Qualitative and Quantitative Analysis of Bacterial Aerosols

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Abstract

The objective of this study was to investigate qualitatively and quantitatively the bacterial aerosols before, during, and after clinical work sessions in different areas within a multichair dental clinic, an isolation clinic, the sterilization center, and the prosthetic laboratory in the College of Dentistry, King Saud University. Also, the contributions of aerosols generated by different types of dental procedures were investigated. Air sampling using blood and heart infusion agar plates at four selected areas was performed three times per day over a 2-week period before, during, and after clinical sessions. The concentration of total bacterial aerosols was 5 times higher in the multichair clinic, 3.6 times higher in the prosthetic laboratory, 2 times higher in the sterilization center and isolation clinic during working sessions as compared to before the working sessions. At the end of the working day, aerosols decreased 50-70% in all areas. Staphylococcus epidermidis had the highest prevalence (37.12%) of colony composition of bacteria examined. This study demonstrates that aerosols increase during and after work sessions and, therefore, increase the chance for infectious agent transmission. Preventive measures should be instituted to reduce or disrupt aerosols as a transmission route in the multichair dental clinic, sterilization center, prosthetic laboratory, and isolation clinic.

Keywords: Aerosols, airborne, bacteria, dental clinics, air sampling

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Introduction
The spread of infection has long been considered one of the main concerns in the dental community. Indeed, infectious agents may be transmitted to patients and dental staff via several vectors, including instruments and air. Bioaerosols are an important consideration for infection control and occupational health, as these particles may consist of or convey potentially hazardous microorganisms, allergens, or other toxic substances. Infective causative agents may include bacteria, viruses, fungi, and possibly even prions. Since many procedures in the dental operatory generate aerosols, dental professionals are exposed to a wide range of microorganisms that may cause diseases such as the common cold, pneumonia, tuberculosis, herpes, hepatitis B, and acquired immune deficiency syndrome (AIDS).

Although the existence of dental and microbiologic aerosols has been known for a long time, the scientific analysis of the role they have in dentistry has been investigated only recently. Furthermore, data concerning bacterial contamination of air in multichair dental clinics, such as those found in dental schools, hardly exists.

In 1995 Grenier studied the quantity of bacterial air contamination during dental treatments in both a closed dental operatory and a multichair dental clinic. He concluded that dental treatments significantly increased the levels of bacterial air contamination in both the closed dental operatory and the multichair dental clinic. Whether such levels of contamination have any influence on infection rates is not known.

Miller et al. have demonstrated the distribution of tracer organisms when a patient’s denture was polished in an office dental laboratory. Aerosols can remain airborne for extended periods of time, can contaminate air conditioning systems, and may be inhaled by all dental staff and patients.

In this study the levels of aerosols as well as the composition of such aerosols within the College of Dentistry, King Saud University, Riyadh, Saudi Arabia were examined.

Methods and Materials
After acquiring permission from the Director of Clinics, four sites were selected within the dental college (King Saud University, Darraiyah): (1) a multichair dental clinic (24x14x3m with 20 dentists and 2 hygienists), (2) the main sterilization center (9x5x3m with a two-way instrument handling system), (3) the prosthetic dental laboratory (19x10x3m with 25 dental technicians), and (4) an isolation clinic (52m where infectious patients are treated one morning per week).

An equal number of culture medium plates (blood agar and brain heart infusion) were placed 30 minutes prior to the initiation of work sessions in the selected areas. Using methods similar to Johnston et al. a 15-20 minute exposure of blood agar and brain heart infusion culture medium plates was used to collect airborne bacteria. Johnston, et al. proved such plates are a valid medium for collecting airborne bacteria. The same procedure was repeated 2 hours after the working session began. Different dental procedures were conducted, most notably the use of ultrasonic scalers and high-speed handpieces. In the dental clinics culture medium plates were placed 2-3 ft away from the patient's mouth, since this is the area where bacterial aerosol concentration is at its highest, as reported by Micik et al.

The third set of culture medium plates were placed after the working period (i.e., after 6 hours of dental treatments and laboratory procedures). This was performed on Saturday, Monday, and Wednesday (i.e., beginning, middle, and last day of the week). In the isolation clinic it was performed during the working session as mentioned previously. Unlike the other areas, only 3-4 hours of dental procedures are conducted. This was performed over a two-week period and the number of plates used and the position of the plates were the same for the before, during, and after analyses. After collection of the samples, the blood agar and brain heart infusion medium plates were incubated aerobically at 37°C in a B&T incubator for 48 hours. The laboratory technician performed bacterial colony counting. The number of colonies was expressed as colonies.
per media plate (c/plate). The research technician was unaware of the culture medium plate’s time of exposure or location. This was followed by a microscopic examination using a Reichert-Jung Series 150 light microscope to determine bacterial cell morphology.

Data collected were statistically analyzed using Statistical Package for Social Sciences (SPSS). One-way analysis of variance (ANOVA) was performed to test the overall differences between means. Pairwise multiple comparisons were used to test the differences between specific means.

Results
The mean value of the number of organism c/plate in each of the specified areas (before, during, and after working hours) is shown in Table 1. The composition of isolated colonies, including organism types and percentages of distribution, are shown in Figure 1.

Following the initial culturing results, a choice was made to continue with blood agar plates only; as the c/plate of both culture media was comparably similar.

As expected, the bacterial counts before the start of the procedures in all four tested areas were low. In the multichair clinic during the two-hour period of treatment there was a 5x increase in bacterial colonies. In the prosthetic laboratory, there was a 3.6x increase. A moderate increase of 2x was noted both in the sterilization center and the isolation clinic. (Figure 2)

<table>
<thead>
<tr>
<th>AREA</th>
<th>DAY</th>
<th>BEFORE</th>
<th>DURING</th>
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<td>SAT</td>
<td>1 Diphtheroids</td>
<td>2 Diphtheroids</td>
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<td>2 Micrococcus</td>
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<td>2 Staph. epidermidis</td>
<td>2 Staph. aureus</td>
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<td>MON</td>
<td>1 Micrococcus</td>
<td>4 Diphtheroids</td>
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<td>7 Staph. epidermidis</td>
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<td>2 Diphtheroids</td>
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<td>MON</td>
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<tr>
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<td>5 Staph. epidermidis</td>
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<td></td>
<td>2 Micrococcus</td>
<td>6 Dipherorphis</td>
<td>3 Micrococcus</td>
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</table>

Table 1. The mean values of the number and type of organism colonies per media plate (c/plate) in each of the specified areas. The same number of plates (one blood agar and one brain heart infusion plate) where used before, during, and after the dental work in each area.
Thirty minutes after the procedures were stopped, the levels of bacterial contamination decreased (range of 50-70%) in all areas, as seen in Table 1.

The total mean bacterial counts remained relatively stable, with a slight increase towards the end of the week (i.e., Saturday to Wednesday), which was not statistically significant ($\alpha=0.562$).

There were no statistical differences observed when comparing the bacterial counts in the multichair clinic, sterilization center, or isolation clinic. A highly significant difference was observed when comparing the previously mentioned areas to the prosthetic laboratory results ($\alpha=0.001$).

Figure 3 shows the prosthetic dental laboratory overall gave substantially higher results of bacterial contamination. Surprisingly, bacterial counts from the isolation clinic were relatively low (Figure 4).

Three main organism constituents with a total of seven types of species were isolated and identified. As demonstrated in Figure 5, *Staphylococcus epidermidis* had the highest presence (37.1%), followed by *Micrococcus* (32.6%), and *diphtheroids* (28.2%). In the multichair clinic...
2 c/plate of *S. aureus* (0.6%) were identified; in the sterilization center 2 c/plate of *Pseudomonas* (0.6%) were isolated; and 3 c/plate of *Fungi* (0.9%) were identified in the dental laboratory.

**Discussion**

A safe environment is an important consideration for all dental personnel and patients. Previous studies have demonstrated that to ensure a safe office environment, universal precautions must be used with all patients as well as the need for adequate control of the transmission of infectious diseases associated with an indoor environment whether airborne or otherwise.

The numbers presented as c/plate are relative values representing only aerobic bacteria capable of growth on blood agar media plates. It is likely actual microbial content in the specified areas was much higher than that reported here, as the culture medium and growth conditions used did not allow the identification of all types of organisms including viruses, anaerobic bacteria, and organisms requiring specialized medium.

The multichair clinic studied was the interns’ clinic (as opposed to undergraduate clinics) because of the multidiscipline nature of the interns’ daily clinical routine. Most dental clinics within the school are similar in design and are open to each other, sharing the same air space. The sterilization center and dental laboratory were both main areas of interest and concern and, therefore, chosen for evaluation. As the data demonstrates, the levels of bacterial air contamination rose during dental treatment and laboratory procedures. The subsequent decrease of bacterial air contamination was noticed 30 minutes after the end of the working period. In some cases it remained at similar levels. This is in agreement with results reported by different investigators who suggested the decrease in levels could be referred to as the rapid settling of bacterial-laden particles. Lorato and others reported that when droplets containing organisms from the mouth are forced into the air, they react in two ways. Heavy droplets fall to the floor and become part of the floor dust. Aerosol particles light in weight remain suspended in the air, leaving a residue called droplet nuclei. This residue can remain suspended in the air for extended periods and may eventually reach the respiratory passages of anyone exposed to the residue. They hypothesized these organisms, including possible viable pathogenic organisms, were still suspended in the air 30 minutes after the dental procedure was completed.

Another factor that would also explain the relative stability of levels throughout the working weeks was the air conditioning, air handling, and ventilation systems. Within the King Saud University dental school, the air environment is regulated by a series of air conditioners, ventilation pumps, and exhaust fans. These not only supply the area with cold air but also work to pump out retained air. These function from 7 a.m. till 5 p.m. in all areas within the school. Monthly maintenance is performed on these units.

The dental technician’s laboratory workplace is of a more congested, closed nature, and situated on the ground floor with no windows. Twenty-five technicians perform laboratory procedures including pouring impressions, die trimming, polishing, sand blasting, and others. This may explain the comparatively high levels of bacterial contamination, especially before dental procedures are initiated.

The isolation clinic, although a closed operatory in nature, is regulated by the same air conditioning and ventilation systems as the multichair clinic. Patients are received only once a week for a 4-hour period encompassing 3 to 4 patients only. This could explain the relatively low contamination levels. Overall reports by Grenier in 1995 showed the bacterial air contamination levels were similar when comparing closed to open operators. Lorato et al. observed a similar air microbial contamination pattern (before, during, and after an operative treatment) in a closed dental operatory. When evaluating the bacterial contamination composition, the main components were *S. epidermidis*, *Micrococcus*, and *diphtheroids*, *S. epidermidis* is located on the skin and spreads through contact. It is a normal commensal of the skin causing infection only when an opportunity arises, hence, it is considered an opportunistic pathogen. It is, however, the most common cause of infection in patients with implanted prosthetic devices such as heart valves, artificial joints, and catheter related sepsis. *Micrococci*, found in abundance on the lingual surface within the oral cavity, often with
predilection for the tongue surface, are similar to Saphylococci. However, their role in disease, if any, is unknown. Diphtheroids, normally inhabiting the skin and conjunctiva, are occasionally opportunistic pathogens in compromised patients (e.g., endocarditis in prosthetic valves and bacteremia). Of the remaining isolated organisms, the main cause for concern is S. aureus. It normally inhabits human skin and mucous membrane, especially the anterior nares and the perineum and is usually transmitted via the hands. It is a common cause of a variety of diseases including wound infections, abscesses, septicemia, osteomyelitis, endocarditis, and a number of respiratory infections. Pseudomonas is also known to cause a number of serious infections including eye infections and pneumonia.

It is important to note Saphylococcus species and Peudomonas usually demonstrate a resistance (multiresistance) to a number of drugs including penicillin and methicillin. The previously mentioned types are frequently found as contaminating bacteria in clinical specimens and may cause infections like pneumonia and eye infection and are always present in the hospital and clinical environments.12

**Conclusions**

An important consideration and focus point for all dental personnel and patients is a safe office environment. In dental clinics dental staff and patients could contract several infectious agents through airborne transmission. In addition dental aerosols containing opportunistic pathogens should be considered hazardous for immuno-compromised patients4 who could develop serious infections. It is the dentist’s responsibility to recognize susceptible patients and take needed protective measures to prevent any possibility of cross infection. Constant revision and updating of medical history is essential, as many researchers16,17,18 have shown most patients do not offer a truthful medical history. Routine immunizations of all dental auxiliary staff should be up-to-date according to the relevant national immunization schedule.19 Our data confirms a potential transmission route for infectious agents and supports the importance of protecting against cross infection agents contained in aerosols. It is, therefore, emphatic for all dental staff to reduce the spread of such opportunistic pathogens through cleanliness, hand washing, pre-procedural antiseptic mouthwash, sterilization of dental instruments, and electrostatic devices.5,17 As revealed in our study, the potential hazard of infection in dental clinics is not confined to dental personnel in direct contact with patients. Control of airborne transmission of infectious diseases associated with indoor environments is important to all personnel working in the clinics. Based on this, all personnel should abide by infection control guidelines7,2 such as wearing protective masks, gloves, and eyewear. Because of the high risk of cross contamination in dental clinics, research should be directed toward developing an effective means for controlling and removing dental aerosols. Additional epidemiological surveys of dental personnel and auxiliary mortality and morbidity are needed to define actual infection rates and the influence of such contamination levels on infection rates.
References
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