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## **CLINICAL RESEARCH**

Aerosols Generated during Endodontic Treatment: A Special Concern during the Coronavirus Disease 2019 Pandemic



# SIGNIFICANCE

Aerosols generated during dental procedures are of concern during the COVID-19 pandemic. This clinical study revealed a lower number of aerosolized microorganisms generated during pulpotomy than in pulpectomy and nonsurgical root canal therapy.

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# ABSTRACT

Introduction: The aims of this study were to investigate aerosolized microorganisms generated during endodontic emergencies and nonsurgical root canal therapy (NSRCT), to assess the spread of airborne microbes, and to verify the spatial distribution of airborne microbial spread. Methods: A total of 45 endodontic procedures were sampled, including full pulpotomy (n = 15), pulpectomy (n = 15), and NSRCT (n = 15). Samples were collected during room resting and after treatment. The passive air sampling technique using settle plates was applied. Agar plates were set at different locations in the operatory. The colonyforming unit (CFU) was counted in brain-heart infusion blood agar plates. A set of agar plates containing selective chromogenic culture media was used for the isolation and presumptive identification of target microorganisms. Fungi were investigated using Sabouraud dextrose agar. **Results:** Pulpotomy generated the lowest mean CFU count (P < .05). There was no difference between the mean CFU counts found in pulpectomy and NSRCT (P > .05). A higher mean CFU count was found close to the patient's mouth (0.5 m) than at a 2-m distance in pulpectomy and NSRCT (P < .05). There was no difference between the mean CFU count found in front of the patient's mouth versus diagonal in pulpectomy and NSRCT (P > .05). Staphylococcus aureus (22/45, 48.8%) was the most frequent bacteria species. Longer treatment times were associated with higher CFU counts. Conclusions: Our findings indicated that pulpotomy generates less aerosolized microorganisms than pulpectomy and NSRCT. The proximity to the patient's mouth and the treatment duration were implicated in the level of contamination. (J Endod 2021;47:732-739.)

# **KEY WORDS**

Aerosol; coronavirus disease 2019; endodontic procedures; microorganism; root canal

Aerosols generated during dental procedures have recently taken the forefront of discussion in dentistry because of the coronavirus disease 2019 (COVID-19) pandemic<sup>1,2</sup>. As a result, there is likely a risk of transmission of acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in dental practice<sup>3</sup>. The primary mode of SARS-CoV-2 transmission is aerosol/droplet spread and contact with virus-contaminated surfaces acting as fomites<sup>4</sup>. Because of the dual risk of high amounts of aerosols generated in dentistry and saliva-borne SARS-CoV-2 in both symptomatic and asymptomatic individuals, dental associations immediately implemented guidelines restricting aerosol-generation procedures at the early stage of the COVID-19 pandemic. However, dental associations' responses to curb the clinic-associated nosocomial transmission of SARS-CoV-2 varied at that time<sup>5,6</sup>. Despite guidance, practitioners were reluctant and fearful of disease transmission and cross contamination within the dental clinic environment<sup>7</sup>.

Recommendations to avoid aerosol-generating procedures at the early stages of the pandemic posed significant challenges for managing dental emergencies, particularly to endodontists<sup>3,8,9</sup>. To avoid aerosol-generating procedures, palliative care with pharmacologic management of pain became the primary treatment rather than treating the endodontic emergencies with definitive root canal treatment (eg, nonsurgical root canal treatment [NSRCT])<sup>3</sup>. The secondary management<sup>3</sup> for endodontic emergencies, in particular for symptomatic irreversible pulpitis, the most common endodontic

emergency<sup>9</sup>, and symptomatic apical periodontitis, became full pulpotomy<sup>10,11</sup>. The selection of full pulpotomy as secondary management was due to an advantageously reduced treatment time, which could minimize endodontists' risk of being exposed to SARS-CoV-2 infection. At that time, endodontists raised the question of whether definitive root canal treatment or full pulpotomy generates more aerosolized microorganisms.

Over the years, most studies in dentistry have investigated bacterial aerosols generated during restorative and periodontal procedures<sup>1,2</sup>. However, current research has not assessed aerosolized microorganisms during root canal treatment. A systematic review and meta-analysis<sup>1</sup> suggested that studies are needed to measure aerosol contamination during dental procedures. The lack of studies evaluating aerosolized microorganisms in endodontic procedures, especially this meta-analysis, raised concerns among endodontists.

Because of this lack of evidence, our study focused on investigating aerosolized microorganisms generated during different root canal treatments. First, we successfully investigated the aerosolized microorganisms generated during endodontic emergency procedures (pulpotomy and pulpectomy) and NSRCT, describing the microbial load and composition; second, we assessed how far the airborne microbes spread during endodontic procedures and the level of contamination; and third, we verified the spatial distribution of airborne microbial spread during endodontic procedures and the level of contamination.

## MATERIALS AND METHODS

This study was approved by the local institutional review board at the University of Maryland, Baltimore, MD (#HP-00092103).

The passive air sampling technique using "settle plates" was applied to investigate microbial fallout during pulpotomy, pulpectomy, and NSRCT. This sampling technique has been widely used in different fields<sup>12-14</sup>. Microbial fallout samples were collected from a total of 45 endodontic procedures, including full pulpotomy (n = $(15)^{10}$ , pulpectomy (n = 15), and NSRCT (n = 15) 15). The sampling was performed in maxillary and mandibular teeth with primary root canal infection and symptomatic apical periodontitis undergoing the aforementioned treatment. Nonsurgical retreatment and periapical surgery were excluded from this study. The root canals were irrigated with 2.5% sodium hypochlorite.

#### **Sampling Procedures**

All samples were collected in the endodontic resident's operatory in a  $4 \times 4 \text{ m}^2$  room with closed doors. Samples were obtained first thing in the morning after overnight room resting. A high-efficiency particulate air (HEPA) filter (OSO Pure ADP-70 Air Disinfecting Purifier; Skaare Enterprises Inc, Glendale, AZ) was left on overnight and throughout the procedure.

For the first sample (s1), the room resting sampling, a set of agar plates (Table 1) was exposed to air for 30 minutes in the operatory before treatment. The plates were then closed and incubated accordingly (Table 1). This s1 sample was used to determine the colony-forming unit (CFU) count.

For the second sample (s2), treatment sampling, a set of new agar plates was opened concurrent to the start of the access cavity. To avoid traffic air turbulence, the dentist, assistant, and patient were already seated. The dentist and the dental assistant were positioned at the 11 and 1 o'clock position, respectively. Both the dentist and the dental assistant were wearing personal protective equipment, including a 6000 Series Half Facepiece Respirator (3M, St Paul, MN) and iMask + Face Shield (i-MAX Protective Eyewear Pty. LTD., Tasmania, Australia). The plates were spatially distributed at 1-m high from the floor at 4 different sites:

- 1. 0.5 m directly in front of the patient's mouth,
- 2. 2 m directly in front of the patient's mouth,
- 3. 0.5 m directly diagonal of the patient's mouth, and
- 4. 2 m directly diagonal of the patient's mouth.

Before rubber dam isolation and access cavity, patients were requested to use a 0.12% chlorhexidine digluconate antiseptic rinse for 60 seconds. The access cavity was performed under rubber dam isolation with a high-speed handpiece in 40,000 rpm with water spray. The flow rate for water was set at 6, on a 0 (no flow) to 100 (maximum) scale, for the Planmeca Compacti5 dental unit (Planmeca, Hoffman Estates, IL), The ADS EOS Extraoral Suction System (ADS Dental System Inc, Ontario, CA) was used immediately in front of the patient's mouth ( $\approx$ 20 cm). The plates were left open until the completion of the treatment. This s2 sample was used to determine the microbial contamination levels (CFU/plate) and the composition of target bacterial species.

TABLE 1 - The Set of Agar Plates Used for the Investigation of Microbial Fallout during Root Canal Procedures

| Outhing Madia                                    | la cubatica   | Townshipson   | color  |
|--|---|---|--|
| Culture Media                                    | Incubation  | l'arget bacteria  | appearance   |
| Brain-heart infusion<br>agar + 5% sheep<br>blood | 37°C in aerobic<br>conditions for 48<br>hours         | Variety of organism<br>types, including<br>bacteria, yeasts, and<br>filamentous fungi   | Various  |
| CHROMagar<br>Staphylococcus                      | 37°C in aerobic<br>conditions and read<br>at 24 hours | Staphylococcus aureus<br>Staphylococcus<br>epidermidis<br>Staphylococcus<br>saprophyticus<br>Other staphylococci<br>Streptococci inhibited<br>Gram-negative bacteria<br>inhibited | Pink to mauve<br>Colorless to<br>pinkish<br>Turquoise<br>blue<br>Various           |
| CHROMagar<br>Pseudomonas                         | 30°C in aerobic<br>conditions read at 24<br>hours     | Pseudomonas spp.<br>Most of<br><i>Enterobacteriaceae</i><br>Gram-positive bacteria<br>inhibited   | Blue green<br>Mauve to<br>violet or<br>inhibited                                   |
| CHROMagar<br>Strep A                             | Incubate at 37°C and read at 24 hours                 | Group A Streptococcus<br>Other oral streptococci<br>Other gram-positive<br>bacteria<br>Yeasts<br>Gram-negative bacteria   | Orange to red<br>Colorless or<br>steel blue<br>Inhibited<br>Inhibited<br>Inhibited |
| Sabouraud dextrose<br>agar +<br>chloramphenicol  | 25° ± 2°C in aerobic conditions for 7 days            | Yeasts and molds<br>Bacteria inhibited  | Various  |

### Measure of Microbial Fallout (Microbial Load/CFU Count)

To measure the fallout microorganisms, standard Petri dish plates 9 cm in diameter containing brain-heart infusion agar + 5% sheep blood were used. After the incubation period (Table 1), the number of CFUs was counted in the plate using a stereomicroscope with  $1000 \times$  magnification (VWR, Radnor, PA). The mean number of CFUs was calculated.

# Investigation of Microbial Composition

For the investigation of microbial composition, plates containing selective chromogenic culture media (CHROMagar, DRG International, Inc., Springfield, NJ, U.S.A.) were used for the isolation and presumptive identification of target Staphylococcus spp, Streptococcus spp, and Pseudomonas spp (Table 1). Additionally, Sabouraud dextrose agar + 0.05 g chloramphenicol was used for fungi investigation (Table 1). All culture media were prepared according to the manufacturer's instructions. A set of standard Petri dish plates 9 cm in diameter containing the aforementioned culture media were left open throughout the treatment spatially distributed as described previously. The plates were closed and incubated accordingly (Table 1). After the incubation period, the colony's presence was verified in the agar plate, and the presumptive identification was performed according to the typical colony appearance described by the manufacturer (Table 1). American Type Culture Collection (Manassas, VA) strains were tested as a positive control before presumptive identification of the samples.

Figure 1 shows the typical color colony appearance of bacteria species in

CHROMagar media. Time to complete endodontic treatment was measured in minutes by stopwatch. Time recorded began with the initiation of access cavity preparation with a high-speed handpiece and ended with placement of a temporary restoration.

## **Statistical Analysis**

Data were expressed as the mean  $\pm$  standard deviation. After the Shapiro-Wilk test, data were analyzed by 1-way analysis of variance (ANOVA) to assess differences between groups followed by a Tukey test for multiple comparisons. Two-way ANOVA was applied to determine the differences within groups (position and distance factors). Kruskal-Wallis 1-way ANOVA on ranks was used to evaluate the duration of the treatment (time in minutes). To explore the possible association between time and bacteria levels, Pearson correlation analyses were performed. Correlation analyses were performed for the overall study sample. For all tests, a significance level of 5% was used.

# RESULTS

## Microbial Fallout after Room Resting (s1) and during Endodontic Procedures (s2) (CFU Count and Level of Contamination)

At s1, bacteria were detected in only 3 of 45 room resting samples with a low mean CFU value of 0.177  $\pm$  0.386. No difference was found among the treatment modalities at s1 (*P* > .05). There was a significant difference between the mean CFU values found in s1 versus s2 for all treatments (*P* < .05). At s2, pulpotomy generated the lowest mean CFU count (*P* < .05) (Table 2). There was no significant difference between the mean CFU

count found in NSRCT and pulpectomy at s2 (P > .05) (Table 2).

### Spatial Distribution of Airborne Microbial Spread (CFU Count and Level of Contamination)

There was a significant difference between the level of contamination encountered close to the patient's mouth (0.5 m) than at a 2-m distance both in pulpectomy and NSRCT (P < .05) (Table 2). Furthermore, there was no significant difference between the CFU count set directly in front of the patient's mouth versus diagonal (P > .05), irrespective of the distance (0.5 or 2 m distant), both in pulpectomy and NSRCT (Table 2).

## **Microbial Fallout Composition**

Overall, the most frequent bacteria species detected was *Staphylococcus aureus* (22/ 45, 48.8%) followed by *Staphylococcus epidermidis* (19/45, 42.2%) and oral streptococci (15/45, 33.1%). Oral streptococci were only detected close to the patient's mouth at a 0.5-m distance but not at 2 m. *Pseudomonas aeruginosa* and fungi were not detected.

# Treatment Duration (in minutes) and Level of Contamination

The mean time required to complete the endodontic procedures was  $34.8 \pm 3.3$  minutes for pulpotomy,  $73.7 \pm 13.7$  minutes for pulpectomy, and  $108 \pm 16.8$  minutes for NSRCT, respectively. There was a positive correlation between the procedure duration and the level of contamination (CFU count) (Table 3). Longer treatment times were associated with higher CFU counts. Dispersion graphs (Fig 2) show the correlation between



FIGURE 1 – Typical color colony appearance of bacteria species presumptively identified in CHROMagar media (a stereomicroscope with 1000 × magnification).

**TABLE 2** - The Mean  $\pm$  Standard Deviation of Microbial Fallout (Colony-forming Unit/Plate) according to the Treatment,Distance, and Spatial Distribution

| Distance                         | In front of the patient's mouth  |   | Diagonal from the patient's mouth   |  |
|----------------------------------|--|---|---|--|
| Treatment                        | BHI (0.5 m close)  | BHI (2 m far)   | BHI (0.5 m close)   | BHI (2 m far)  |
| Pulpotomy<br>Pulpectomy<br>NRSCT | $\begin{array}{l} 0.46 \pm 0.7^{Aa} \\ 2.5 \pm 1.8^{Ba} \\ 2.7 \pm 2.1^{Ba} \end{array}$ | $0.26 \pm 0.4^{Aa}$<br>$1.5 \pm 1.6^{Bb}$<br>$1.5 \pm 0.8^{Bb}$ | $0.5 \pm 0.5^{Aa}$<br>2.46 ± 2.5 <sup>Ba</sup><br>2.6 ± 1.3 <sup>Ba</sup> | $0.2 \pm 0.4^{Aa}$<br>$1.4 \pm 1.2^{Bb}$<br>$1.4 \pm 0.8^{Bb}$ |

BHI, brain-heart infusion; NRSCT, nonsurgical root canal treatment.

Different superscript uppercase letters indicate a statistically significant intergroup difference (within the same position and distance) (1-way analysis of variance/Tukey post hoc test). P < .05 was applied for BHI (.5-m close), and Kruskal-Wallis 1-way analysis of variance on ranks was applied for BHI (2 m far), BHI (diagonal .5 m close), and BHI (diagonal 2 m far). Different superscript lowercase letters indicate statistically significant intragroup difference within the same position (.5 m vs 2 m) (2-way analysis of variance/Tukey post hoc test, P < .05).

the duration of the procedure and the level of contamination.

## DISCUSSION

Data obtained in the present study revealed that pulpotomy generated the lowest mean CFU count compared with pulpectomy and NSRCT (P < .05). There was no difference between the mean CFU counts found in pulpectomy and NSRCT (P > .05). We found a higher level of contamination close to the patient's mouth (0.5 m) than at a 2-m distance both in pulpectomy and NSRCT (P < .05). Additionally, there was no difference between the mean CFU count found in front of the patient's mouth versus diagonal in pulpectomy and NSRCT (P > .05). Furthermore, longer treatment times were associated with higher CFU counts.

In this study, to achieve our results, we investigated aerosolized microorganisms using the passive air sampling technique with "settle plates." This sampling technique has been widely used in dentistry<sup>12–14</sup>. This method quantifies the viable microorganisms that can settle, grow, and multiply in a plate<sup>15</sup>. Some authors have listed several advantages of passive air sampling<sup>15</sup>. It uses Petri dishes containing culture media exposed to the air for a given time to collect biological particles<sup>15</sup>. These biological particles "sediment" out and are subsequently incubated, and results are expressed in CFUs. Besides collecting

microbial fallout onto agar plates, it provides a valid risk assessment to measure the airborne population's harmful part<sup>16</sup>. One of the disadvantages of this passive air sampling technique is the lack of standardization across the studies, limiting the comparison of the results. For example, Petri dishes of different diameters, exposure times, nutrient media, incubation temperatures, and times make it difficult to compare data. Here, we followed the most common parameters described in the literature for the CFU count with Petri dishes 9 cm in diameter, brain-heart infusion agar supplemented with 5% sheep blood, and incubation at 37°C for 48 hours.

We found almost no cultivable airborne microorganisms for the room resting samples, indicating good air quality. It is worth pointing out that the HEPA filter was left on overnight. According to the Institute of Environmental Sciences and Technology, HEPA filters can capture 99.97% of contaminants 0.3  $\mu$ m in size and larger.

To minimize the risk of exposure to SARS-CoV-2 during the COVID-19 pandemic, besides the use of regular personal protective equipment including a KN95 mask and face shield, we combined different interventions to reduce contaminated aerosols during endodontic procedures, including interventions to prevent the contamination of aerosols in the mouth (chlorhexidine mouthwash), interventions to prevent contaminated aerosols from escaping the

**TABLE 3** - The Correlation between the Overall Duration of the Procedure (Time in Minutes) and the Level of

 Contamination (Colony-forming Unit Count)

|  | R    | P value |
|--|------|---------|
| BHI agar 0.5 m from the patient's mouth          | 0.46 | .001    |
| BHI agar 2 m from the patient's mouth            | 0.56 | .0000   |
| BHI agar 0.5 m diagonal from the patient's mouth | 0.65 | .00000  |
| BHI agar 2 m diagonal from the patient's mouth   | 0.58 | .0000   |

BHI, brain-heart infusion.

mouth (rubber dam isolation and high-volume evacuation system [HVE]), and interventions to reduce the overall concentration of aerosols in the dental operatory (HEPA filter). Surprisingly, despite using all of the aforementioned preventive measures to reduce contaminated aerosols produced during root canal therapy, we detected bacteria in the majority of the samples collected after pulpectomy and NSRCT. Although bacteria were still recovered from the majority of the samples after pulpectomy and NSRCT, it was recovered in a low mean value (<3 CFU/plate count). Monteiro et al.<sup>14</sup> reported a mean CFU/plate count of 21.5  $\pm$  12.1 at 0.5 m and 17.8  $\pm$  9 at a 2-m distance from the patient's head position during endodontic treatment. The lower levels of contamination (CFU/plate count) found here compared with Monteiro et al.<sup>14</sup> are attributed to combining the interventions mentioned previously to reduce aerosols during endodontic procedures.

To prevent contamination of aerosols in the mouth, all patients used chlorhexidine antiseptic rinse for 60 seconds. Preprocedural mouth rinses can reduce the salivary concentration of microorganisms, reducing the number of viable microorganisms on aerosolgenerating procedures<sup>17,18</sup>. The use of mouth rinses in the context of COVID-19 is currently being evaluated<sup>19–22</sup>. Recent studies have shown the anti–SARS-CoV-2 efficacy of mouth rinses<sup>21,22</sup>.

To prevent contaminated aerosols from escaping the mouth, we placed the rubber dam isolation before starting the access cavity. Although rubber dam isolation is the standard of care for endodontic treatment, controversy exists whether its use can reduce bacterial atmospheric contamination during dental procedures<sup>23–27</sup>. The majority of the studies<sup>24–27</sup> produced a broad consensus that rubber dam isolation during aerosolgenerating procedures effectively reduces the spread of spatter by 33% and reduces surface contamination with bacteria at 80%-99% at a distance up to 1 m. However, Al-mad et al.<sup>23</sup> suggested that the rubber dam could deflect spatter onto the dentist's head. More recently, in a systematic review, Kumbargere Nagraj et al.<sup>1</sup> revealed a lack of clinical evidence on the ability of rubber dam isolation in reducing the spread during aerosol-generating procedures. It is worth pointing out that most dental aerosol studies reported in the literature are in restorative and periodontal procedures with a lack of evidence in endodontic procedures. To the best of our knowledge, this is the first study to compare aerosols generated during pulpotomy, pulpectomy, and NSRCT.

The HVE system used here to prevent contaminated aerosols from escaping the



**FIGURE 2** – Dispersion graphs showing the correlation between the duration of the procedure (time in minutes – independent variable) and the overall (all groups) data of the CFU count. (A) 0.5 m and (B) 2 m from the patient's mouth; (C) 0.5 m and (D) 2 m diagonal from the patient's mouth.

mouth can draw a large air volume within a short period<sup>18,28</sup>. Studies evaluating the use of HVE have shown varying results, with a 90.8% reduction of aerosols<sup>29</sup> to no significant differences<sup>30</sup>, between the use and no use of HVE. Kumbargere Nagraj et al.<sup>1</sup> revealed that HVE might reduce bacterial contamination in aerosols less than 1 ft but not at a long distance. Additionally, Noro et al.<sup>31</sup> found that an extraoral vacuum aspirator effectively reduced streptococci spread and recommended treating patients with infectious diseases.

How far the aerosols spread and what level of contamination are of concern<sup>13,14</sup>. Here, for pulpectomy and NSRCT, the contamination level (CFU count) was significantly higher at 0.5 m than at 2 m. In agreement, Monteiro et al.<sup>14</sup> reported a higher mean bacteria CFU count at 0.5 m (21.5 ± 12.1) at a 2-m (17.8 ± 9) distance from the patient's head position during endodontic treatment. Besides investigating how far the aerosols can spread during endodontic procedures, we also investigated the spatial distribution of airborne microbial spread during endodontic procedures and the contamination level. Our data indicated no difference in the contamination level found in front and diagonal of the patient's mouth. According to Bentley et al.<sup>32</sup> there is an extremely variable distribution of bacterially contaminated aerosols and spatter. However, it seems to be a consensus that the highest contamination is found close to the patient's mouth and at the patient's chest area<sup>13,14,32</sup>. Studies on dental aerobiology reveal that, depending on the size of the airborne particles, they can remain suspended as aerosols or fall rapidly and splatter on objects in their trajectory<sup>33</sup>. Airborne particles larger than 50–100  $\mu$ m in diameter have initial forces greater than the frictional air forces and are ballistic in nature. True aerosol particles are usually less than 50 μm in diameter, invisible, and airborne for longer periods<sup>33</sup>. Infective agents are bacterial aerosol particles in the 0.5- to 10-µm diameter range, which can be inhaled and impinged in

the terminal bronchioli and alveoli of the human lung<sup>33</sup>.

For the investigation of microbial composition, we used agar plates containing selective chromogenic culture media (CHROMagar) for the presumptive identification of Staphylococcus spp, Streptococcus spp, and Pseudomonas spp. CHROMagar media is widely used for the presumptive identification of pathogens<sup>34,35</sup>. This chromogenic culture media technology is based on soluble colorless molecules (called chromogens) composed of a substrate (targeting a specific enzymatic activity) and a chromophore for microbial investigation<sup>36</sup>. When the target organism's enzyme cleaves the colorless chromogenic conjugate, the chromophore is released. There are a couple of advantages to this method. As a colorbased differentiation method it is easy to read and under normal light conditions, it is distinguishable with the naked eye. Moreover, it allows for easy differentiation of microorganisms<sup>36</sup>. We examined

Staphylococcus spp, Streptococcus spp, and *Pseudomonas* spp based on previous investigations reporting their occurrence in aerosols generated during dental procedures<sup>13,37–41</sup>. *Pseudomonas* was investigated because it is an opportunistic pathogen present in biofilm in dental unit waterlines and may also be aerosolized during dental procedures<sup>39</sup>. Additionally, we investigated the presence of fungi using Sabouraud dextrose agar supplemented with chloramphenicol, which was previously reported in dental aerosols<sup>13</sup>.

The most frequent bacteria species identified in the present study was S. aureus followed by S. epidermidis. Previous studies have demonstrated that Staphylococcus spp are frequently identified in dental aerosol studies<sup>13,14,37,38,40</sup>. We detected oral streptococci only close to the patient's mouth (at a 0.5-m distance) but not at 2 m. The presumptive identification of oral streptococci found here is a relevant indicator of salivary contamination of air. Such a finding, in consonance with other results<sup>41</sup>, supports the concept that saliva is 1 of the sources of pathogens in dental aerosols. Despite plausible evidence suggesting that dental unit waterlines might contribute to a large fraction of the microbial load in dental

aerosols, mainly *Pseudomonas* spp.<sup>39</sup> we verified no colony growth in the CHROMagar *Pseudomonas* media. Furthermore, we recovered no fungi with Sabouraud dextrose agar supplemented with chloramphenicol. It is worth noting that it is expected to find a certain microbial heterogeneity across clinical studies.

There is no direct evidence indicating that the spread of microorganisms during dental treatment is a major cause of infectious disease in dentists and patients. However, the possibility cannot be ignored, especially during the current COVID-19 pandemic in which symptomatic and asymptomatic patients carrying SARS-CoV-2 can be a source of infection in dental practices.

In light of the current COVID-19 pandemic situation, our data showed that fewer aerosolized microorganisms are generated during pulpotomy than in pulpectomy or NSCRT. Additionally, our results indicated a higher level of contamination closest to the patient's mouth with no difference in spatial distribution directly in front or diagonal to the mouth. However, it is important to highlight that our study evaluated bacterial contamination. Viruses are much smaller, and it can be speculated that viruses suspended in the air in small airborne particles can reach greater distances from the patient's mouth than was found here.

One of the limitations of this study is that because of the current COVID-19 pandemic, we could not assess individual interventions adopted to verify their single effectiveness in reducing dental aerosols during endodontic treatment. However, while waiting for more researchers to share their preventive measures to reduce aerosolized microorganisms generated during endodontic procedures during the current COVID-19 pandemic, our study shows that the preventive measures adopted at our institution resulted in an overall low number of CFU counts for all treatment modalities.

In conclusion, our findings indicate that pulpotomy generates less aerosolized microorganisms than pulpectomy and NSRCT. Moreover, the proximity to the patient's mouth and the treatment duration were implicated in the level of contamination.

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