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Waterborne pathogens and dental waterlines

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For centuries, waterborne disease has been a persistent scourge of civilization. The endemicity of diseases such as cholera and typhoid fever in the world's cities was assured by the lack of adequate sanitation and ignorance of the means by which these diseases were transmitted [1]. Snow's famous "Broad Street pump-handle" experiment in nineteenth-century London demonstrated that contaminated water was associated with the spread of cholera and led to the development of public health measures that now assure that safe drinking water is available in most areas of the industrialized world [2]. Although modern water and sewer systems have rendered outbreaks of typhoid and cholera newsworthy aberrations in today's world, water remains a potential reservoir for the transmission of disease [3]. Within the last quarter of a century, outbreaks of waterborne disease caused by previously obscure or unknown organisms such as *Legionella* [4], *Cryptosporidium* [5], and *Escherichia coli* O157:H7 [6] have served as potent reminders of our vulnerability.

To most people, the dental office may seem an unlikely place to acquire a waterborne infection. A quick perusal of a typical dental treatment room, however, reveals how greatly the dentist and staff rely on water in the performance of their duties. In addition to the familiar cup filler and cuspidor, dental handpieces, ultrasonic scalers, and other devices rely on water to cool and irrigate operative sites. Because the design characteristics of most dental equipment render them particularly vulnerable to the growth of microbial biofilms on water-bearing surfaces, patients and staff may be exposed to

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water that may exhibit thousands or millions of colony-forming units (CFUs) of bacteria per milliliter of water [7–15]. Because much of the water used in dentistry is delivered in the form of spray, both the airborne and enteric routes can potentially spread organisms. Contaminated water used during surgical procedures exposes previously uninfected tissues and the vascular system.

Although most of the flora recovered from dental equipment are gram-negative heterotrophic water bacteria with little known pathogenic potential, human pathogens including *Pseudomonas aeruginosa* [15,16], *Legionella* species [14,17,18], and nontuberculous mycobacteria [8,9] may be present. Protozoa [10,19] and fungi [12,13,20] that have been associated with human disease have also been identified in biofilms and water from dental devices. Despite efforts to design equipment that does not retract oral fluids during treatment, organisms consistent with human oral flora have also been recovered from dental waterlines [8].

To understand why water from dental units contains such high levels of microbial contamination when the tap water supplying the dental office meets standards for safe drinking water, it is first necessary to have a basic understanding of the nature of microbial biofilms and the engineering features of dental units.

The nature of biofilms

Biofilms are complex, symbiotic microbial communities that almost certainly evolved early in Earth's history and existed for billions of years before the rise of eukaryotes [21]. Biofilms flourish wherever water and solid surfaces exist, ranging from abyssal ocean depths to temporary ponds and rivulets on snow-clad mountain peaks. Human beings are themselves naturally colonized by biofilms (eg, dental plaque) [21,22].

Bacteria are the basic building blocks of biofilms and provide essential structural elements in the form of attachment organs and the extruded polysaccharide matrix that protects the biofilm from dessication, predation, or chemical insults. Using advanced techniques such as scanning electron microscopy, confocal laser microscopy, and advanced staining techniques, researchers have revealed the extraordinary cooperation among organisms required to generate a biofilm [21]. Donlan and Costerton [22] described how the definition of biofilm has evolved as research has revealed the architectural complexity and physiologic attributes of biofilms. These authors propose a new definition of biofilm as a

> microbially derived sessile community characterized by cells that are irreversibly attached to a substrate or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription [22].

Biofilms are remarkably resilient, responding quickly to environmental changes. Biofilm organisms are very different in gene expression than their planktonic (free-living) counterparts. Adhesion to surfaces results in expression of genes that transform the planktonic cell into a productive member of biofilm society—one that contributes materials necessary for adhesion and the production of exopolymer. As the now-sessile cells divide and form microcolonies, they communicate with one another using acyl homoserine lactone signaling compounds. These bacterial pheromones regulate a wide range of physiologic processes including elaboration of virulence factors and increased production of exopolymer [22–24]. Employing this process, known as *quorum sensing*, biofilms achieve a level of structural and physiologic sophistication that, in many respects, mimics the functionality attained by multicellular life. Quorum sensing also appears to play a role in the breakup of a biofilm, dispersing individual cells or cell clusters to colonize distant sites [25].

Although principally bacterial in nature, biofilms may also provide a haven for other organisms including fungi [8], protozoa [19,20], and nematode worms [10]. In the clinical setting, biofilms exhibit increased resistance to both antibiotics and chemical germicides due to delayed penetration of antimicrobial agents through the diffusional barrier formed by the extracellular matrix [26,27]. Nutrient-limited physiologic changes in the mode and rate of growth of bacteria also contribute to their ability to withstand chemical attack [22].

Biofilm-mediated infections of implanted medical devices and indwelling catheters account for thousands of serious infections each year. The annual cost for treating community-acquired urinary tract infections in the United States alone has been estimated at 1.6 billion dollars [28]. The estimated expenditure on dental treatment, much of which is directed toward the effort to combat biofilms in the form of dental plaque and treat biofilm-mediated periodontal disease and caries, amounts annually to over 60 billion dollars [29].

Biofilm and dental equipment design

Dental waterlines provide an environment conducive to rapid proliferation of biofilm. Biofilm-forming bacteria appear to favor hydrophobic nonpolar surfaces [25]. Planktonic organisms suspended in the bulk fluid quickly colonize the chemically inert waterlines [7,15]. Motile bacteria respond to chemical stimuli and move toward attractants consisting of low-molecular-weight organic matter [30]. Organic conditioning films quickly form on water-bearing surfaces and serve as a locus for bacterial attachment [25,30]. After contact with a substrate, the cells express genes associated with adhesion and begin the formation of biofilm [21,23,25].

Intuitively, low flow rates, periods of stagnation, and the low shear stresses associated with laminar flow regimes characteristic of water in narrow-bore tubing would seem to favor the formation of biofilm in dental water lines. Biofilm engineering research, however, suggests that biofilms also appear to flourish under high–shear stress conditions. In fact, areas of turbulent flow and high shear forces are often sites that exhibit more extensive biofilm formation [22]. This data may help explain why flushing waterlines, even for extended times, has little lasting impact on attached biofilm [10,31,32]. Costerton and colleagues [33] described the viscoelastic nature of the biofilm polymers and proposed that this contributes to the resistance of biofilms to dislodgement. This viscoelastic nature may also help explain how fragments of biofilm and metabolic by-products are shed into the bulk fluid, contaminating the effluent discharged from dental instruments.

To understand why water sprayed from a dental handpiece contains thousands or millions of bacterial CFUs when the same water entering the dental unit meets federal drinking water standards that permit only several hundreds of colonies, we must turn to the geometry of the dental water system. A typical dental unit water system consists of 10 m or more of 0.5 mm diameter tubing. The volume of fluid contained in this system, exclusive of large-volume water reservoirs or heaters, rarely exceeds 60 mL. For a fixed volume of fluid, the surface area in a cylinder (the water line) increases geometrically as the diameter decreases. Therefore, the surface area available for growth of biofilms in a dental unit is large compared with the surface area in a larger diameter line such as a water main. Water used in dental treatment must therefore run a lengthy gauntlet of biofilm-colonized surfaces, collecting detached clumps of biofilm and microbial byproducts on its way to the unsuspecting patient.

United States drinking water standards focus on eliminating coliform bacteria that serve as sentinels for fecal contamination. The surface water rule however, permits up to 500 CFUs of heterotrophic bacteria in potable water [34,35]. Although residual chlorine levels introduced at the water treatment facility help suppress the growth of biofilm and survival of planktonic bacteria in the bulk fluid, the interior surfaces of water mains and plumbing often remain colonized by biofilms that can release viable planktonic cells [36]. Even the purest municipal water therefore, may contain measurable numbers of bacteria. Protozoa (including potentially pathogenic amoebae) may also be delivered to the unit in water that is, in all respects, considered safe to drink [19].

Inside the dental unit, the chlorine residual that suppresses planktonic growth in the bulk fluid is quickly exhausted by contact with the biofilm and its exopolymer matrix [37]. Even high concentrations of potent germicides can often be ineffective for controlling biofilms in dental units [8].

Dental waterlines and health

As a consequence of the processes previously described, water used for dental treatment becomes contaminated with microorganisms and their metabolic by-products. From the earliest observation of bacteria in water produced by dental units, researchers have expressed concern that patients could become infected by exposure to contaminated water or aerosol [38]. Many of the predominantly gram-negative bacteria isolated from dental water sources are recognized as opportunistic pathogens [22,39]. In addition, aquatic mycobacteria, *Legionella* species, fungi, and protozoa have been recovered. Table 1 provides a list of frequently reported organisms, their potential pathogenicity in humans, and published evidence of diseases associated with dental exposure.

For an infection to occur in a given individual, however, there must be a sufficient number of pathogenic organisms, a susceptible host, and a portal of entry into that host. In the case of dental unit waterlines, the literature supports the presence large numbers of organisms, many of them with potential to infect humans [7,9,11,13–15,17,20,40]. Virulence factors that

Table 1
Frequently isolated waterline organisms and their potential for pathogenicity in humans

Organism	Potential pathogenicity	Dental case reports
Pseudomonas aeruginosa	Wound infection, septicemia, pneumonia [66]	Wound infection [16]
Other *Pseudomonas* species and *Burkholderia*[a]	Wound infection, septicemia, pneumonia [66]	None
Legionella pneumophila and other *Legionella* species	Pneumonia, wound infection, [67,68]	Increased seropositive response to anti-*Legionella* in dental workers [54,55], fatal legionellosis in dentist?[b] [14]
	Pontiac fever [50]	
Aquatic *Mycobacteria*	Wound infection [69,70], pneumonia	None
Moraxella species	Conjunctivitis, endocarditis [71]	Endocarditis?[b] [44]
Flavobacterium (*Chryseobacterium*)	Endocarditis [72]	None
Pathogenic amoebae	Conjunctivitis, gastroenteritis, meningitis [73]	None
Cladosporium (fungus)	Granulomatous pneumonitis [52]	None
Oral flora	Transmission of periodontal pathogens [74]	None

[a] May include *Burkholderia* and other species previously included among the Pseudomonads.

[b] Anecdotal, no peer-reviewed case report available.

may increase the pathogenicity of bacteria include heat tolerance, hemolysis [11], and exopolymer formation [22]. Mayo et al [11] observed that gram-negative bacteria recovered from dental units exhibited the virulence factors of α or β hemolysis when plated on tryptic soy blood agar, whereas organisms recovered from tap water connected to the same municipal water supplies did not [11]. The use of heaters to warm dental water for patient comfort may select for organisms preadapted to growth at body temperature.

Costerton et al [41] proposed that biofilm organisms have increased potential for successful colonization of living tissues due to the protection from assault by the immune system that the enveloping exopolymer provides. The various portals of entry and the types of conditions that may result are summarized in Table 2.

Water used for dental treatment is present not only in liquid form but also as aerosols (small particles that remain suspended in air and can be drawn into the terminal alveoli of the lung during respiration) and spatter (larger particles that settle more rapidly onto surfaces). Fragments of biofilm suspended in liquid water can enter the host and colonize oropharyngeal mucosa and the gastrointestinal tract. Surgical procedures can result in bacterial invasion of exposed tissues. Aerosols containing biofilm fragments may be responsible for bacterial infections of the respiratory system [41]. Spatter may also result in colonization of nasal mucosa or conjunctiva with or without clinical manifestations. Colonization of the oropharynx or upper respiratory tract has been implicated as an antecedent event in the development of bacterial pneumonia due to secondary aspiration of organisms [42].

Waterline bacteria also can theoretically enter the circulatory system during dental procedures. Reinhart et al [43] compared the numbers and types of bacteria recovered from the blood of patients undergoing ultrasonic scaling using either tap water or sterile water irrigation. Although there was

Table 2
Portals of entry and potential diseases and conditions associated with waterline microorganisms

Portal of entry	Form of contaminants	Organisms/agents	Diseases/conditions
Oropharynx	Liquid, spatter	Bacteria, protozoa	Colonization with possible secondary aspiration
Nasal mucosa, upper respiratory tract	Spatter, aerosol	Bacteria, protozoa	Colonization with possible secondary aspiration
Conjunctiva	Spatter	Bacteria, protozoa	Conjunctivitis
Surgical sites	Liquid	Bacteria, protozoa, endotoxin	Wound infection, bacteremia
Lower respiratory tract	Aerosol	Bacteria, fungi, endotoxin	Pneumonia, granulomatous pneumonitis, asthma
Gastrointestinal tract	Liquid	Bacteria, protozoa	Gastroenteritis

no significant difference in numbers of recoverable bacteria from each group, nearly twice the numbers of gram-negative bacteria were seen in the patients treated with tap water [43]. Bacteremia is associated with the development of infectious endocarditis in susceptible individuals. A case of *Moraxella* endocarditis in a dental patient that may have been associated with dental treatment has been reported anecdotally [44].

A number of conditions can increase the susceptibility of individuals to infection by opportunistic microorganisms. Systemic conditions that result in varying degrees of immune suppression include diabetes mellitus, AIDS, organ transplantation antirejection therapy, and cancer chemotherapy. Martin [16] reported two cases of localized *Pseudomonas aeruginosa* infection following routine dental treatment in patients undergoing cancer chemotherapy. The organism isolated from the wound infection sites was matched by pyocine typing to water samples collected from the dental units used for their treatment [16].

Cystic fibrosis patients are particularly susceptible to life-threatening infections caused by *Pseudomonas aeruginosa* and, to a lesser extent, by *Burkholderia cepacia* [22]. Despite the fact that both organisms have been recovered frequently in dental treatment water, a study conducted in a Danish cystic fibrosis treatment facility concluded that dental treatment only resulted in a 1% to 2% risk for acquiring *Pseudomonas aeruginosa* from dental treatment, a risk equivalent to the yearly "natural background" incidence in that clinic [45].

Colonization and infection are not the only potential hazards associated with exposure to microbially contaminated water. Gram-negative bacteria elaborate lipopolysaccharide molecules, also known as *endotoxin*, in their cell wall membranes. Endotoxin can produce potent physiologic effects in humans. A complex of clinical complications in hemodialysis patients known as *pyrogenic reactions* have been linked to elevated levels of endotoxin in water used for hemodialysis. Symptoms commonly observed include chills, fever, and tachycardia but can progress to severe hypotension, septicemia and shock [46]. Putnins et al [47] found levels of endotoxin as high as 2560 endotoxin units (EU) per milliliter in water collected from colonized dental units. Waterline biofilms were the most likely source of this contamination because levels in tap water from the same location never exceeded 66 EU/mL. Because endotoxin has been implicated as a cause of delayed wound healing, Putnins et al [47] speculated that irrigation with contaminated water could have deleterious effects on the postoperative course of patients undergoing periodontal surgery. Although there are no data to suggest what the "safe" level of endotoxin in dental treatment water might be, water used to prepare dialysate should not exceed 0.25 EU/mL [46].

Exposure to inhaled endotoxin can also produce acute physiologic responses including fever, cough, and dyspnea. Long-term exposure to endotoxin among susceptible individuals has been associated with chronic pulmonary disease in workplace settings such as metalworking and fiberglass manufacturing where high levels of bacteria are found in water [48]. Rose et al [49] reported an outbreak of granulomatous pneumonitis among lifeguards working at an indoor swimming pool complex where high levels of gram-negative bacteria and endotoxin were present in water sprays [49]. The etiology of Pontiac fever, an acute condition that produces symptoms including headache, fever, chills, myalgia, shortness of breath, and fatigue, may be related to inhalation of endotoxin produced by *Legionella* rather than by bacterial colonization [50].

Endotoxin also may play a paradoxic role in the pathophysiology of asthma. Although asthma can be exacerbated by inhalation of endotoxin, exposure in infancy may actually promote enhanced tolerance to allergies [48]. A study of the effect of dental treatment on asthmatic children aged 6 through 18 years found a significant decrease in lung function in 15% of the subjects. The investigators, however, were unable to correlate this effect with dental stress or any other specific aspect of dental treatment [51]; they did not consider the possible role of waterborne endotoxin, which left this possibility open to further investigation.

Little is known about the consequences of dental unit–mediated exposure to aquatic fungi. A case of pneumonitis associated with water in a hot tub that was colonized with the aquatic fungus *Cladosporium* has been reported [52]. This organism has been recovered from water collected from dental units, although no case reports of illness in the dental setting have been reported [12,13,20].

Both epidemiologic investigations and case reports of illness associated with microbially contaminated dental treatment water are few in number. Clark [53] evaluated the nasal flora of dentists and found them to have a higher prevalence of gram-negative bacteria than is typically encountered in the general population. It was suggested that this phenomenon arose from chronic exposure to aerosolized water from dental equipment [53].

In 1985, Fotos et al [54] investigated the seroprevalence of anti-*Legionella* antibody in dental workers as a marker for possible occupational exposure. Dental health care workers were nearly two and a half times more likely than a demographically similar control population of nondental workers to exhibit antibody to *Legionella*. There was also a strong correlation between the length of time an individual had worked in dentistry and the likelihood that they would be seropositive [54]. In 1988, Reinthaler et al [55] found that dental workers exhibited six times the seroprevalence of anti-*Legionella* antibody compared with a control group and that dentists had a higher prevalence than ancillary personnel [55]. None of the participants in either study provided a medical history consistent with a retrospective diagnosis of legionellosis or Pontiac fever.

Martin's [16] report of localized *Psuedomonas aeruginosa* infection discussed earlier in this article remains the only formal case report linking dental treatment water to human illness. This investigation also observed transient carriage of the same strain of *Pseudomonas aeruginosa*, without evidence of clinical infection in 78 patients treated using colonized dental units [16]. Atlas et al [14] anecdotally reported a possible case of fatal legionellosis in a dentist in a review article on the prevalence of *Legionella* bacteria in dental units. Although *Legionella* species were recovered from dental office water supplies, they were not matched to isolates from the patient's lungs [14]. No formal report of this case, however, has been published.

Concern over potential health consequences has led to recommendations by government agencies and professional organizations intended to improve the quality of water used in dental treatment. The Centers for Disease Control and Prevention, in "Recommended Infection Control Practices for Dentistry: 1993" [56], urged dentists to install and maintain antiretraction valves on dental units and flush units at the beginning of the day and between patients. They further stated that only sterile solutions should be used for procedures that involve the cutting of bone [56]. In 1996, the American Dental Association published the recommendations of an expert panel that was convened in 1995 to review the scientific evidence regarding dental waterlines. The American Dental Association statement [57] acknowledged that the quality of water used in dental treatment should be improved and urged dental manufacturers to develop methods that could ensure that water used in dental treatment would contain fewer than 200 CFU/mL of heterotrophic, mesophilic water bacteria in unfiltered output. This goal was consistent with data from the field of hemodialysis that linked systemic reactions in patients to hemodialysate containing greater than 200 CFU/mL [57].

A number of different methods to control or eliminate microbial contamination in dental water systems have been evaluated in the peer-reviewed dental literature. Most investigators have attempted to improve water quality by flushing the lines with fresh water [10,32,58,59], introducing antimicrobial chemicals [12,13,17,37,38,60,61], or filtering output water [62,63]. A number of commercially available agents or devices are now available that purport to control or eliminate biofilms in dental equipment. A complete discussion of the currently available technology is beyond the scope of this article. Recent literature reviews by Mills [44] and Pedersen et al [64] and a report of a workshop on dental water lines sponsored by the National Institutes of Health [65] provide more detailed discussion of this topic.

Summary

Humans, like every other living thing on Earth, have evolved in a world dominated by many billions of microscopic life forms. Most of the time, we live in a state of harmony (or even mutualism) with our invisible coinhabitants. When this balance becomes disturbed however, the consequences can be devastating. Infectious diseases including malaria, tuberculosis, and AIDS remain the world's greatest mass murderers.

Dental workers strive to reduce infection risks for their patients through infection control measures that reduce or eliminate potentially pathogenic agents in the clinical environment. As increasing numbers of patients with varying degrees of immune suppression present for dental treatment, the need to ensure an aseptic treatment environment will become a higher priority for the dental profession. The possibility that exposure to aerosols contaminated with endotoxin might exacerbate asthma or cause chronic respiratory problems in dental health care workers should be investigated. Although direct evidence of widespread complications among patients or occupationally acquired illness among dental workers is presently lacking, reducing the numbers of microorganisms present in dental treatment water is consistent with other empiric measures that form the basis of infection-control practice.

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