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Aerosolized bacteria and microbial activity in dental clinics during cleaning procedures

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ABSTRACT

Use of sharp instruments, handpieces, water-air sprays, and high speed rotary devices during dental cleaning procedures can release oral bacteria, which may cause significant occupational bioaerosol exposure risks. This study aimed at sampling of airborne bacteria and identification of prevalent bacterial genera and testing of overall microbial activity in settled splatter over clinic floors in several US dental clinic rooms during dental cleaning procedures (n = 15). Culturable airborne bacteria were measured by a Biostage impactor and the diversity and relative abundance of the airborne culturable bacterial community were evaluated by pyrosequencing of 16s rRNA genes. ATP levels were determined in swabbed splatter samples collected from floor surfaces for understanding overall microbial activity and estimating the general cleanliness of the clinic surfaces. Concentrations at the beginning, during, and after dental cleaning procedures were 671 ± 525, 917 ± 1203, and 899 ± 823 CFU/m^3, respectively for airborne bacteria and 91 ± 101, 243 ± 129, and 139 ± 77 RLU/sample, respectively for ATP levels on floors. The dominant bacterial phylum was *Proteobacteria*. A total of 45 bacterial genera were detected, notable among them included *Psychrobacter, Pseudomonas, Sporosarcina,* and *Streptococcus*. Several pathogenic bacterial species such as *Psychrobacter* sp. (including *P. pulmonis*, and *P. faecalis*), *Streptococcus* sp. (including *S. thermophiles*, *S. parasanguinis*, and *S. oralis*), *Pseudomonas* sp. (including *P. graminis*) were identified in air samples collected at different stages of the dental cleaning procedures. The concentration of airborne bacteria in dental clinic rooms did not increase significantly during the cleaning procedures. The diversity of culturable bacteria, however, changed. This change in the diversity and the similarity in major taxa detected in our study to the bacterial taxa reported recently from acute or chronic root canal infections and supragingival plaque samples indicate that oral bacteria from patients can significantly contribute to airborne bacterial load in dental clinics during cleaning procedures.

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1. Introduction

Human oral cavity serves as a natural habitat and reservoir for a wide variety of microorganisms. Different sharp instruments, drilling machines, turbine burs, water-air sprays, ultrasonic scalers, air polishers, abrasion units, and high speed rotary or other aerosol forming devices are routinely used in dental clinics during dental cleaning procedures. Therefore, these procedures can release a lot of oral microorganisms including bacteria from patients' oral cavity in the clinic room air (Al Maghlouth, Al Yousef, & Al Bagieh, 2004; Bentley, Burkhart, & Crawford, 1994; Grenier, 1995; Harrel & Molinari, 2004; Micik, Miller, Mazzarella, & Ryge, 1969; Rautemaa, Nordberg, Wuolijoki-Saaristo, & Meurman, 2006; Shpuntoff & Shpuntoff, 1993; Timmerman, Menso, Steinfort, van Winkelhoff, & van der Weijden, 2004) causing significant occupational bioaerosol exposure risks for dentists, dental hygienists, and other dental clinic employees. In addition, bacteria from the biofilms may produce aerosols in the dental surgery and cleaning rooms (Harrel, 2004). *Pseudomonas aeruginosa*, *P. cepacia*, *Legionella pneumophila*, and *Mycobacterium chelonae* were previously identified in biofilms in this type of settings (Miller & Palenic, 1998). According to Gowtham and Deepthi Padma (2014), there are four basic routes of entry for spreading of infectious microorganisms including bacteria in a dental clinic setting through bioaerosols, which are blood-borne; saliva-droplet (from patient); direct contact (patient and/or contaminated equipment); and water-droplet (from biofilms and contaminated dental water supply system). Although a few reports are available addressing these problems, mostly from several European countries, to our knowledge, there is no report available on the volumetric airborne concentration levels of bacteria in dental clinics in the United States. The study of Grenier (1995), which was conducted a long ago in Canada and an old model of bioaerosol sampler – Slit-to-Agar biological air sampler – was used by the author. This small-scale study was conducted in four locations of a dental clinic and samples were collected from closed dental operatory and a multi-chair dental clinic during ultrasonic scaling treatment only. Two studies were conducted in Italy where Castiglia et al. (2008) and Pasquarella et al. (2012) measured airborne bacterial concentrations in Italian dental clinics 'during clinical practice – when the dentist, assistant and patient were present' and during 'dental treatment' using a Surface Air System (SAS) sampler (International PBI, Milan, Italy). Szymańska and Dutkiewicz (2008) investigated the effect of dental unit waterline disinfection on airborne bacterial concentrations in the dental clinics of Poland and they used an RCS Plus sampler. A recent study in Germany (Kimmerle et al., 2012) compared airborne bacterial load in dental clinics versus public area in Germany. Another recent study from India reported airborne bacteria from dental surgery rooms (Jimson, Kannan, Jimson, Parthiban, & Jayalakshmi, 2015) but they used settle agar plates and volumetric air sampling method was lacking. Therefore, adequate data on airborne bacteria in dental clinics during dental cleaning procedures are still lacking, particularly from the US dental clinics. Besides that, to our knowledge, overall microbial activity in this type of clinic occupational environment was never examined. Some researchers measured bacterial aerosol compositions in dental clinics previously using settle plates where quantitative information on inhalable exposure levels was not obtained (Decraene, Ready, Pratten, & Wilson, 2008). However, the authors found *Enterococcus faecalis* on 10% of plates placed in dental treatment rooms, which supported the possibility of nosocomial infection risks in this type of setting. Although a few previous studies explored overall bacterial concentrations or concentrations of specific bacterial species, to our knowledge, the community compositions of airborne bacteria in dental clinics were never determined using high-throughput sequencing methods. Shifts in bacterial community composition during different time points of dental cleaning procedures were never investigated as well. These data are important and required for better characterization of airborne bacterial flora and evaluating the risk of exposure to both patients and dental clinic staff to potential human pathogens. Therefore, the major aim of our study was sampling of airborne bacteria using a volumetric bioaerosol sampler (Biostage impactor) from several US dental clinics, identification of prevalent culturable bacterial genera, and estimation of airborne bacterial community composition and diversity among collected culturable bacteria during different time points of dental cleaning procedures. Usually typical environmental bacterial community compositions are evaluated in total bacteria and often specific detection of bacteria is accomplished by targeted methods (e.g., PCR, ELISA, biochemical assays); however, here we examined community compositions in culturable airborne bacteria using next generation sequencing (NGS) based tool because potential infections from culturable bacteria is more relevant with respect to indoor air quality and potential infection risks in dental clinics.

Microbial aerosols released during dental cleaning procedures can contaminate floors and various surfaces in the clinic rooms. In addition to bioaerosols, sharp instruments, drilling machines, turbine burs, ultrasonic scalers, air polishers, abrasion units, and high-speed rotary instruments can generate large particles too during cleaning procedures. Adequate data on overall microbial activity and general surface cleanliness of dental clinic rooms are lacking in the scientific literature. Particles of > 50 μm in diameter generated in dental clinics are referred to as splatter (Gowtham & Deepthi Padma, 2014). These splatters may carry microorganisms and due to their large aerodynamic size they cannot suspend in the air and deposit quickly on different dental clinic surfaces and the clothes, hair, skin, eyes and mucous membranes of dental personnel as well as on the floors of clinic room. Because overall microbial activity and microbial load in settled splatter over clinic floors were never examined, we intended to measure this in terms of total ATP level. Food and beverage processing industries routinely use ATP systems to rapidly verify if surfaces have been cleaned adequately so that new product runs are not contaminated by preceding product runs, and to confirm that biofilms are not developing on the surface that could affect quality. ATP released by microbial cells produces light in the presence of luminescence enzymes and the intensity of the released light can be measured with a luminescence instrument and quantified as Relative Light Units or RLU. The RLU data can be used as a measure of microbial activity or overall metabolically active microbial burden. Recently ATP levels as the measures of total microbial activity or microbial load were explored by several researchers in different kind of clinical or hospital environmental settings (Aycicek, Oguz, & Karci, 2006; Cooper, Griffith, Malik, Obee, & Looker, 2007; Griffith, Cooper, Gilmore, Davies, & Lewis, 2000) as well as in field experiments on bioaerosol monitoring and evaluation of bioaerosol samplers (Lee & Chang, 2000; Seshadri, Han, Krumins, Fennell, & Mainelis, 2009). Therefore, we used this uncommon approach for understanding microbial activity during dental cleaning procedures in the clinic room floors, where deposition of bacteria in large droplets and splatter originating from saliva, tooth debris, supra- and sub-gingival dental plaque, and calculus are very likely.