

MICROBIOLOGICAL QUALITY OF ULTRASONIC
DENTAL SCALER OUTPUT WATER.

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by
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ABSTRACT

Objectives: Since ultrasonic dental scalers contain internal narrow-diameter waterline tubing susceptible to bacterial biofilm growth, this study determined the cultivable prevalence of aerobic mesophilic heterotrophic bacteria in ultrasonic scaler output water subjected to one of two commercial biocides favorably reviewed by the American Dental Association as effective for microbial biofilm control in dental unit waterlines, and assessed the potential antimicrobial effect on output water of an ultrasonic scaler tip activated at a maximum power frequency.

Methods: Aseptically-collected water samples were obtained, and neutralized for residual chlorine with sodium thiosulfate, from 12 ultrasonic scalers provided municipal tap water treated with an iodine resin cartridge (Group A), and from eight ultrasonic scalers provided distilled water treated with a 0.78% silver ion-based disinfectant (Group B), all within a dental school clinic setting. The latter eight ultrasonic scalers were additionally sampled after output water was passed through an inserted scaler tip activated to a maximum power setting similar to its potential use in clinical patient care. All water samples were evaluated for aerobic mesophilic heterotrophic bacteria using nationally-accepted analytical procedures detailed in *Standard Methods for the Examination of Water and Wastewater, 23rd Edition* (2017), with sample aliquots spread-plated onto R2A agar and aerobically incubated at 28°C for seven days. Aerobic mesophilic heterotrophic bacteria were considered elevated when ≥ 500 CFU/ml of the species were recovered per water sample. Predominant cultivable isolates of aerobic mesophilic heterotrophic bacteria in the water samples were identified using matrix-

assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry and Bruker MALDI Biotyper analytic software.

Results: Aerobic mesophilic heterotrophic bacteria averaged 610 ± 380 (SD) CFU/ml per ultrasonic scaler in Group A ultrasonic scalers, with eight of 12 (66.7%) evaluated ultrasonic scalers yielding elevated counts ≥ 500 CFU/ml. Similar mean values of 667 ± 340 (SD) CFU/ml per ultrasonic scaler were found in Group B ultrasonic scalers, with five of eight (62.5%) evaluated ultrasonic scalers yielding elevated aerobic mesophilic heterotrophic bacteria counts ≥ 500 CFU/ml. *Acidovorax temperans*, *Novosphingobium subterraneum*, *Cupriavidus metallidurans*, and *Sphingobium yanoikuyae* were the most prevalent cultivable species identified from ultrasonic scaler waterlines where the source water was municipal tap water treated with molecular iodine. In contrast, nearly pure growth of *Cupriavidus metallidurans* alone dominated cultivable waterline isolates from ultrasonic scalers where the source water was distilled water treated with a 0.78% silver ion disinfectant. No statistically significant impact was found on aerobic mesophilic heterotrophic bacterial counts after ultrasonic scaler output water passed through an inserted scaler tip activated to maximum power ($P > 0.05$, Wilcoxon signed-rank test).

Conclusions: Approximately two-thirds of ultrasonic scalers in a dental school clinic setting yielded output water that failed to meet potable water regulatory standards in the United States with regard to aerobic mesophilic heterotrophic bacterial counts, even when subjected to disinfection with one of two American Dental Association favorably-reviewed biocide products, or exposed to high-frequency ultrasonic scaler tip oscillations. These findings question the effectiveness of infection control procedures

used to manage ultrasonic scaler waterlines, and raise concerns about the safety of ultrasonic scaler output water introduced into the oral cavity of dental patients during dental treatment procedures.

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CHAPTER 1

INTRODUCTION

High-speed handpiece drills, air-water syringes, and ultrasonic scalers attached to dental operatory chair units all require water for cooling and/or removal of debris in dental patient care procedures. Water is delivered to and through these dental devices by narrow-diameter plastic tubing connected either to a municipal tap water outlet, or a self-contained water bottle reservoir attached to the dental operatory chair unit.

The microbiological quality of dental operatory chair unit output water has been a concern for several decades (Williams et al. 1995, 1996, Barbeau et al. 1998, DePaola et al. 2002, Canares & Allison 2020). High numbers of aerobic mesophilic heterotrophic bacteria, and more pathogenic microorganisms, have been recovered from dental operatory chair unit waterlines left untreated by adjunctive chemical disinfectants (Williams et al. 1994, 1995, Barbeau et al. 1996, 1998, Walker et al. 2000, Canares & Allison 2020). The increased inner-surface area of narrow-diameter dental unit tubing relative to the relatively small volume of fluid passing through provides a specialized ecologic niche that permits microorganisms normally present in low abundance in municipal tap water to form dense multi-species bacterial biofilms adhering to the inner lumen of the narrow-diameter tubing (Canares & Allison 2020). The waterline biofilms may then seed high numbers of individual planktonic bacterial cells into dental operatory chair unit output water that feeds into the unit's attached high-speed handpiece drills, air-water syringes, and ultrasonic scalers, and ultimately, into the oral cavity of dental patients during dental treatment procedures (Canares & Allison 2020).

At present, a < 500 CFU/ml upper threshold value exists as the acceptable regulatory standard by the American Public Health Association, the American Water Works Association, and the United States Environmental Protection Agency, for the presence of aerobic mesophilic heterotrophic bacterial counts in potable drinking water (Baird et al. 2017, United States Environmental Protection Agency 1999).

Since ultrasonic dental scalers contain internal narrow-diameter plastic waterline tubing susceptible to bacterial biofilm growth, similar to dental chair operatory units, water emitted from ultrasonic scalers may also contain high numbers of aerobic heterotrophic bacteria. Interestingly, relative little attention has been given to the study of bacterial populations colonizing ultrasonic scaler waterlines. Ballinger et al. (1976) found bacterial levels in output water from four ultrasonic scalers to be too-numerous-to-count in sample dilutions up to 10^{-9} , and detected Gram-negative, aerobic, *Pseudomonas* and *Alcaligenes* species forming a mucous mass clogging a water control volume on one ultrasonic scaler. However, the tap water source for the ultrasonic scalers also revealed high bacterial counts, so it was not clear whether the ultrasonic scaler waterlines themselves were colonized by bacterial biofilms. Gross et al. (1986) and Dayoub et al. (1978) found bacterial growth on trypticase soy agar incubated at 35-37°C for a week averaged, respectively, 509,000- 587,000 and 499,000 colony forming units (CFU)/ml in output water from ultrasonic scalers attached to dental operatory chair units supplied from a municipal tap water source. Williams et al. (1993) found a mean 19,800 CFU/ml of aerobic mesophilic heterotrophic bacteria in output water from 12 ultrasonic scalers sampled in dental clinics in California, Oregon, and California, with light and electron

microscopic examinations revealing 30-50 micron thick, mature, highly-structured, bacterial biofilms lining the lumen of functioning dental unit waterlines (Williams et al. 1993). Wirthlin & Marshall (2001), in evaluating 15 ultrasonic scalers used for five or more years with a municipal tap water source, and subjected to a two minute flush with sterile water supplied separately from a dental operator chair unit, found a mean 582 CFU/ml, with a range of 110 to 1,870 CFU/ml, of aerobic mesophilic heterotrophic bacteria in the ultrasonic scaler output water. These findings suggest that ultrasonic scaling devices used frequently in human periodontal disease treatment often emit bacterially-contaminated coolant water that fails to meet United States potable drinking water standards (Baird et al. 2017, United States Environmental Protection Agency 1999).

Various approaches have been suggested for improving the microbiological quality of water emanating from dental operator chair units and ultrasonic scalers, including, prolonged water flushing, air desiccation, microbial filters, fabrication of waterline materials with antimicrobial properties, and application of various chemical disinfectants (O'Donnell et al. 2011). However, little data is available which specifically evaluates, independent of dental operator chair unit water, the effectiveness of these approaches on ultrasonic scaler output water (Wirthlin & Marshall 2001).

To further advance knowledge in this area, the purpose of this study was to determine the cultivable prevalence of aerobic mesophilic heterotrophic bacteria in ultrasonic scaler output water subjected to one of two commercial biocides favorably reviewed by the American Dental Association as effective for dental unit waterlines, and

to assess the potential antimicrobial effect on ultrasonic scaler output water of an ultrasonic scaler tip activated at a maximum power frequency.

CHAPTER 2

MATERIALS AND METHODS

Laboratory Facilities

This study was performed in the Oral Microbiology Testing Service (OMTS) Laboratory at Temple University School of Dentistry, Philadelphia, Pennsylvania. The OMTS Laboratory is state-licensed for high-complexity bacteriological analysis by the Pennsylvania Department of Health (Clinical Laboratory Permit Number 021872) as an oral microbiology reference laboratory. The OMTS Laboratory is also federally certified by the United States Centers for Medicare and Medicaid Services to be in compliance with Clinical Laboratory Improvement Amendments (CLIA) regulations (CLIA Certificate Number 39D0707385), and fulfills all proficiency testing, quality control, patient test management, personnel requirements, and quality assurance standards required of clinical laboratories engaged in diagnostic testing of human specimens in the United States (Rauch & Nichols, 2007).

All of the present study data was obtained from non-human biologic water specimens from ultrasonic dental scaling instruments, without intervention or interactions with any living humans, and not involving any identifiable private human patient information. Because the study was laboratory-based, and did not involve human subjects, as defined by United States Department of Health and Human Services regulations at 45 CFR part 46.116(f), it did not require review and approval from the Temple University Institutional Review Board.

Ultrasonic Scalers

A total of 20 magnetostrictive ultrasonic dental scalers, where the physical vibratory movement of the tip is in an elliptical pattern (Krishna & De Stefano 2016), were included and microbiologically sampled in this study.

A group of 12 of these ultrasonic scalers (Group A), were comprised of eight Cavitron Select SPS and four Cavitron Plus models (both Dentsply Sirona, Charlotte, North Carolina) (Figure 1), and were in routine daily use in the predoctoral dental clinic clusters at Temple University School of Dentistry, Philadelphia, Pennsylvania.



Figure 1. Example of a Cavitron Select SPS ultrasonic scaling instrument with a blue inserted scaling tip for clinical application.

The ultrasonic scalers were selected at random from the dispensaries within each of the three floors of predoctoral dental clinic clusters located in the dental school building. The source water for these ultrasonic units was provided through dental operatory chair units with a water reservoir bottle. For a period of approximately six months prior to ultrasonic scaler waterline sampling, the source water for the instruments

was municipal tap water treated by passage through a molecular iodine resin-loaded cartridge (DentaPure cartridge, Crosstex International, Inc., Hauppauge, New York) attached to the water reservoir bottle output straw leading into the dental operator chair unit.

A separate group of eight ultrasonic scalers (Group B), all comprised of Cavitron Plus models (Dentsply Sirona, Charlotte, North Carolina), were employed in the graduate periodontology clinic at Temple University School of Dentistry, and were similarly served source water by a reservoir bottle system attached to the dental operator chair unit. However, the source water for Group B ultrasonic scalers over a period of approximately four years was distilled water treated continuously with a 0.78% silver ion disinfectant (Citrisil daily blue tablets, Sterisil, Inc., Palmer Lake, Colorado).

Both DentaPure cartridges and Citrisil tablets are favorably reviewed by the American Dental Association as effective for microbial biofilm control in dental unit waterlines (American Dental Association Council on Scientific Affairs 2014). Neither Group A nor Group B ultrasonic scaler waterlines were subjected at their installation nor periodically to adjunctive “shock treatment” (Mills 2000) with sodium hypochlorite or another biocide, with the aim of eliminating or reducing bacterial counts at or below standard limits in waterlines with already established bacterial biofilms (American Dental Association Council on Scientific Affairs 2007), as is often recommended for intermittent application to dental operator chair unit waterlines (American Dental Association Council on Scientific Affairs 2007, Bowen et al. 2015, Pankhurst et al. 2017).

Ultrasonic Scaler Output Water Samples

All ultrasonic scaler water samples were collected using nationally-accepted water sampling procedures detailed in *Standard Methods for the Examination of Water and Wastewater, 23rd Edition* (Baird et al. 2017). Ultrasonic scaler water samples were collected in the morning before clinic use after two minutes of waterline flushing through the ultrasonic scaler waterlines with distilled water delivered via a Cavitron Dual Select dispensing system (Dentsply Sirona, Charlotte, North Carolina) (Figure 2), as previously described (Wirthlin & Marshall 2001), instead of water from the dental operator chair unit.



Figure 2. Example of Cavitron Dual Select dispensing system used to deliver distilled water to ultrasonic scaler instruments.

The Cavitron Dual Select dispensing system was disinfected prior to use similar to procedures described by Wirthlin & Marshall (2001). The dispensing system bottles

and connection lines were flushed with a 50% sodium hypochlorite solution, with a full load passed through both of the dispensing system's bottles into connecting lines. This was followed by full loads of distilled water flushed through both bottles and connecting lines, and then introduction of compressed air for 30 minutes to dry all internal components.

For water sampling of ultrasonic scalers, the ultrasonic scaler handle orifice was first disinfected with a 70% alcohol wipe. Then, a 100 ml aliquot of distilled water was passed from the Cavitron Dual Select dispensing system into and through each ultrasonic scaler and collected from the instrument handle orifice without an inserted tip into a sterile, 125 ml, Security-Snap™ BacT plastic bottle (item number: 411610R27; Thermo Fisher Scientific, Rockwood, Tennessee) with a 100 ml fill line and a 10 mg sodium thiosulfate tablet. After sample collection and closure of the cap, the bottle was inverted several times to facilitate dissolving of the sodium thiosulfate tablet into the ultrasonic scaler waterline sample to neutralize residual chlorine that may be present in the water sample.

For Group B ultrasonic scalers, a second water sample was collected from each of them after insertion of a scaler tip and activation to maximum power similar to its potential use in clinical patient care. A 100 ml aliquot of ultrasonic scaler output water was collected as described above from the water spray created by output water passing through the inserted scaler tip (Figure 3).

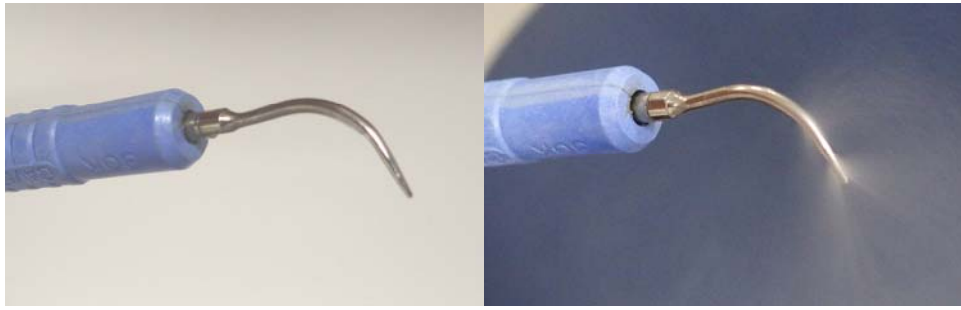


Figure 3. Ultrasonic scaling tip not activated (left), and activated with spray created from coolant water (right).

Transport to the oral microbiology laboratory, and plating of all water specimens, was completed within 90 minutes of collection, during which the samples were kept at room temperature.

Microbiological Methods

All water samples were evaluated by microbial culture for aerobic mesophilic heterotrophic bacteria using nationally-accepted analytical procedures detailed in *Standard Methods for the Examination of Water and Wastewater, 23rd Edition* (Baird et al. 2017). Upon delivery to the oral microbiology laboratory, the samples were vortexed for 15 seconds, and aliquots spread-plated with a sterile bent glass rod onto the surface of Reasoner's low-nutrient R2A agar in 100 x 15 mm plastic Petri plates (BBL™ Prepared Plated R2A Agar Media, Thermo Fisher Scientific, Rockwood, Tennessee). Reasoner's R2A agar was designed for selective cultivation and enumeration of slow-growing aerobic mesophilic heterotrophic bacteria in potable water, and was comprised of 0.05% proteose peptone, 0.05% casamino acids, 0.05% yeast extract, 0.05% dextrose, 0.05% soluble starch, 0.03% dipotassium phosphate, 0.005% magnesium sulfate, 0.03% sodium

pyruvate, and 1.5% agar (Reasoner & Geldreich 1985). All inoculated plates were incubated in an inverted position at 28°C in an aerobic incubator (Heratherm™ IMC 18 Incubator, Thermo Fisher Scientific, Rockwood, Tennessee). Total aerobic mesophilic heterotrophic bacterial colony counts were enumerated after seven days of incubation with an automated colony counter system (AccuCount™ 1000 Macroscopic Automated Colony Counter, BioLogics, Inc., Manassas, Virginia), validated to reliably detect bacterial surface colonies ≥ 0.3 mm in diameter (Goss et al. 1974), to determine the number of microbial colony-forming units (CFU)/ml in ultrasonic scaler output water, and to determine the prevalence of samples yielding a threshold of ≥ 500 CFU/ml of aerobic mesophilic heterotrophic bacteria in ultrasonic scaler output water.

Bacterial Identification

Predominant cultivable isolates of aerobic mesophilic heterotrophic bacteria in ultrasonic scaler output water were identified using matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry and Bruker MALDI Biotyper analytic software (Bruker Daltonics, Billerica, Massachusetts), following procedures previously described (Rams et al. 2016, 2018). In brief, single colonies of isolated aerobic mesophilic heterotrophic bacteria were picked with a sterile wooden toothpick from the surface of R2A agar culture plates, and individually direct-spotted as a thin film into separate circles on the surface of a polished steel MALDI-TOF mass spectrometry target plate, and allowed to dry at room temperature. A 1.0 μ l overlay of a 98-100% formic acid solution was placed and allowed to air dry over the colony smears to facilitate on-plate extraction of bacterial cell proteins. Each spot was then subjected to a

second overlay solution with 1.0 μ l of a matrix mixture, comprised of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid, and allowed to dry at room temperature. A positive control bacterial test standard, containing *Escherichia coli* DH 5 alpha strain ribosomal proteins, was prepared according to manufacturer instructions, and processed similar to the other bacterial isolates. Negative control spots on the target plate were left blank or with the dried matrix solution alone.

Mass spectra for each bacterial isolate were acquired using a Microflex LT benchtop mass spectrometer equipped with a 20-Hz nitrogen laser (Bruker Daltonics, Billerica, Massachusetts). The instrument was operated with Bruker Daltonics FlexControl software (version 3.4) in a linear positive mode within a 2-20 kDa mass range, with voltage settings of 20 kV for ion source one, 18.05 kV for ion source two, 6.0 kV for the lens, and 2,560 V for the linear detector. Mass spectra were automatically obtained using an average 500 laser shots, and analyzed with Bruker Daltonics MALDI Biotyper 3.1 software and the MBT 6903 MSP Library, to determine the most likely microbial species identification of cultivated aerobic mesophilic heterotrophic bacteria. A MALDI Biotyper score, generated as a level of probability by the software, of ≥ 1.7 was utilized as a threshold for reliable species identification, as recommended by Hsu & Burnham (2014). Scores of < 1.7 were considered to provide less reliable bacterial identification. A MALDI Biotyper log score ≥ 2.0 was considered to represent definitive species identification.

Study Personnel

Ultrasonic scaler waterline specimens were collected by Dr. Thomas E. Rams and Dr. Joseph L. Mustazza. R2A culture media preparation and specimen inoculation were carried out in a standardized fashion by a single oral microbiology laboratory technician (Jacqueline D. Sautter), with participation and supervision by the OMTS Laboratory director (Dr. Rams). Dr. Rams carried out all culture analysis and MALDI-TOF mass spectrometry laboratory procedures.

Data Analysis

Mean total viable counts and standard deviation values of aerobic mesophilic heterotrophic bacteria/ml recovered in ultrasonic scaler waterline samples were calculated for both Group A and Group B ultrasonic scalers. The prevalence of elevated levels of aerobic mesophilic heterotrophic bacterial counts among sampled ultrasonic scalers was determined to be the proportion of all water samples within each ultrasonic scaler group yielding ≥ 500 of aerobic mesophilic heterotrophic bacteria CFU/ml. The proportional recovery of each predominate aerobic mesophilic heterotrophic bacterial species identified by MALDI-TOF mass spectrometry was ascertained per ultrasonic scaler waterline sample by calculating the percentage of the identified aerobic mesophilic heterotrophic bacteria species CFU relative to total viable counts as determined on R2A agar culture plates. A Wilcoxon signed-rank test, and a *P*-value threshold of ≤ 0.05 for statistical significance, compared changes in \log_{10} -transformed levels of aerobic mesophilic heterotrophic bacterial counts/ml determined prior to use of, and after, insertion of an ultrasonic scaler tip activated to maximum power. The PC-based

STATA/SE 16.0 for Windows (StataCorp PL, College Station, Texas) 64-bit statistical software package was used in the data analysis.

CHAPTER 3

RESULTS

Bacterial Counts in Ultrasonic Scaler Output Water

Aerobic mesophilic heterotrophic bacteria averaged 610 ± 380 (SD) CFU/ml in output water samples from Group A ultrasonic scalers, with eight of 12 (66.7%) Group A ultrasonic scalers yielding elevated counts ≥ 500 CFU/ml (Figure 4).

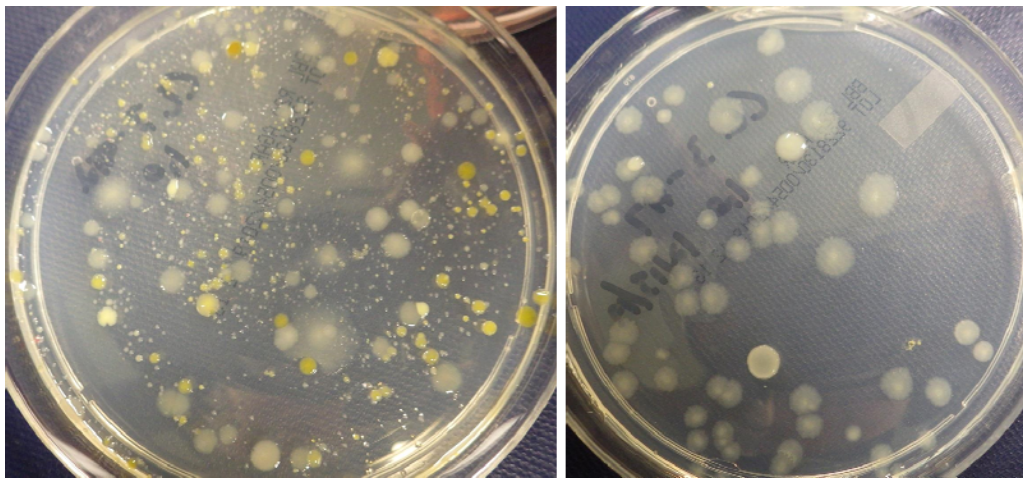


Figure 4. Examples of ultrasonic scaler output water samples with elevated (826 CFU/ml) (left) and acceptable (78 CFU/ml) (right) levels of aerobic mesophilic heterotrophic bacteria. The source water for both instruments was municipal tap water treated with molecular iodine (Group A).

Similar mean values of 667 ± 340 (SD) CFU/ml per ultrasonic scaler were found in output water from Group B ultrasonic scalers, with five of eight (62.5%) Group B ultrasonic scalers yielding elevated counts ≥ 500 CFU/ml.

Predominant Isolated Bacterial Species

Table 1 lists predominant cultivable species in output water from Group A ultrasonic scalers as identified by MALDI-TOF mass spectrometry.

Table 1. Species Identified From Group A Ultrasonic Scaler Waterlines

| Species: | No. of positive ultrasonic scalers | % species recovery (SD) |
|--------------------------------------|---------------------------------------|----------------------------|
| <i>Acidovorax temperans</i> | 6 | 28.2 (33.2) |
| <i>Novosphingobium subterraneum</i> | 5 | 28.4 (35.5) |
| <i>Cupriavidus metallidurans</i> | 5 | 19.7 (29.0) |
| <i>Sphingobium yanoikuyae</i> | 4 | 8.3 (3.4) |
| <i>Cupriavidus pauculus</i> | 3 | 32.0 (51.1) |
| <i>Sphingomonas paucimobilis</i> | 2 | 16.1 (3.8) |
| <i>Methylobacterium rhodesium</i> | 2 | 1.6 (0.1) |
| <i>Sphingobium</i> species | 2 | 50.2 (69.8) |
| <i>Cupriavidus necator</i> | 1 | 29.0 |
| <i>Cupriavidus</i> species | 1 | 87.7 |
| <i>Sphingomonas parapaucimobilis</i> | 1 | 0.4 |
| <i>Sphingomonas xenophegum</i> | 1 | 0.8 |
| <i>Sphingomonas</i> species | 1 | 87.7 |
| <i>Neisseria macacae</i> | 1 | 1.0 |

Acidovorax temperans, *Novosphingobium subterraneum*, *Cupriavidus metallidurans*, and *Sphingobium yanoikuyae* were the most prevalent cultivable species identified in output water from Group A ultrasonic scalers, where the organisms were each recovered from four to six of the 12 evaluated ultrasonic scaler waterlines. Average cultivable proportions of these microbial species ranged from 8.3% to 28.4% of total viable aerobic mesophilic heterotrophic bacterial counts. Other less frequently identified species included other *Sphingobium* species, other *Cupriavidus* species, several species belonging to the *Sphingomonas* genus (including *S. parapaucimobilis* and *S. xenophegum*), *Methylobacterium rhodesium*, and *Neisseria macacae*. A number of additional bacterial isolates were not able to be successfully identified with the MALDI-TOF mass spectrometry database used in the present study. An example of MALDI-TOF mass spectrometry identification of a cultivable isolate in Group A ultrasonic scaler output water samples is provided in Figure 5.

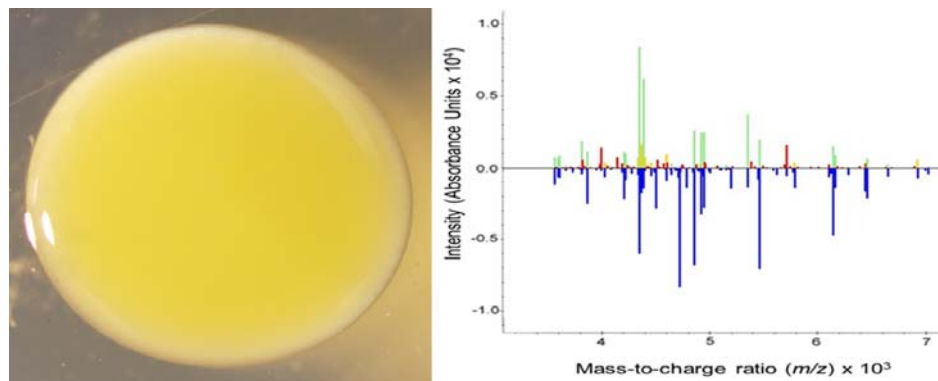


Figure 5. Colony morphology (left) and MALDI-TOF mass spectrometry match (right) for *Novosphingobium subterraneum*. Blue peaks in bottom half of mass spectra match with inverted intensity scale indicate the reference MALDI Biotyper protein profile for

the species, with color of peaks in upper half reflecting the degree of matching of the water isolate with the reference library protein profile (green = full match, yellow = partial match, red = no match).

Table 2 lists predominant cultivable species in output water obtained from Group B ultrasonic scalers as identified by MALDI-TOF mass spectrometry.

Table 2. Species Identified From Group B Ultrasonic Scaler Waterlines

| Species: | No. of positive ultrasonic scalers | % species recovery (SD) |
|-----------------------------------|------------------------------------|-------------------------|
| <i>Cupriavidus metallidurans</i> | 8 | 97.2 (2.4) |
| <i>Methylobacterium rhodesium</i> | 3 | 0.7 (0.2) |
| <i>Micrococcus luteus</i> | 1 | 0.5 |

Cupriavidus metallidurans was the most predominant cultivable species in Group B ultrasonic scaler waterlines, with all eight evaluated ultrasonic scalers in Group B positive for the species (Figure 6).

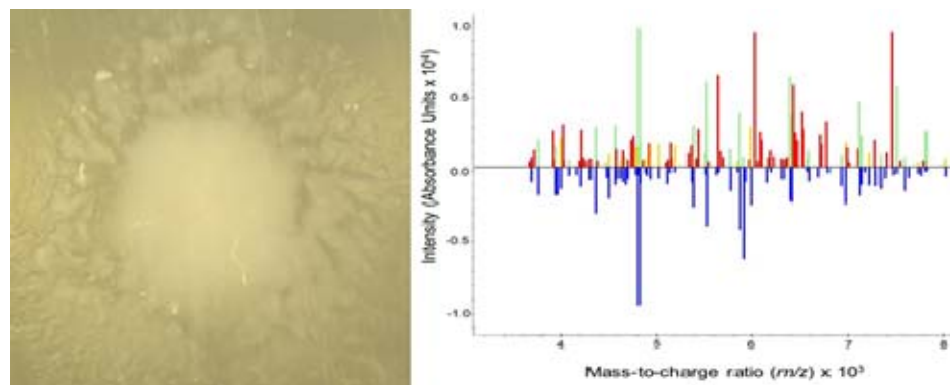


Figure 6. Colony morphology (left) and MALDI-TOF mass spectrometry match (right) for *Cupriavidus metallidurans*. Blue peaks in bottom half of mass spectra match with

inverted intensity scale indicate the reference MALDI Biotyper protein profile for the species, with color of peaks in upper half reflecting the degree of matching of the water isolate with the reference library protein profile (green = full match, yellow = partial match, red = no match).

The mean cultivable proportional recovery of *Cupriavidus metallidurans* was 97.2% of total viable aerobic mesophilic heterotrophic bacteria isolated on R2A medium. Only trace levels of *Methylobacterium rhodesium* and *Micrococcus luteus* were found in waterlines from three of evaluated Group B ultrasonic scalers, and one Group B ultrasonic scaler, respectively.

The MALDI-TOF mass spectrometry testing in this study provided satisfactory test results on the evaluated positive and negative controls, as *E. coli* DH 5 alpha strain ribosomal proteins used as positive controls appropriately produced MALDI Biotyper log scores ≥ 2.0 , with target spots left blank or with the dried matrix solution alone as negative controls appropriately not producing any mass spectra peaks.

Effect of Activated Ultrasonic Scaler Tip on Bacterial Counts

A mean 527 ± 330 (SD) CFU/ml of aerobic mesophilic heterotrophic bacteria were recovered from Group B ultrasonic scaler output water samples collected after passage through an inserted scaler tip activated to maximum power. These levels were numerically less, but not statistically different ($P > 0.05$, Wilcoxon matched pair signed-rank test), from average values of 667 ± 340 (SD) CFU/ml found for output water from the same ultrasonic scalers when no tip was inserted and activated.

CHAPTER 4

DISCUSSION

The major finding from this study is that 13 of 20 (65.0%) ultrasonic dental scalers in routine clinical patient care use at a United States dental school emitted coolant output water with levels of bacterial contamination (aerobic mesophilic heterotrophic bacteria counts ≥ 500 CFU/ml) that violated regulatory drinking water standards in the United States, and would thus be considered by the United States Environmental Protection Administration to be unfit for human consumption (United States Environmental Protection Agency 1999). Aerobic mesophilic heterotrophic bacteria averaged 610 CFU/ml in output water from 12 ultrasonic scalers supplied municipal tap water treated with an iodine resin cartridge, and averaged 667 CFU/ml in output water from eight ultrasonic scalers provided distilled water treated with a 0.78% silver ion-based disinfectant. Eight of 12 (66.7%) ultrasonic scalers supplied with municipal tap water treated with an iodine resin cartridge, and five of eight (62.5%) ultrasonic scalers provided distilled water treated with a 0.78% silver ion-based disinfectant, yielded elevated aerobic mesophilic heterotrophic bacteria counts ≥ 500 CFU/ml.

These findings are novel in that the bacterial contamination in ultrasonic scaler output water originated from waterlines specific to the ultrasonic scalers, and not from source water coming from either a municipal tap water connection, or a dental operator chair unit attached to the ultrasonic scalers. In this study, a Cavitron Dual Select dispensing system was used, as previously described in an earlier investigation (Wirthlin & Marshall 2001). Use of this device enabled evaluation of bacterial contamination

specific and limited to waterline tubing within the ultrasonic scalers, separate from bacterial populations colonizing dental operator chair units waterlines that otherwise supply ultrasonic scalers with coolant water. By introducing distilled water into ultrasonic scaler waterlines through the Cavitron Dual Select dispensing system, instead of feeding potentially contaminated water from dental operator chair units, all bacterial growth isolated from the sampled ultrasonic scaler output water could then be attributable exclusively to the ultrasonic scaler waterlines, and not to water inputted from a dental operator chair unit. Thus, the present study findings indicate that approximately two-thirds of evaluated ultrasonic scalers have internal waterlines with bacterial biofilms that, by themselves, have the ability to heavily contaminate water passing through the instruments into the mouths of dental patients.

Importantly, this high prevalence of ultrasonic scaler output water samples with unacceptably high aerobic mesophilic heterotrophic bacterial counts was found despite the use on the ultrasonic scaler source water of one of two commercial biocides favorably reviewed by the American Dental Association as effective for dental unit waterlines. Neither municipal tap water treated with an iodine resin cartridge, nor distilled water treated with a 0.78% silver ion-based disinfectant, was uniformly effective in adequately suppressing bacterial populations within the ultrasonic scaler waterlines. This disappointing product performance may be due to the absence of any initial or periodic antimicrobial “shock treatment” (Mills 2000) with sodium hypochlorite or another biocide, administered to the ultrasonic scalers in order to suppress any pre-existing established bacterial biofilms in their waterlines. In this regard, the “Instructions for

Use” for the iodine resin cartridge states “To obtain maximum bacteriological reduction, dental unit waterlines are to be cleaned with an approved dental unit waterline agent prior to initial installation of Dentapure[®] cartridges.” Similarly, the “Directions for Use” for the 0.78% silver ion disinfectant also specify that the product’s shock tablet must be initially used for an overnight time period prior to daily use of the regular disinfectant, and then at least monthly afterward. While these measures were performed at Temple University School of Dentistry on dental operator chair unit waterlines, no such steps were carried out on ultrasonic scaling devices, which are stored disconnected to dental operator chair units at Temple University School of Dentistry when not in clinical patient care use. This omission likely accounts for most of the impaired performance of the two biocides, which are marketed as being effective in maintaining, rather than establishing, low aerobic mesophilic heterotrophic bacterial counts in dental waterlines. Additional studies are needed to determine the impact of adding initial and/or periodic antimicrobial “shock treatment” (Mills 2000) with sodium hypochlorite or another biocide to specific management of ultrasonic scaler waterlines.

In addition to the inability of the two chemical disinfectants to adequately suppress ultrasonic scaler waterline bacterial populations, this study additionally found that no statistically significant impact was found on aerobic mesophilic heterotrophic bacterial counts after ultrasonic scaler output water passed through an inserted scaler tip activated to maximum power. This indicates that there are no or little antimicrobial effects exerted on waterline bacteria briefly exposed to high-frequency ultrasonic scaler tip oscillations. These findings are consistent with a previous study finding that

magnetostrictive ultrasonic scalers did not show any in vitro bactericidal effects on *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Campylobacter rectus*, and *Parvimonas micros* over a 2.5 minute exposure time period, and concluded that the primary clinical effect of ultrasonic scalers on dental plaque biofilms is mechanical disruption, rather than a microbial killing effect (Schenk et al. 2000). Consequently, one cannot expect to obtain a marked reduction in aerobic mesophilic heterotrophic bacterial counts in ultrasonic scaler waterlines as a result of mere passage through an activated ultrasonic scaler tip at a maximum power setting.

Among R2A culture medium isolates, a noteworthy difference was found between the two evaluated biocides in regard to predominate microbial species identified in waterline samples. In ultrasonic scaler waterlines where the source water was municipal tap water treated with molecular iodine, *Acidovorax temperans*, *Novosphingobium subterraneum*, *Cupriavidus metallidurans*, and *Sphingobium yanoikuyae* were the most prevalent cultivable species identified with MALDI-TOF mass spectrometry. In comparison, nearly pure growth of *Cupriavidus metallidurans* alone dominated cultivable waterline isolates from ultrasonic scalers where the source water was distilled water treated with the 0.78% silver ion disinfectant, with only trace levels of *Methylobacterium rhodesium* and *Micrococcus luteus* additionally recovered from some evaluated water samples. All of these microbial genera have been previously recovered in other studies of dental unit waterlines (Singh et al. 2003, Costa et al. 2015, Zhang et al. 2018, Yoon & Lee 2018). Interestingly, the dominance of *Cupriavidus metallidurans* in ultrasonic scaler waterlines supplied by a distilled water source treated with the 0.78% silver ion

disinfectant is likely due to resistance of the organism to the silver ion biocide, which has been shown to result in dental operator chair unit waterline overgrowth of the species, as previously reported (Rams et al. 2019).

Questions have been raised as to the clinical significance of elevated numbers of aerobic mesophilic heterotrophic bacteria in dental operator chair unit and ultrasonic scaler output water. A review published in 2007 concluded that “There is no definitive evidence of a widespread public health hazard from exposure to [dental unit waterlines]” (Pankhurst & Coulter 2007). However, a number of microbial species recovered in high numbers from ultrasonic scaler waterlines in this study, such as *Cupriavidus metallidurans*, have been implicated as pathogenic species in systemic medical infections in immunocompromised patients (Langevin et al. 2011, D’Inzeo et al. 2015). In this regard, the Organization for Safety, Asepsis and Prevention in a recent position paper on dental waterline safety concluded that “the presence of high numbers of potentially pathogenic microorganisms in procedural water used for dental treatment is inconsistent with best health-care practices” (Mills et al. 2018). Based on this principle and the present study findings, the effectiveness of infection control procedures used at Temple University School of Dentistry to manage ultrasonic scaler waterline microbial populations must be questioned, and concerns raised about the safety of ultrasonic scaler output water introduced from these instruments into the oral cavity of dental patients during dental treatment procedures. Additional research on these issues is urgently needed.

CHAPTER 5

CONCLUSIONS

Approximately two-thirds of ultrasonic scalers in a dental school clinic setting yielded output water that failed to meet potable water regulatory standards in the United States with regard to aerobic mesophilic heterotrophic bacterial counts, even when subjected to disinfection with one of two American Dental Association favorably-reviewed biocide products, or exposed to high-frequency ultrasonic scaler tip oscillations. *Acidovorax temperans*, *Novosphingobium subterraneum*, *Cupriavidus metallidurans*, and *Sphingobium yanoikuyae* were the most prevalent cultivable species from ultrasonic scalers where the source water was municipal tap water treated with molecular iodine. In contrast, nearly pure growth of *Cupriavidus metallidurans* alone dominated cultivable waterline isolates from ultrasonic scalers where the source water was distilled water treated with a 0.78% silver ion disinfectant. These findings question the effectiveness of infection control procedures used to manage ultrasonic scaler waterline microbial populations, and raise concerns about the safety of ultrasonic scaler output water introduced into the oral cavity of dental patients during dental treatment procedures.

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