various dental procedures. It is evident that most professional hygiene modalities did not trigger increased contamination values. In essence, the use of the air-polishing device, the ultrasonic device as well as the use of hand instruments and prophylaxis rubber cups did not result in any additional contamination besides the background evaluated by the baseline assessment. It is interesting to notice that those procedures traditionally considered at much higher risk for aerosol production (air-polishing, ultrasonic scaling), and therefore banned in many countries during the COVID-19 pandemic, did not seem to increase the environmental contamination significantly more than other supposedly safer procedures like cavity preparation, or even simply polishing with a rubber cup and manual instrumentation. The traditional professional oral hygiene procedure for general patients involves the use of ultrasonic instrumentation followed by polishing with a rubber cup and abrasive paste or air-polishing in case of tough stains. If the patient presents periodontal involvement, normally manual instrumentation is applied as well. A novel minimally invasive protocol has been introduced, involving the prominent use of air-polishing followed by ultrasonic instrumentation, known by the name of GBT (Guided Biofilm Therapy) [17]. The results of the present study, suggest that air-polishing and ultrasonic instrumentation do not increase air contamination significantly compared to manual scaling and polishing with rubber cups and abrasive paste, and can safely and confidently be utilised. However, it is also important to highlight the relevance of proper use of a pre-procedural rinse [10] and HVE in conjunction with air-polishing, as recommended by the manufacturers.

Substantially higher aerosol contamination than that noticed for the professional hygiene devices was observed after the use of rotary drilling instruments used for cavity preparation. In many dental offices, turbines with abundant water cooling are in use with or without the application of HVE. In the latter case, the air contamination was significantly and substantially elevated compared to all other methods tested. On the other hand, air contamination following the use of a turbine but with the HVE yielded similar outcomes as after the use of the red contra-angle hand-piece. When the procedure was performed with a red 1:5 contra-angle hand-piece with HVE, a moderate increase in air contamination was seen when compared to the prophylactic instruments. Apparently, the air contamination triggered by the

water spray with the red contra-angle can be rather limited, provided that a HVE is simultaneously applied. The observed four-fold increase in air contamination with the use of a turbine with Low Volume Evacuator (LVE) might constitute a higher contamination risk that could be avoided. It is, therefore, advisable to combine abundant cooling with HVE for restorative procedures, as it collects larger volumes of aerosols [9].

Unfortunately, HVE does not seem to be used as much as it should. Whilst no specific surveys of European clinicians are available, a survey of American hygienists, King & Muzzin [18] reported that, even if most of the surveyed hygienists think that is very important to minimise dental aerosols, very few used HVE with air-polishers and ultrasonic scalers. Aurangzeb et al. [19] surveyed Indian dental surgeons and found that only 3.8% of surgeons used HVE routinely. Yuzbasioglu et al. [20] showed that 41.6% of Turkish dentists used HVE. However, chances are that after the COVID-19 outbreak, these numbers may have increased dramatically, together with the awareness of the importance of aerosol control.

Pre-procedural rinses with chlorhexidine and cetylpyridinium chloride mouthwashes are also recommended to decrease the oral microbial load [10] that may become aerosols [21].

The results of the present study are in agreement with a historical study by Micik et al. [6] These authors investigated the amount of aerosol produced during various dental procedures by placing agar plates in the test room, and measuring the contamination via the number of CFU per minute of dental procedure. Activities such as breathing, speaking, shouting, coughing and sneezing were also analysed. CFU counts are very useful because they account only for the viable microorganisms in the sampled aerosol. However, there are sensitivity limitations due to the fact that many bacterial species cannot grow on standard agar plates, and viruses are not detected at all [9]. Regardless, CFU counts may be used as a good index of airborne contamination. In agreement with the present study, cavity preparation was amongst the procedures causing the biggest amount of contamination, and the application of HVE significantly reduced it. Interestingly, coughing also seemed to produce a considerable amount of aerosols [22].

A recent study by Zemouri et al. [23] measured a higher CFU/m<sup>3</sup> count higher than the present study. However, a comparison is difficult as this study does not specify which procedure was sampled

and for how long. Another recent study by Matys et al. [11] investigated the aerosol production during different dental procedures and with different suction devices. The colleagues came to the same conclusion that HVE allows a significant control over the produced aerosol when a high-speed hand piece is used for caries removal. Moreover, still in accordance with the present study, ultrasonic scaling did not seem to produce more aerosol than caries removal. However, a major difference between this study and Matys et al. [11] comes from the fact that the colleagues used manikin models instead of real patients. Also, measurements were taken with a laser particle counter. As the laser particle counter cannot distinguish between biological and non-biological materials, no information about the actual pathogenic potential of the aerosol in that study can be determined.

Whilst bacterial air contamination is important, most of the recent focus has been around aerosol contamination with viral particles, due to the COVID-19 pandemic. Adenosine triphosphate (ATP) is present in the cells of all living micro-organisms (bacteria, fungi and protozoa), but viruses cannot generate or store energy in the form of ATP. Therefore, one might argue that the present or the aforementioned studies cannot assess fully aerosol contamination. However, a study from Sifuentes et al. [24] demonstrated that ATP measurements could be useful for evaluating the effectiveness of hygiene interventions aimed at preventing viral spread in the workplace. In their study, reduction in ATP reflected reduction in viral concentration.

A limitation of the present study was the fact that the area was sampled for only 10 min during treatment. Professional oral hygiene sessions or decay removal may often last longer possibly creating more contamination. Moreover, this study did not take into account potential surface contamination by splatter. The reduced air flow speed and the 20 cm position from the mouth of the collection funnel allowed the entry of lighter particles only (aerosol), but was too slow and far to attract heavier splatter droplets. Given the bigger particle size of splatter ( $> 50 \mu\text{m}$ ), it settles on surfaces quite fast and it is able to contaminate only a small area around the dental chair [4]. Splatter control is performed through suction, proper Personal Protective Equipment (PPE), clear demarcation between dirty and clean areas and surface disinfection after each and every patient [8]. Another limitation of the study lays in the single sample used for ATP calibration and CFU calculation. Whilst some studies report a good quantitative correlation between ATP bioluminescence measurements and culture results [14, 15, 22], many factors might have influenced the sensitivity of the applied essay potentially leading to under or overestimation of the CFUs, such as the inclusion of extracellular ATP derived from dead cells [24], the fact that bacteria have different levels of intra-cellular ATP based on their species [25] and ATP levels can rise in the patient's mouth after a meal [26]. Therefore, whilst the reproducibility of such an essay appears satisfactory, alternative traditional methods such as culture-based sampling might be recommended to confirm the results of the present study [27].

## 5 | Conclusion

In conclusion, the results of the present study indicate that common dental and hygiene procedures do not trigger air contamination via aerosol spreading provided that proper suction

devices and pre-procedural disinfection mouth rinses are applied. However, if LVE is used in combination with drilling procedures an increased concentration of contaminants has to be expected. The highest contamination was observed during caries excavation with turbine and LVE.

## 6 | Clinical Relevance

### 6.1 | Scientific Rationale

Investigate the microbial load in the aerosol generated during various everyday dental procedures, in view of current reviewed concerns about infection control.

### 6.2 | Principal Findings

Common dental and hygiene procedures do not trigger air contamination via aerosol spreading provided that high-volume suction devices and pre-procedural disinfection mouth rinses are applied.

### 6.3 | Practical Implications

High volume suction and pre-procedural mouth rinses should be mandatory before aerosol-generating procedures.

### Author Contributions

M.M. and N.P.L. designed the study; E.S., M.S. and L.M. collected the samples and organised the data; M.D. designed the methods and performed the sample analysis; S.C. performed the statistical analysis; A.S. and N.P.L. wrote the article.

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### Conflicts of Interest

M.M. reports grants, consulting fees and non-financial support from EMS—Electro Medical Systems outside the submitted work; E.S. reports grants, consulting fees and non-financial support from EMS—Electro Medical Systems outside the submitted work; M.D. is employed by EMS - Electro Medical Systems which supported this study; A.S. reports consulting fees from EMS—Electro Medical Systems outside the submitted work; S.C. reports no conflicts of interest. L.M. reports no conflicts of interest. M.S. reports no conflicts of interest. N.P.L. reports no conflicts of interest, and received consulting fees from EMS—Electro Medical Systems outside the submitted work.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Mensi Magda, upon reasonable request.

## References

1. X. Peng, X. Xu, Y. Li, L. Cheng, X. Zhou, and B. Ren, "Transmission Routes of 2019-nCoV and Controls in Dental Practice," *International Journal of Oral Science* 12, no. 1 (2020): 9, <https://doi.org/10.1038/s41368-020-0075-9>.

2. C. M. C. Volgenant and J. J. de Soet, "Cross-Transmission in the Dental Office: Does This Make You Ill?," *Current Oral Health Reports* 5, no. 4 (2018): 221–228, <https://doi.org/10.1007/s40496-018-0201-3>.
3. T. Takeshita, S. Kageyama, M. Furuta, et al., "Bacterial Diversity in Saliva and Oral Health-Related Conditions: The Hisayama Study," *Scientific Reports* 6 (2016): 22164, <https://doi.org/10.1038/srep22164>.
4. R. L. Miller, R. E. Micik, C. Abel, and G. Ryge, "Studies on Dental Aerobiology. II. Microbial Splatter Discharged From the Oral Cavity of Dental Patients," *Journal of Dental Research* 50, no. 3 (1971): 621–625, <https://doi.org/10.1177/00220345710500031701>.
5. A. M. Bennett, M. R. Fulford, J. T. Walker, D. J. Bradshaw, M. V. Martin, and P. D. Marsh, "Microbial Aerosols in General Dental Practice," *British Dental Journal* 189, no. 12 (2000): 664–667, <https://doi.org/10.1038/sj.bdj.4800859>.
6. R. E. Micik, R. L. Miller, M. A. Mazzarella, and G. Ryge, "Studies on Dental Aerobiology. I. Bacterial Aerosols Generated During Dental Procedures," *Journal of Dental Research* 48, no. 1 (1969): 49–56, <https://doi.org/10.1177/00220345690480012401>.
7. C. Zemouri, H. de Soet, W. Crielaard, and A. Laheij, "A Scoping Review on Bio-Aerosols in Healthcare and the Dental Environment," *PLoS One* 12, no. 5 (2017): e0178007, <https://doi.org/10.1371/journal.pone.0178007>.
8. WHO, *Infection Prevention and Control of Epidemic and Pandemic Prone Acute Respiratory Infections in Health Care* (Geneva, Switzerland: WHO, 2014).
9. S. K. Harrel and J. Molinari, "Aerosols and Splatter in Dentistry: A Brief Review of the Literature and Infection Control Implications," *Journal of the American Dental Association* 135, no. 4 (2004): 429–437, <https://doi.org/10.14219/jada.archive.2004.0207>.
10. V. C. Marui, M. L. S. Souto, E. S. Rovai, G. A. Romito, L. Chambrone, and C. M. Pannuti, "Efficacy of Preprocedural Mouthrinses in the Reduction of Microorganisms in Aerosol: A Systematic Review," *Journal of the American Dental Association* 150, no. 12 (2019): 1015–1026.e1, <https://doi.org/10.1016/j.adaj.2019.06.024>.
11. J. Matys and K. Grzech-Leśniak, "Dental Aerosol as a Hazard Risk for Dental Workers," *Materials* 13, no. 22 (2020): 5109, <https://doi.org/10.3390/ma13225109>.
12. L. Veronesi, M. E. Colucci, C. Napoli, et al., "Air Microbial Contamination in Dental Clinics: Comparison Between Active and Passive Methods," *Acta Biomed* 91, no. 3–S (2020): 165–167, <https://doi.org/10.23750/abm.v91i3-S.9440>.
13. A. Watanabe, N. Tamaki, K. Yokota, M. Matsuyama, and S. Kokeguchi, "Use of ATP Bioluminescence to Survey the Spread of Aerosol and Splatter During Dental Treatments," *Journal of Hospital Infection* 99, no. 3 (2018): 303–305, <https://doi.org/10.1016/j.jhin.2018.03.002>.
14. A. Watanabe, N. Tamaki, K. Yokota, M. Matsuyama, and S. Kokeguchi, "Monitoring of Bacterial Contamination of Dental Unit Water Lines Using Adenosine Triphosphate Bioluminescence," *Journal of Hospital Infection* 94, no. 4 (2016): 393–396, <https://doi.org/10.1016/j.jhin.2016.08.001>.
15. Y. S. Cho, H. R. Kim, H. S. Ko, S. B. Jeong, B. Chan Kim, and J. H. Jung, "Continuous Surveillance of Bioaerosols on-Site Using an Automated Bioaerosol-Monitoring System," *ACS Sensors* 5, no. 2 (2020): 395–403, <https://doi.org/10.1021/acssensors.9b02001>.
16. I. Vouras, G. N. Antonoglou, S. Anoixiadou, and S. Kalfas, "A Novel Biofilm Removal Approach (Guided Biofilm Therapy) Utilizing Erythritol Air-Polishing and Ultrasonic Piezo Instrumentation: A Randomized Controlled Trial," *International Journal of Dental Hygiene* 20, no. 2 (2021): 381, <https://doi.org/10.1111/idh.12533>.
17. J. Koch-Heier, H. Hoffmann, M. Schindler, A. Lussi, and O. Planz, "Inactivation of SARS-CoV-2 Through Treatment With the Mouth Rinsing Solutions ViruProX® and BacterX® Pro," *Microorganisms* 9, no. 3 (2021): 521, <https://doi.org/10.3390/microorganisms9030521>.
18. T. B. King and K. B. Muzzin, "A National Survey of Dental Hygienists' Infection Control Attitudes and Practices," *Journal of Dental Hygiene* 79, no. 2 (2005): 8.
19. A. M. Aurangjeb, T. Zaman, and M. Badruddoza, "Practice of Dental Surgeons About Dental Splatter and Aerosol," *City Dental College Journal* 10, no. 2 (2013): 10.
20. E. Yüzbaşıoğlu, D. Saraç, S. Canbaz, Y. S. Saraç, and S. Cengiz, "A Survey of Cross-Infection Control Procedures: Knowledge and Attitudes of Turkish Dentists," *Journal of Applied Oral Science* 17, no. 6 (2009): 565–569, <https://doi.org/10.1590/s1678-77572009000600005>.
21. X. Xie, Y. Li, H. Sun, and L. Liu, "Exhaled Droplets due to Talking and Coughing," *Journal of the Royal Society Interface* 6, no. Suppl 6 (2009): S703–S714, <https://doi.org/10.1098/rsif.2009.0388.focus>.
22. L. Y. Sifuentes, S. L. Fankem, K. Reynolds, A. H. Tamimi, C. P. Gerba, and D. Koenig, "Use of ATP Readings to Predict a Successful Hygiene Intervention in the Workplace to Reduce the Spread of Viruses on Fomites," *Food and Environmental Virology* 9 (2017): 14–19, <https://doi.org/10.1007/s12560-016-9256-2>.
23. C. Zemouri, C. M. C. Volgenant, M. J. Buijs, et al., "Dental Aerosols: Microbial Composition and Spatial Distribution," *Journal of Oral Microbiology* 12, no. 1 (2020): 1762040, <https://doi.org/10.1080/20002297.2020.1762040>.
24. S. Fazilat, R. Sauerwein, M. L. Jennifer, et al., "Application of Adenosine Triphosphate-Driven Bioluminescence for Quantification of Plaque Bacteria and Assessment of Oral Hygiene in Children," *Pediatric Dentistry* 32, no. 3 (2010): 195–204, <https://doi.org/10.1016/j.ajodo.2008.12.002>.
25. A. Watanabe, N. Tamaki, K. Yokota, S. Kokeguchi, H. O. Ito, and M. Matsuyama, "Use of ATP Bioluminescence Assay to Evaluate Oral Streptococci," *Biocontrol Science* 27 (2022): 229–233.
26. C. Bill, J. A. Danielson, and R. S. Jones, "Salivary Intercellular Adenosine Triphosphate Testing in Primary Caretakers: An Examination of Statistical Significance Versus Diagnostic Predictability," *Clinical and Experimental Dental Research* 3, no. 6 (2017): 244–250, <https://doi.org/10.1002/cre2.95>.
27. M. R. Fulford, J. T. Walker, M. V. Martin, and P. D. Marsh, "Total Viable Counts, ATP, and Endotoxin Levels as Potential Markers of Microbial Contamination of Dental Unit Water Systems," *British Dental Journal* 196, no. 3 (2004): 157–159, <https://doi.org/10.1038/sj.bdj.4810943>.