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ABSTRACT

Background. The use of dental handpieces produces aerosols containing microbial agents, bacteria and viruses representing a high-risk situation for airborne cross-infections. This study aimed to map and quantify the biological contamination of a dental operatory environment using a biological tracer.

Methods. *Streptococcus mutans* suspension was infused into the mouth of a phantom, and an operator performed standardized dental procedures using an air turbine, a contra-angle handpiece or an ultrasonic scaler. The presence of the tracer was measured at 90 sites on the dental unit and the surrounding surfaces of the operatory environment.

Results. All tested instruments spread the tracer over the entire dental unit and the surrounding environment, including the walls and ceiling. The pattern and degree of contamination were related to the distance from the infection source. The maximum distance of tracer detection was 360 cm for air turbine, 300 cm for contra-angle and 240 cm for scaler (11.8, 9.8 and 7.9 ft, respectively). No surface of the operative environment was free from the tracer after the use of the air turbine.

Conclusions. Attention should be paid to minimize or avoid the use of rotary and ultrasonic instruments when concerns for the airborne spreading of pandemic disease agents are present.

Practical Implications. The present study supports the recommendations of dental associations to avoid treatments generating aerosols, especially during pandemic periods. Guidelines for the management of dental procedures involving aerosols are urgently needed, as well as methods for the aerosols modification aimed to inactivate the infective agent.

Key Words. Aerosols; Air Microbiology; Bacteria; Communicable Disease Control; Cross-Infection; Decontamination; Dental Equipment; Disease Transmission, Patient-to-Professional; Streptococcus; Equipment contamination.

INTRODUCTION

The risk of airborne contamination in dentistry is considered high[1] due to the unique characteristics of the dental equipment. The cooling spray of dental handpieces is one of the primary sources of splatter and aerosol in surgery.[2-4] Splatters are air suspensions of liquid or solid particles having a particle size of approximately 100 µm or more, while aerosol particles have a smaller diameter (<50 µm).[5] Splatters are too large to be inhaled but can contaminate skin, eyes, hair, and clothing, in addition to the dental working area. On the contrary, aerosol particles can remain suspended for a relatively long time (up to 30 min) after the end of an operative procedure and are easily spread throughout the operative environment by air currents.[6-8] There is evidence that dental aerosol can reach a distance of 1 through 3 meters from its source.[9, 10] From this point of view, they are vectors of infective agents that show potential for contamination not only of the dental personnel and patients but also of all exposed surfaces of the dental unit and the operatory environment.[5] In addition to microbial species from non-pathogenic oral flora, aerosols may contain pathogenic bacteria (such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Staphylococcus* spp) and viruses (such as HIV, HBV, HCV, HSV, influenza virus, and rhinovirus).[11-14] The problem of airborne contamination in the dental operatory environment recently returned to the spotlight due to the outbreak of coronavirus disease 2019 (SARS-CoV-2). This infective agent owes its virulence to high contagiousness related to airborne transmission and to the fact that it can survive on surfaces for up to 72 h.[15] The spreading of SARS-CoV-2 places tremendous stress on health systems worldwide.[16] For this reason, additional preventive measures are introduced and continuously updated in all health care settings, including dentistry, to reduce further dispersion of this disease.[4, 17-22]

In the last 50 years, attempts have been made to determine the topographical distribution of the airborne contamination caused by the different rotary and vibrating-oscillating dental instruments.[3, 19, 20] The relevance of these data is profound in terms of trying to rationally direct the disinfection procedures on areas of higher contamination, and to control the spreading of diseases.

Different approaches have been used to map the contaminants, from dye tracers to microbiological evaluation of air and surface contamination.[2, 10, 14, 23] The literature shows that dental treatments significantly increase biological contamination of dental operatories to a higher level than public areas.[14, 24] Nevertheless, to our knowledge, no study has determined the topographical distribution of surface contamination in the dental operatory, though this information is essential to implement protocols for disinfection procedures in areas with critical levels of contaminants.

In the present study, we aimed to evaluate the contamination resulting from the use of rotary and vibrating oscillating instruments in a dental operatory, using a biological tracer. The null hypotheses were that the presence of the tracer would be uniformly detected on the dental unit and the operatory environment surfaces, and the spread of the tracer would not be different when using different handpieces.

METHODS

Operatory room. A 598 x 376 x 270 cm (length x width x height, corresponding to 19.6 x 12.3 x 8.9 ft) operative environment located in the Dental Clinic, San Paolo Hospital, University of Milan, was used for the study. Both raised floor and false ceiling were made of 60 x 60 cm-sized PVC panels (2 x 2 ft). The air-conditioning system of the room was isolated by sealing the inlet. The room was equipped with a dental unit (Skema 4, Castellini, Bologna, Italy), two dental stools and a four-door cabinet located behind the dental chair.

The presence of a biological tracer was measured in 22 sites of the dental unit and 68 sites of the operatory room, as follows. A total of 14 sites were located on the dental chair, one on the assistant pad, one on the instrument tray, one on the cuspidor cup, one on the water glass tray, three on the overhead dental unit light and one on the foot pedal. In the operative room, we located 48 sites on the floor, 4 on the wall in front of the dental unit, 1 on the lateral column, 5 on the back wall, 6 on the ceiling and 4 on the cabinet (Fig. 1, 2). The same sites were used throughout all the experiments.

Bacteria. All reagents and culture media were obtained from Becton–Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, USA). A wild strain of *Streptococcus mutans* was isolated

on Mitis Salivarius Bacitracin (MSB) agar from the dental operator who performed all dental procedures. Biochemical identification of the isolated strain was performed using an automatic device (Vitek 2, BioMerieux, Marcy-L'Etoile, France). Then, a pure suspension of *S. mutans* in Trypticase-soy broth was obtained from a single colony grown on the selective medium after a 12-h incubation at 37 °C in a 5%-supplemented CO_2 environment. Cells were harvested by centrifugation (1.500 x g, 19°C, 5 min), washed twice with sterile phosphate-buffered saline (PBS), and resuspended in the same buffer. The cell suspension was subjected to sonication (Sonifier model B-150; Branson, Danbury, CT, USA; 7W energy output, 30 s) to disperse bacterial chains and adjusted to 1.0 McFarland standard. A fresh cell suspension in the logarithmic phase was obtained before the beginning of each experiment.

Experimental setup. A phantom head was adapted to the chair headrest in a standard working position (Fig. 1A). The jaws inside the phantom were equipped with resin teeth (Columbia Dentoform Corp., Long Island City, NYC, USA). The drainpipe of the phantom was connected to a high-speed suction. After that, the operator performed a total of three standardized dental procedures on the lower right first molar, as follows. For the first procedure, the operator prepared a Class I cavity using an air turbine handpiece (Bora Led, Bien-Air Dental SA, Bienne, Switzerland) equipped with a cylindrical diamond bur (835KR.314.016, Komet Italia Srl, Milan, Italy). The air pressure was 3.3 atm, and the speed was 320.000 r.p.m. For the second procedure, a contra-angle handpiece (1:1) (CA 1:1, Bien-Air) was used with a round tungsten carbide bur (H1SM.204.020, Komet) inside the already prepared cavity at a speed of 50.000 r.p.m, to mimick removal of deep carious tissue and cavity refining. In the third procedure, an ultrasonic scaler (Suprasson, Satelec - Acteon, Merignac, France, operating at 28 kHz oscillation frequency) equipped with an A2 insert, reached below the gumline of the labial, lingual and interproximal surfaces of the same tooth. Each procedure lasted 240 s, and the resin tooth was replaced after performing the three procedures. A continuous flow of *S.mutans* suspension (30 ml/min) was infused in the mouth of the phantom on the lingual surface of the lower right second molar throughout all procedures using a drip device. The operator wore biohazard protective full suit,

including cover shoes, gloves, FFP2/N95 mask without valve, and face shield to protect himself against possible infections by the tracer agent.

Operatory and microbiological procedures. Before the beginning of each procedure, 90 MSB agar plates were coated and placed one in each of the corresponding sites, keeping the lid closed. The operator took their position and then a coworker, equipped with the same biohazard protections as the operator, opened the lids of every plate. After that, the operator opened the suspension drip and performed a 4-min procedure. The plates were closed by the coworker 26 min after the end of the procedure to allow aerosols to settle. Then, he immediately transferred the plates to the microbiological laboratory. The plates were incubated at 37 ◦C for 48h in a 5% supplemented CO_2 environment. At the end of the incubation, colonies were counted, and results were expressed as colony-forming units (CFU)/cm^2.

The procedure was repeated 15 times for each handpiece. Between procedures, the environment was disinfected overnight using an ambient decontamination device (Phileas 75, Devea, Granchamp-des-Fontaines, France). The operator repeated the same procedures once without using the bacterial suspension, considering the results for the tested dental unit and the operative environment as the tracer's blank.

Statistical analysis. The statistical software (JMP 10.0, SAS Institute, Cary, NC, USA) was used to analyze microbiological data belonging to the tracer presence on the dental unit and the operatory room. Shapiro-Wilk's test was applied to check the normality of the data distribution, and Bartlett's test was used to check homogeneity of variances preliminarily. Since the data distribution was not normal, data were log-transformed to approach a normal distribution. A two-way ANOVA was used considering the handpiece and the topography as fixed factors, and Tukey's HSD post-hoc test was used to highlight significant differences between groups, at a level of significance (α) of 0.05.

RESULTS

Figures 2 and 3 show the topographical distribution of the tracer. Mean tracer levels ±1SE are shown in Figure 4.

Tracer presence on dental chair unit. ANOVA results showed a highly significant difference in tracer levels between the tested handpieces ($p<0.01$). The mean levels of tracer were as follows: air turbine (0.51±0.17) > scaler (0.47±0.14) > contra-angle handpiece (0.41±0.14). No significant difference was found between different sites on the dental unit when considering the distance from the infection source ($p>0.05$). Also, an interaction between the considered factors was not found ($p=0.08$). When considering the two sides of the dental chair, the left -side showed higher tracer levels than the right side when the air turbine was used ($p=0.01$). No significant differences in tracer presence were noticed when using contra-angle or scaler ($p=0.98$ and $p=0.48$, respectively).

Tracer presence in the operatory room. The use of all handpieces spread the tracer all over the surrounding environment of the dental operatory. The mean levels of tracer were as follows: air turbine (0.26±0.38) > contra-angle (0.20±0.26)> scaler handpiece (0.17±0.27). The pattern and the tracer levels were related to the distance from the infection source. A highly significant interaction was found between factors ($p<0.0001$). This result shows that the topographical distribution of the tracer varied depending on the tested handpiece. When the ultrasonic scaler was used, the tracer reached a maximum distance of 240 cm (7.9 ft), while during the use of the contra-angle handpiece the tracer reached 300 cm (9.8 ft). When using the air turbine, however, no site remained free from tracer presence, reaching the maximum distance recorded, i.e. 360 cm (11.8 ft). Sites located on the floor behind the operator showed low tracer presence. The tracer presence on the walls and the ceiling was low, except for the ceiling area just over the dental unit, where we found a high tracer presence (Figure 3). Morphological observation of plates after incubation showed high variability in the distribution of the colonies on the surfaces of the plates located within 150 cm from the tracer source.

DISCUSSION

The danger of cross-infection through splatters and aerosols has long been considered one of the main concerns in the dental practice.[2, 3, 14, 20, 24] Air-spray cooled handpieces, such as air turbines, contra-angles, and scalers, produce splatters and aerosols that can reach a considerable distance,

carrying potentially infective agents.[6, 14, 19, 22] Despite being necessary, the use of air-spray cooling is recognized as one of the primary sources of contamination in the dental setting.[23] In fact, even before the outbreak of SARS-CoV-2, the potential airborne spreading of life-threatening infections was well recognized.[13, 14, 25] Nevertheless, there is very little data available on the topographical distribution of contamination induced by aerosol-generating devices. The SARS-CoV-2 outbreak highly increased the need for such experimental data,[22, 26, 27] in order to rationally address the operative and disinfection procedures yielding the lowest possible contamination levels.

The contamination usually produced directly by the patient themself (talking, breathing, sneezing or coughing) or during high-risk medical procedures (tracheal intubation, manipulation of the oxygen mask, bronchoscopy, non-invasive ventilation, insertion of a nasogastric tube) shows a high variability due to interindividual differences.[14, 28] Furthermore, aerosols produced by the patient show different behavior depending on the particle size. Indeed, it was observed that the size of a pathogen dictates the size of the particle that is carrying that pathogen. For instance, aerosol particles that carry viral particles are much smaller than particles carrying larger pathogens such as bacteria.[12] This may not be the case in the dental setting since aerosols and splatters are mechanically produced and thus have a particle size that depends on the functioning parameters of each handpiece. The current study showed that dental handpieces generate a contamination pattern with relatively low variability. The reason for this phenomenon is due to the direct production of the aerosol by handpieces in a standardized way following defined operating parameters. Studies demonstrated that aerosols and splatters produced by dental handpieces are able to carry and diffuse any pathogen that is present in the oral environment and in saliva. These pathogens include bacteria and viruses from the nose, throat, and respiratory tract.[6] SARS-CoV-2 is an infective pathogen that is mainly harbored in these locations, and therefore it is prone to be carried by aerosol-generating dental procedures.

The present findings allowed to reject both null hypotheses, implying that the presence of the tracer was not uniformly detected on the dental unit and the operatory environment surfaces, and the spread of the tracer was significantly different when the tested handpieces were used. In fact, the present results revealed the existence of heavy contamination involving the whole dental unit

as well as the surrounding surfaces of the operative room. Values higher than 0.10 CFU/cm^2 exceeded the guideline value for good hygiene, indicating moderate contamination[29]. Values higher than 0.20 CFU/cm^2 were arbitrarily considered as a high contamination level. Furthermore, the area contaminated by the biological tracer via splatters and aerosols was surprisingly wide, reaching a maximum distance of 360 cm from the infection source when we operated the air turbine. Subsequently, no surface of the operative environment was left free from the biological tracer after the tested dental procedures involving air turbine.

When looking at the contamination in the dental operatory, the contra-angle yielded lower tracer levels than the air turbine, and the scaler showed the lowest tracer levels overall. The same sequence was evidenced when considering the maximum distance at which the tracer was detected. High variability in colonies distribution of the sites within 150 cm from the infection source after using the handpieces likely suggests that splatters were the primary vector of the tracer. This result may suggest that the primary source of contamination for both dental operators and dental unit surfaces may be splatters rather than aerosols. On the contrary, relatively regular distribution of the bacterial colonies at a higher distance suggests aerosols as the primary vector of the tracer, and this finding was not dependent on the type of handpiece.

Walls and ceiling showed a relatively regular distribution of the colonies, being seemingly reached by aerosols. This result is relevant since no study in the literature demonstrated the possibility for aerosols to reach such surfaces. These findings suggest the need for disinfection protocols to include such surfaces. Regarding the topographical distribution of the tracer on the dental chair, the distance from the infection source did not influence tracer levels, except for air turbine, that caused a higher degree of tracer presence on the left side of the chair, likely due to the fact that the operator was righthanded. Also, lower tracer presence on the floor behind the operator was probably due to the barrier effect caused by the operator's position.

Considering airborne transmission, bacterial and viral infectious agents may be carried by aerosols which can remain suspended for a significant amount of time and travel relatively long distances[5, 6, 14, 18, 27, 30]. However, there is no evidence in the literature that bacteria behave differently from viruses when spread by an aerosol. In the present study, a biological tracer was used to simulate

clinical conditions as closely as possible and to allow both quantitative and topographical evaluation of aerosol diffusion. The bacterial tracer (*S. mutans*) was selected to simulate the diffusion of any infective agent by aerosol. The choice of a relatively low pathogenic microorganism as a tracer and of a passive method of sampling was motivated by health risk concerns and ethical reasons. Due to the peculiar characteristics of the analysis techniques that were used in the present study, relatively large variability of the data was seen, as expected. A high number of replications (15) of the experiments were made to control such effects.

We have to distinguish between studies using an active sampling method and the studies using a passive one. An active sampling method is based on suction systems coupled with filters or agar plates that collect the infective agents in specific sampling locations. This technique has been extensively used to characterize the different types of infective agents of an aerosol[30, 31]. However, it does not allow to map the surface spreading of the contamination. Passive methods are mainly based on detection of surface contamination by aerosols, most often by agar plates or sampling filters, that collect the droplets coming into contact with the surface after a specified amount of time. The latter method, therefore, allows for precise mapping and evaluation of the variability of the contamination at a specific site.

Very few studies mapped the operatory room surfaces reached by aerosols produced by dental handpieces, and, to our knowledge, none are based on the use of a biological tracer under standardized conditions. Miller and coworkers.[3] used a setup similar to that of the present study, and they demonstrated the presence of a high degree of bacterial contamination at about 240 cm (the measured maximum distance) using an air turbine. Hackney and coworkers used Viridans streptococci as biological indicators of oral contamination of the operatory since they are known to be abundant in human saliva[32]. These bacteria were detected on operatory surfaces after dental treatments were finished, and surfaces were disinfected, confirming the validity of using a biological tracer, though. The approach is quite similar to the one used in the current study, yet it was performed without a true standardization of the infection source. Contrarily to the setup of that study, the experimental conditions applied in this study allowed us to define the topographical

distribution of the contamination and to measure this parameter reliably. A comparison between the contaminating effect of the different tested handpieces was therefore possible.

Rautemaa and coworkers collected fallout samples on blood agar plates (measured maximum distance: 200 cm) in the operatory after using air turbine.[20] The results showed significant contamination at all sampled distances. These findings are in agreement with those of the present investigation on air turbine contamination. Using a similar experimental setup as in the present study, Purohit and coworkers evaluated the effect of rinses with an antibacterial mouthwash on the reduction of airborne contamination measured at a maximum distance of 60 cm.[33] Contrarily to our results, the ultrasonic scaler produced significantly higher contamination than the air turbine. Higher variability in contamination data at the recorded distance may explain the differences between the findings. The results of Chuang and coworkers showed that bacterial aerosols could reach a 100 cm horizontal and a 50 cm vertical distance (measured maximum distances) from a patient's oral cavity, remaining suspended for 20 minutes.[34] The findings of the present study show that the distances reached by dental aerosols are severely underestimated.

Possible limitations of the present investigation are related to the operatory that provided space constraints to the source of infection, and the absence of data regarding the operator contamination. When looking at the topographical distribution of the tracer, it is reasonable that the operator was exposed to highest tracer levels. Also, the air-conditioning intake was blocked in the present setup; therefore, the effect of air currents on aerosols are not known.

Further research is needed to find alternative approaches to the threat represented by aerosol generation in dentistry. A possible solution could be represented by modifying the composition of the aerosols produced by handpieces. This could be achieved by the addition of water spray with a disinfectant agent able to inactivate the pathogen while avoiding deterioration of dental unit waterlines and having very low toxicity, such as for instance 0.5% hydrogen peroxide for coronaviruses. In this way, the disinfectant agent could be active both on the aerosol spreading phase and once deposited on surrounding surfaces within one minute[35]. Another topic for future research should be to study the influence of additional protection procedures, such as the use of high-volume suction systems and rubber dams on the spread of contamination.

CONCLUSIONS

Dental procedures involving rotary and oscillating handpieces spread the biological tracer throughout the dental operatory. Therefore, attention should be paid to minimize their use, especially during a pandemic by an airborne spreading agent. The results of the present study highlight the need to disinfect all surfaces of the dental operatory within 360 cm of the infection source (patient's oral cavity). Furthermore, since the maximum contamination was found in the dental unit area, the highest attention must be paid to the use of personal protection equipment and decontamination procedures of the operators.

Figure legends

Figure 1. The dental unit inside the operative environment is shown. Opened agar plates that were used to evaluate the blank can be seen. The phantom head was mounted in a working position, and the bacterial suspension was attached with a drip. Arrows in Fig. 1B show the locations of two of the six plates that mapped tracer presence on the ceiling. Fig. 1C shows the location of the detection sites on the floor, at 60 x 60 cm distance.

Figure 2. Schemes representing the topographic distribution and the tracer levels after the dental procedures using A: air turbine, B: contra-angle, and C: scaler handpieces. Each red dot represents a measurement site on the dental unit. The three sites on the dental light source were averaged. The dimension of the dot represents the tracer level. The spreading pattern of splatter and aerosols close to the tip of each handpiece is displayed under each corresponding scheme. Air turbine produced the finest and farthest spreading microparticles.

Figure 3. Schemes representing the topographic distribution and the tracer levels after the dental procedures using A: air turbine, B: contra-angle, and C: scaler handpieces. Each red dot represents a measurement site of the operatory, including the ceilings, depicted below the main schemes. Values higher than 0.20 CFU/cm^2 were considered as high contamination levels, and the corresponding surfaces were displayed in red. Lower values indicated moderate contamination and

the corresponding surfaces were displayed in orange. White dots and surfaces represented no detection of the tracer.

Figure 4. Tracer presence on the different locations of the dental unit and in the operating environment expressed as CFU/cm^2. Means $\pm$ 1SE are indicated, and different superscript letters indicate significant differences between groups (Tukey test, $p<0.05$). An apparent decrease in tracer presence with an increasing distance from the infection source can be seen in the operating environment, independently from the tested handpiece. However, air turbine spread the tracer at a significantly higher distance than contra-angle, which in turn spread the tracer farther than the scaler. All sites of the dental unit generally obtained very high tracer presence; the highest one was found on the cuspidor cup when we used the air turbine, while the lowest one was found on the same site when we operated the contra-angle handpiece.

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