Molecular analysis for bacterial contamination in dental unit water lines

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Dental chair units are equipped with a narrow-bore, flexible, plastic tubing called dental unit waterlines (DUWLs) that supply water to all dental instruments (air/water syringe, turbine, rinsing equipment) (O’Donnell et al., 2011). Bacterial contamination is often observed in the complex dental unit. Therefore, biofilms containing multiple bacterial species easily form within DUWLs (Cobb et al., 2002). Bacterial numbers per ml in DUWLs can reach several million overnight (Depaola et al., 2002). Heavy microbial contamination of DUWLs is thought to be the result of biofilm formation within small-bore plastic tubes used to supply water to dental equipment (Pankhurst et al., 1998). Opportunistic bacteria such as Legionella pneumophila and Pseudomonas aeruginosa have previously been revealed in biofilms within DUWLs (Petti et al., 2004). In addition, Staphylococcus sp. isolated from DUWLs are resistant to several antibiotics (Lancellotti et al., 2007; Gungor et al., 2014). Thus, bacterial contamination in DUWLs is a recent growing concern in dentistry. Several human pathogens in the environment can change to a “viable but non-culturable” state and are not detectable by culture methods (Oliver 2005; Su et al., 2013). The aim of this study was to monitor bacterial contamination in DUWLs using molecular techniques based on bacterial 16S ribosomal RNA gene (16SrDNA) and antibiotic-resistance genes. In this study, bacterial contamination in the 8 DUWLs located at a dental hospital in Japan were investigated by molecular techniques in addition to the conventional culture method. Water samples were collected at the end of the working day, after 3.5 days, and at the beginning of the working day before use. After appropriate dilutions, the water samples were plated on R2A agar (DAIGO®, Nihon Pharmaceutical Co. Ltd, Tokyo, Japan), selective for heterotrophic bacteria and incubated at 25°C for 7 days, to obtain total bacterial counts in terms of colony forming units (CFU) per ml. Abundant white and off-white colonies were observed on the R2A agar plates, from each DUWL water sample. The average bacterial count at end of the working day, for each DUWL water sample was as follows: 1) 390 CFU/ml, 2) 3,020 CFU/ml, 3) 3,780 CFU/ml, 4) 552 CFU/ml, 5) 435 CFU/ml, 6) 175 CFU/ml, 7) 1,245 CFU/ml, 8) 3,320 CFU/ml. Thus, only 3 chair units were found to meet the Centers for Disease Control and Prevention (CDC) recommended water quality standard in terms of the maximum permissible level of contamination by heterotrophic bacteria in DUWL water at the end of the working day, at 500 CFU/ml (Kohn et al., 2004). On the other hand, the average bacterial count at end of the working day, for each DUWL water sample was as follows: 1) 390 CFU/ml, 2) 3,020 CFU/ml, 3) 3,780 CFU/ml, 4) 552 CFU/ml, 5) 435 CFU/ml, 6) 175 CFU/ml, 7) 1,245 CFU/ml, 8) 3,320 CFU/ml. Thus, only 3 chair units were found to meet the Centers for Disease Control and Prevention (CDC) recommended water quality standard in terms of the maximum permissible level of contamination by heterotrophic bacteria in DUWL water at the end of the working day, at 500 CFU/ml (Kohn et al., 2004). On the other hand, the average bacterial count for each DUWL water sample at beginning of the working day was as follows: 1) 6,090 CFU/ml, 2) 6,325 CFU/ml, 3) 3,925 CFU/ml, 4) 4,915 CFU/ml, 5) 5,122 CFU/ml, 6) 795 CFU/ml, 7) 2,810 CFU/ml, 8) 6,345 CFU/ml.

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Additionally, after 3 and a half days of not being in use, the bacterial count in each DUWL was highly increased. Thus, the quality of water from the DUWLs sampled did not fulfill the water standards criteria set by the CDC. Several previous reports have indicated that flushing with water for several minutes at the beginning of the working day effectively reduces the bacterial load in DUWLs (Pankhurst et al., 1998; Cobb et al., 2002; Watanabe et al., 2008). However, biofilm formation within the DUWLs presents a challenge for complete elimination of bacteria by flushing with water (O’Donnell et al., 2011). O’Donnell et al. concluded that chemical DUWL cleaning, dental chair unit design, and development of automated DUWL cleaning control systems provide effective long-term solutions to DUWL maintenance. Water from DUWLs and hospital wastewaters is not sterile and contains high numbers of bacteria. Several studies have reported the presence of opportunistic pathogens and antibiotic-resistant bacteria in DUWLs (Petti et al., 2004; Al-Hiyasat et al., 2007; Mäayeh et al., 2008; Arvand et al., 2013) and hospital wastewaters (Schwartz et al., 2003; Yomoda et al., 2003; Thompson et al., 2013).

In this study, contamination by antibiotic-resistant pathogens and Legionella species in DUWLs was further examined by conventional polymerase chain reaction (PCR) method. Each 50-ml water sample collected at the beginning of the working day was centrifuged at 10,000×g for 30 min at 4°C. The supernatant was discarded and the pellet was suspended in 200 μL InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). Bacterial DNA was extracted according to the manufacturer’s instructions and used for PCR. Antibiotic-resistant pathogens, namely methicillin-resistant S. aureus (MRSA), multi-drug resistant Pseudomonas, extended-spectrum beta-lactamase (ESBL) producing bacteria, and vancomycin-resistant Enterococci were identified by PCR, by amplification of respective bacterial genes, mecA, blaIMP, blaVIM, blatem, vanA, and vanB, as previously described (Murakami et al., 1991; Kariyama et al., 2000; Heneriques et al. 2006). Contamination by Legionella sp. was also investigated using PCR for specific 16SrDNA (Yamamoto et al., 1993). Neither bacterial antibiotic-resistance genes nor Legionella-specific 16SrDNA were detected in any of the DUWL samples analyzed by PCR. Moreover, the profiles of bacterial contamination in DUWLs were evaluated by PCR-denaturing gradient gel electrophoresis (DGGE) in this study. The PCR-DGGE method has become one of the new analytical tools for microflora (Fujimoto et al. 2003). A forward primer with a 40-bp GC-rich sequence added to the 5′-end (5′-CGCCGCCGC-GCCCCCGCGCGCTCCCGCGCCTCCCGCCCGCCTAC-GGAGGACGAGC-3′) and a reverse primer (5′-CCGT-CATTCTTTRATGTT-3′) were used to amplify by PCR, approximately 585-bp 16SrDNA fragments corresponding to nucleotide positions from 341 to 926 in the genome sequence of Escherichia coli. PCR was performed using EX-Taq® kit (TaKaRa Shuzo Ltd., Otsu, Japan) as previously described (Fujimoto et al., 2003). DCode Universal Mutation Detection System (Bio-Rad Lab.) was used for sequence-specific separation of the PCR products on 6% (w/v) polyacrylamide gel containing a linear gradient denaturant, ranging from 20% to 50%. The gel was run for 4 h at 200 V in 0.5X Tris-acetate-EDTA (TAE) buffer (1X TAE buffer contains 0.04 mol/l Tris base, 0.02 mol/l acetic acid, and 1.0 mmol/l EDTA, pH 8.5) at a constant temperature of 60°C. After staining with ethidium bromide, the gel was viewed under UV transillumination. The dominant 16SrDNA bands on the denaturing gradient gel were excised, purified, and re-amplified using PCR as previously described (Fujimoto et al., 2003). The re-amplified DNA bands were cloned into a vector (TOPO TA Cloning kit®, Invitrogen, Carlsbad, CA, USA), and the DNA inserts were sequenced using a kit (ABI Prism BigDye® terminator v1.1 cycle sequencing ready reaction kit, Perkin Elmer, Foster City, CA, USA) and an automated DNA sequencer (ABI PRISM 3100, Perkin Elmer). The sequence data were subjected to the BLAST sequence homology search program at GenBank and analyzed. Bacterial species were identified at similarity values above 99%. PCR-DGGE analysis revealed that polycyclic aromatic hydrocarbon-degrading bacterium, Novosphingobium sp., were the most prevalent and dominant contaminating bacteria in all DUWL samples tested. Dechloromonas sp., Blastobacter sp. and Sphingomonadaceae sp. were also identified as other, minor contaminants (Figure 1, Table 1). Novosphingobium sp. and Sphingomonadaceae sp. were identified in all DUWL water samples, by both, culture method and PCR-DGGE analysis. Additionally, PCR-DGGE analysis revealed the profiles of bacterial contamination in the DUWLs. Moreover, polycyclic aromatic hydrocarbon-degrading bacterium, Novosphingobium sp., was the most prevalent and dominant, whereas, Dechloromonas sp., Blastobacter sp., and Sphingomonadaceae sp.
were identified as minor contaminating bacteria in the DUWL samples tested in this study. The pathogenicity of *Novosphingobium* sp., *Dechloromonas* sp., *Blastobacter* sp. and *Sphingomonadaceae* sp. in healthy and immunocompromised subjects is still unknown (Rutebemberwa et al., 2014). These bacteria might be useful for one of indicators of water quality in DUWL. Hospitalized patients with infectious diseases and the elderly requiring nursing-care are immunocompromised and more likely to visit dental clinics for dental care and treatment, where both patients and clinic staff could be exposed to microorganisms in DUWL output water and contaminated aerosols. Although the case of infection resulting from exposure to water from the contaminated DUWL is limited (Ricci et al., 2012), it is necessary to monitor the level of bacterial contamination in DUWLs and maintain the water quality.

In conclusion, PCR-DGGE was shown to be a potentially useful molecular analysis tool to monitor DUWL bacterial contamination. Conventional PCR for antibiotic-resistance genes and *Legionella* sp. 16SrDNA could also be used for rapid monitoring and tracing of the source of nosocomial infections via DUWLs.

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References


