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MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINES

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JOSE IVAN SANTIAGO SANTIAGO

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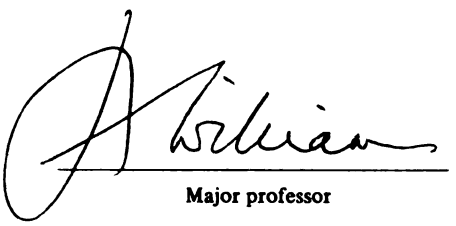

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MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINES

By

José Iván Santiago Santiago

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ABSTRACT

MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINES

By

José Iván Santiago Santiago

Specimens of dental unit water line (DUW) samples were collected from different dental instruments and their microbiological quality assessed. Extensive contamination of DUW was found and comparisons with other potable water sources emphasize the relatively high concentrations of microorganisms in DUW. Evidence of the presence of bacteria, amoebae, and nematodes in DUW points up the need for further studies of these components of DUWL biofilm, as well as health risks posed to personnel, patients, and immunocompromised individuals.

The data confirmed the short term value of two minute flushes, but these findings were offset by first, occasional increases in bacterial concentrations, rather than decreases, followed flushing, and second, reductions in bacterial numbers were often trivial. Microbial contamination was frequently restored to pre-flush levels or higher after brief stasis or use. Additional prophylactic measures are needed to limit DUW microbial contamination.

Le dedico este trabajo científico a mi familia,
pero en especial a mis abuelos que no presenciaron esta parte de mi vida,
en memoria de José Santiago Rivera y Ana Santiago.

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ADA = American Dental Association
AUX = air water syringe - auxiliary lines
AWS = air water syringe
CDC = The Centers for Disease Control
cfu = colony forming units
CWS = Clean Water Systems
DHCP= dental health care professionals
DU = dental unit
DUW = dental unit water
DUWL= dental unit water line
EPA = Environmental Protection Agency
EPS = extracellular polymeric substance
HBV = hepatitis B virus
HIV = human immunodeficiency virus
HSH = high speed handpiece
min = minute
mL = milliliter
nm = nanometer
ppm = parts per million
SBA = sheep blood agar
TSA = trypticase soy agar
uL = microliter
um = micrometer
US = ultrasonic scaler

LITERATURE REVIEW

It may appear from current accounts of the controversy in the press that the rigorous evaluation of dental office infection control practices was brought about entirely by the rapid increase of human immunodeficiency virus (HIV)-infected individuals in the population. Much emphasis has been given to the possible transmission of HIV by an HIV-positive dentist to six of his patients, none of whom was considered to be at high risk of exposure (CDC, 1990a, 1991). However, better infection control procedures in the dental office have been developing for years in parallel with a greater understanding about communicable diseases in general, and their possible transmission using dental instruments (Stevens, 1963; Belting *et al.*, 1964; Hausler and Madden, 1964; ADA, 1978, 1984, 1986, 1988a, 1988b; Holbrook *et al.*, 1978; Bagga *et al.*, 1984; Miller and Palenik, 1985; CDC, 1986, 1993; Crawford and Broderius, 1988, 1990; Christensen, 1991; Cottone and Molinari, 1991; Anonymous - Lancet, 1992; Epstein *et al.*, 1992; Lewis *et al.*, 1992; Lewis and Boe, 1992; Faecher *et al.*, 1993; Mandel, 1993; Miller, 1993; Mills *et al.*, 1993; Pankhurst and Philpott-Howard, 1993; and Watson and Whitehouse, 1993).

The need to develop firm guidelines on infection control

has become apparent because dental professionals and their patients are clearly at risk of exposure to a variety of microorganisms. The Centers for Disease Control (CDC) states that dental patients and dental health care professionals can be exposed to:

> ".. cytomegalovirus, hepatitis B virus (HBV), HIV, herpes simplex virus type 1 and 2, *Mycobacterium tuberculosis*, staphylococci, streptococci, and other viruses and bacteria- specifically, those that infect the upper respiratory tract. Infections may be transmitted in the dental operatory through several routes, including direct contact with blood, oral fluids, or other secretions; indirect contact with contaminated instruments, operatory equipment, or environmental surfaces; or contact with airborne contaminants present in either droplet spatter or aerosols of oral and respiratory fluids." (CDC, 1993).

There is also an emerging recognition of additional serious contributors to the infective hazards present in the dental unit: the formation of a stable biofilms in cooling and irrigation lines (Kelstrup *et al.*, 1977; Oppenheim *et al.*, 1987; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; and Williams *et al.*, 1993) and the unusually high number of pathogenic and opportunistic bacteria present in the water delivered by the instruments to patients and to dental health care professionals with the production of contaminated aerosols by the instruments (Williams *et al.*, 1993). The need for better infection control procedures in the dental clinic is stressed by the more than 200,000,000 dental procedures performed annually (ADA, 1992).

In the following review an account is provided of infectious disease hazards and contemporary regulatory practices for infection control in the dental office. A

history is documented of those studies which have characterized dental water microbiota, their origins, and potential significance in infectious disease transmission in dental operatories.

Introduction:

Infection control practices in dentistry have come under great scrutiny in recent years. This thesis concerns microbiological studies of the much-neglected area of dental unit water contamination, a potential contributor to infection transmission in the dental practice. It also examines several important features of the origins of these contaminants and the typical contamination levels of microbes in dental water.

The literature review covers the background to the current regulatory environment on dental infection control procedures. Infection control practices that have been brought to the forefront by recent episodes of disease transmission in dental offices are reviewed, with special reference to the significance of microbial contamination of water.

Early work, reviewed here, on infection control in the dental office was concerned with infection risks to the dentists and dental staff, and focused on aerosolization of potential pathogens present in the mouths of patients (Stevens, 1963; Belting *et al.*, 1964; and Holbrook *et al.*, 1978). More recent developments have centered upon blood-borne pathogens such as human immunodeficiency virus (HIV),

hepatitis B virus (HBV), and others. The realization that extensive biofilms form within the coolant and irrigant water lines of hand-held dental instruments (high speed drills, ultrasonic scalers, and air-water syringes) has led to the analysis of the microbiota involved in their production. Investigators in the United States and Europe have identified a variety of pathogens, some of them opportunistic, some of them primary, that reside in the dental unit water lines, and are commonly dispensed in high numbers into patients' mouths, including onto exposed lesions and surgical sites. The literature reviewed suggests that some of these microorganisms are derived from the very low level of contaminants in municipal water supplies, while others contaminate the lines from water retraction, or "suck back", of oral fluids from the patient's oral cavity which may harbor microorganisms (Bagga *et al.*, 1984; Miller and Palenik, 1985; Crawford and Broderius, 1988, 1990; Lewis *et al.*, 1992; Lewis and Boe, 1992; and Mills *et al.*, 1993).

Health risks associated with contamination of dental unit water is emphasized by reports in the literature of unusual nasal microbiota of dentists, characterized by the presence of frequent dental water contaminants like *Pseudomonas* and *Proteus* (Clark, 1974). Colonization of the oro/nasal mucosa by aquatic bacteria is apparently not limited to dentists. After the report of infection and production of abscesses by *Pseudomonas* in two immunocompromised patients following dental treatment, a unique study subsequently demonstrated that

Pseudomonas from dental water frequently colonizes the mouths of recipient patients and dentists working with tainted dental units (Martin, 1987). Also, common water contaminants have been determined to be among the primary contributors to severe adult periodontitis (Slots *et al.*, 1988).

Another cause for concern is the mounting evidence of widespread and extensive contamination of dental water lines with *Legionella* species (Rheinthaler and Mascher, 1986; Oppenheim *et al.*, 1987; Michel and Borneff, 1989; Pankhurst *et al.*, 1990; Lück *et al.*, 1993; and Williams *et al.*, 1993). There are indications that dental personnel are at increased risk of exposure to *Legionella* (Fotos *et al.*, 1985; Rheinthaler *et al.*, 1987,1988; and Lück *et al.*, 1993), and one fatality of a dentist with legionellosis is circumstantially linked to this pathogen (J.F. Williams *et al.*, submitted for publication).

Furthermore, reports on respiratory ailments and upper respiratory tract infections have indicated that dental health care professionals and dental students have a greater number of respiratory ailments than other health care professionals (Carter and Seal, 1953; Burton and Miller, 1963; and Mandel, 1993). However, direct evidence of the extent of the health hazard of dental water organisms has been hard to come by. The potential health risk created by water microorganisms cannot be ignored, given the proven ability of water contaminants to use aerosols as infection vehicles (Macfarlane, 1983; Hambleton *et al.*, 1983; Zuravleff *et al.*,

1983; Muder *et al.*, 1986; Midulla *et al.*, 1987; CDC, 1990b; and Faecher *et al.*, 1993), the large number of dental and hygiene treatments are performed annually, and the increase in the number of immunocompromised persons in the population.

One widely used "so called" infection control practice for water borne disease agents, recommended by the American Dental Association (ADA) and the Centers for Disease Control (CDC), consists of the flushing of water lines each morning to eliminate microbial contamination of water, and again between each patient (CDC, 1993). The procedure is examined thoroughly in the work reported in this thesis, and found ineffective. Literature concerning flushing and other possible preventive approaches is also reviewed, and some preliminary observations on the utility of several measures are presented in the appendices.

The dental profession will inevitably be faced with the adoption of preventive practices for water contamination in the coming years, and a spectrum of chemical and physical solutions seems likely to appear for this purpose. The work reported in this thesis may contribute to defining the needs, and establishing the urgency of attending to this problem in dentistry in the United States and elsewhere.

Infectious disease risks and infection control in the dental office:

The need to establish better infection control procedures in dentistry was emphasized in the late 1970's and 1980's by

sporadic outbreaks of hepatitis B and viral gingivostomatitis in dental practices (Levin *et al.*, 1974; Hadler *et al.*, 1981; Reingold *et al.*, 1982; Manzella *et al.*, 1984; and Shaw *et al.*, 1986). Viral infections, which on one occasion resulted in the death of 2 patients with acute hepatitis (Shaw *et al.*, 1986), were caused by dental professionals who had become viral carriers and had unknowingly exposed and transmitted HBV and herpes simplex virus to patients during dental procedures. Dental health care professionals did not routinely wear gloves during the procedures and it was suspected that repeated and vigorous hand washing between patients, to assure infection prevention, caused breaks in the skin which released infective viruses to patients (Levin *et al.*, 1974; Hadler *et al.*, 1981; Reingold *et al.*, 1982; Manzella *et al.*, 1984; and Shaw *et al.*, 1986). The transmission of infectious diseases by dental health care providers to patients was not limited to viruses; in two dental clinics fifteen patients were unknowingly infected with tuberculosis by a carrier dentist (Roderick Smith *et al.*, 1982). At the time there was no recommendation in effect that a protective mask should be worn during dental procedures, and infection of the patients appeared to have started by the colonization of the tooth socket by *Mycobacterium tuberculosis*.

ADA/CDC infection control guidelines:

As early as 1978, the ADA began making recommendations to decrease the risks of infection transmission during dental

procedures (ADA, 1978). This work culminated in the development of guidelines meant to decrease the possibility of transmission of microorganisms from dental professionals to patients (ADA, 1986). Recommendations were made on the prevention of the transmission of infectious diseases, and on infection control practices in the dental office. These included the need to obtain detailed medical histories of the patients, the use of protective barriers, such as the use of gloves (to be changed after each patient), protective masks, and clothing by dental care providers during all dental procedures, and the use of rubber dams in the patients. It was also recommended that environmental surfaces should be kept clean and disinfected, and instruments were to be sterilized or disinfected after use. Handpieces, irrigation syringes, and ultrasonic scalers were to be flushed 20-30 seconds between patients in order to eliminate potentially infective materials from the inside of the instruments. Also any waste should be treated as a potential health hazard.

In 1988, the rapid increase in the population of HIV-infected individuals, coupled with the number of HBV-infected individuals (CDC, 1985), required modifications to be made in the recommended infection control procedures for dental practices (ADA, 1988a). To insure the safety of patients and dental health care providers, dental professionals were advised to obtain vaccination against HBV, to use disposable instruments when possible, and to follow the guidelines established in 1986.

HIV transmission in a dental setting:

The possible transmission of HIV from an infected dentist in Florida to a patient raised doubts about the extent of adoption of infection control procedures recommended by the ADA/CDC in 1988 in dental clinics (CDC, 1990a). An investigation of the infected dentist's former patients has identified five more HIV infected individuals to date (1994). DNA analysis of the dentist's and patients' HIV strains revealed that it was highly likely that the dentist was the infection focus of the virus due to the similarities between the viruses, and dissimilarities to other HIV strains present in the area. Upon review, the mode of transmission appeared to be the use of contaminated dental instruments. Staff of the office indicated that instruments were not routinely sterilized, instruments were only wiped with alcohol after use, and ADA/CDC guidelines were not followed (CDC, 1991).

Other infection control problems in dental clinics:

The HIV outbreak in Florida, due to an apparent lack of infection control protocols, seems to accentuate the inadequacy of preventive measures in dentistry. A survey on the sterilization of dental instruments in 1989 indicated that less than 50% of dentists who answered the survey sterilized their instruments daily, and only 25% of those sterilized the instruments between patients (Dental Products Report, 1993). A report in 1991 indicated that 80% of dentists continue to

surface-disinfect handpieces, and air-water syringes were virtually never sterilized (Christensen, 1991).

The lack of implementation of suitable measures in the dental office, and the fear of HIV transmission through invasive dental procedures, made necessary continuing reevaluation and modification of ADA/CDC guidelines (CDC, 1993). The Florida case emphasized the need to establish regulated infection control practices in dentistry, and brought home to national public health officials the need to change the classification of dental instruments to invasive medical instruments.

Dental instruments:

Some dental instruments are very much like surgical instruments, in that they become contaminated with the patient's blood and secretions which may contain microorganisms, making these instruments possible vectors in patient to patient transmission of infectious diseases (Bagga *et al.*, 1984; Anonymous - Lancet, 1992; Lewis *et al.*, 1992; Lewis and Boe, 1992; CDC, 1993, and Mills *et al.*, 1993). The practice of external disinfection and cold sterilization of medical instruments is only effective in cleaning instruments which are not internally contaminated with patients' material (Anonymous - Lancet, 1992; Lewis *et al.*, 1992; Lewis and Boe, 1992; Mills *et al.*, 1993; and Epstein *et al.*, 1993). However, in dentistry, the use of water retraction in irrigation syringes and high speed cutting drills of dental units (used

to prevent water from dripping on the patients) raised the possibility of influx of oral fluids and blood into dental water lines. An ADA report revealed that some dental units can retract fluids up to ten inches into the dental instrument water line (ADA, 1988b).

Patient-derived materials may include tooth particles, blood and oral secretions, tissue fragments, and microorganisms present in the oral cavity, and all of these could enter the water line (Bagga *et al.*, 1984; Miller and Palenik, 1985; ADA, 1988b; Crawford and Broderius, 1988, 1990; Lewis, 1991; Lewis *et al.*, 1992; Lewis and Boe, 1992; and Miller, 1993). This influx of patients' materials contaminates internally both the dental instruments in use, and the water line attached to the instrument (Bagga *et al.*, 1984; Miller and Palenik, 1985; Crawford and Broderius, 1988, 1990; Lewis, 1991; and Lewis and Boe, 1992), and creates possible means of patient to patient cross contamination.

Although dental instruments have been known to be contaminated internally with patient debris since 1978 (ADA, 1978), and the recommendation to sterilize dates from 1986 (CDC, 1986), external disinfection of handpieces and irrigation syringes remains common (Christensen, 1991; Anonymous - Lancet, 1992; and Dental Products Report, 1993). The problem of aspiration of patients' material into the instrument and the attached water line is compounded by the presence of biofilm in the dental water line. This could affect the elimination of patient material by flushing of the

water line, by permitting adhesion of patient materials to the biofilm. In these circumstances, external disinfection of instruments and flushing would be ineffective modes of decontamination of dental instruments. The need to establish better disinfection techniques has recently been brought to the limelight by Lewis *et al.* (1992). This research group demonstrated that infective virus particles can contaminate the inside of dental instruments and could potentially be transmitted to the next patient, revealing a manner by which any blood borne pathogen, including HIV, could also be transmitted.

New 1993 CDC guidelines:

CDC has recommended the between-patient sterilization of instruments for infection control (CDC, 1993). Dental instrument use is similar to medical devices associated with hepatitis B outbreaks in clinical settings (Kent *et al.*, 1988; and Polish *et al.*, 1992). The issue of sterilization or disinfection of instruments between patients was addressed by determining how instruments are used; any instrument considered to be used in invasive procedures should be sterilized between patients by heat, and any other instruments should be disinfected by using high level disinfection (CDC, 1993). This CDC document suggests that contamination of dental units by retraction of patients' materials could be eliminated with the sterilization of hand held instruments between patients, and the installation of anti-retraction,

one-way check valves (Bagga *et al.*, 1984; Miller and Palenik, 1985; and Crawford and Broderius, 1988, 1990). Although these recommendations have been in place since May 1993, the practice of sterilization of dental instruments between patients is not always followed (Dental Products Report, 1993). Also, irrigant syringes are, on the whole, not autoclavable and the vast majority are not sterilized at all, and certainly not between patients (Christensen, 1991).

Dental unit water microbial contamination:

The firm position taken by national authorities about the need to sterilize high speed drills and irrigant syringes is at odds with the known significance of dental unit water as a possible source of infective microorganisms (CDC, 1993). Dental unit water is not examined routinely for the presence of microorganisms, and contamination of the water line could be critical because few dental procedures are performed without water.

The development of high speed handpieces created a need for a coolant substance in order to prevent damage to the dental pulp. Water is that coolant. Today, dental unit water is still used to prevent damage of the dental pulp by the heat produced by high speed handpieces, but it is also used in the irrigation of dental sites, cleaning the area and rinsing out debris. Thus, any contamination of water violates the sterility of the procedure and contaminates all instruments through which the water flows.

Dental unit water microbiota:

At first glance it would seem that water transmitted by dental instruments should have only low levels of bacterial contamination, because dental units are supplied with potable water from municipal water sources. Municipal water, due to national and state health regulations, must have low levels of microbial contaminants (EPA, 1989). The US Army defines potable water as any treated water with less than 200 colony forming units (cfu) per milliliter (mL), or raw water with less than 500 cfu/mL (Simmons and Gentzkow, 1955). EPA regulations prescribe limits on the contamination of potable water by coliforms and stipulate zero tolerance for the presence of *Legionella*, *Giardia lamblia*, and viruses in water (1989). The presence of heterotrophic bacteria is also limited to 500 cfu/mL in order to prevent interference by these bacteria in coliform tests (Geldreich, 1986; and EPA, 1989).

The numbers of bacteria present in dental unit water vary from one dental unit to another, and within dental units during the working day (Abel *et al.*, 1971; Tippett *et al.*, 1988; and Williams *et al.*, 1994). A recent report indicated that 72% of the water samples taken at different times during a working day and analyzed for the presence of heterotrophic bacteria could not be considered suitable for drinking by EPA standards (Williams *et al.*, 1993).

The problem of microbial contamination of dental water is not a new one. The presence of bacteria in dental water has

been known for at least 30 years (Blake, 1963). Blake isolated bacteria of the genera *Pseudomonas* and *Klebsiella* from the water reservoir of a dental unit. Characterization of the composition of dental unit water microbiota over the last 30 years has extended Blake's observations to include aquatic bacteria and inhabitants of the skin and oral cavity of humans. Bacteria identified in dental unit water include Gram negative and Gram positive genera, among them: *Flavobacterium*, *Bacteroides*, *Pausterella*, *Acinetobacter*, *Staphylococcus*, *Klebsiella*, *Neisseria*, *Moraxella*, *Klebsiella*, *Streptococcus*, and *Legionella* (Larato *et al.*, 1966; Abel *et al.*, 1971; Clark, 1974; Kelstrup *et al.*, 1977; Holbrook *et al.*, 1978; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Oppenheim *et al.*, Mayo *et al.*, 1990; Pankhurst *et al.*, 1990; Whitehouse *et al.*, 1991; and Williams *et al.*, 1993). Contaminants of dental unit water (DUW) are not limited to prokaryotic organisms, but there are also eukaryotes. Filamentous fungi, free-living amoebas, and nematodes have been isolated from dental water (Kelstrup *et al.*, 1977; Michel and Borneff, 1989; and Williams *et al.*, 1993).

The extent of the problem of tainted dental water has been recently reviewed by Williams *et al.* (1993). In their study, water specimens from 150 dental operatories in the states of Washington, Oregon, & California were analyzed for the composition of the aerobic, microaerophilic, and facultative anaerobic microbiota in the samples, and the concentrations of these bacteria in each sample were

determined. The investigation revealed the presence of great numbers of bacteria in many of the samples, which contained at least 20 different species of bacteria and 4 different genera of fungi at contamination levels exceeding potable water standards.

For many years dental procedures have been associated with the development of endocarditis in dental patients (Bayliss *et al.*, 1983). In controlled experiments oral manipulations of rabbits are known to produce endocarditis in some animals (McGowan and Hardie, 1974). Also, common microbial contaminants of dental water are among the most common isolates in adult severe periodontitis (Slots *et al.*, 1988). Contamination of dental water could be potentially hazardous to patients and staff in a dental office because of the presence of pathogens and opportunistic microorganisms (Blake, 1963; Kelstrup *et al*, 1977; Holbrook *et al.*, 1988; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Martin, 1987; Oppenheim *et al.*, 1987; Mayo *et al.*, 1990; Pankhurst *et al.*, 1990; Whitehouse *et al.*, 1991; and Williams *et al.*, 1993).

A review of the literature from 1970-1993 revealed the occurrence of high bacterial contamination levels in water delivered by dental operatories using an in-line water system connected to a municipal supply (Abel *et al.*, 1971; McEntegart and Clark, 1973; Clark, 1974; Gross and Devine, 1976; Gross *et al.*, 1976; Dayoub *et al.*, 1978; Scheid *et al.*, 1982; Mills *et al.*, 1986; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; J.F. Williams *et al.*, 1993; and H.M. Williams *et*

al., 1994). Contamination in water delivered by ultrasonic scalers was determined to range from 48,100 cfu/mL to 2,600,000 cfu/mL (Gross and Devine, 1976; Gross *et al.*, 1976; Dayoub *et al.*, 1978; and Williams *et al.*, 1993). Bacterial concentrations in water delivered by air/water syringes was from 250 cfu/mL to 1,200,000 cfu/mL (Gross and Devine, 1976; Gross *et al.*, 1976; Mayo *et al.*, 1990; and Williams *et al.*, 1993). High speed handpieces delivered contaminated water that contained from 5,700 cfu/mL to 3,600,000 cfu/mL (Abel *et al.*, 1971; Clark, 1974; Gross and Devine, 1976; Gross *et al.*, 1976; Dayoub *et al.*, 1978; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Mills *et al.*, 1986; and Williams *et al.*, 1993).

Reports in 1993 issues of the Journal of the American Dental Association indicates that the delivery of dental unit water with high bacterial contamination through dental instruments is an accurate representation of typical dental water contamination in clinics today (Mills *et al.*, 1993; and Williams *et al.*, 1993). The numbers of bacterial contaminants in dental unit water in the report by Williams *et al.* were similar to contamination levels previously reported in the literature. They reported that heterotrophic plate counts of bacteria from water delivered by air/water syringes, from different operatories, ranged from less than 30 cfu/mL to 1,200,000 cfu/mL, while contamination of water delivered by high speed handpieces ranged from less than 30 cfu/mL to 550,000 cfu/mL. The degree of contamination found in DUW is remarkable, given that similar bacterial concentrations have

been found only in dilute sewage or in heavily contaminated bodies of water near sewage treatment plants (Gainey and Lord, 1950; and Rheinheimer, 1991). Dental unit water bacterial counts were much higher than the bacterial presence found in unpolluted lakes and streams (LeChevallier *et al.*, 1990; and Rheinheimer, 1991).

The ability of dental unit water contaminants to cause medical problems could be the result of either aerosolization of pathogens or direct injection of pathogens. Aerosolization of *Chlamydia trachomatis*, during a dental procedure of a *Chlamydia* infected patient, is suspected to be the cause of the development of purulent conjunctivitis by a dentist (Midulla *et al.*, 1987). The ability by DUW bacteria to infect humans is demonstrated by the development of dental abscesses, caused by *Pseudomonas,* in the oral cavity of immunocompromised patients immediately after dental treatments (Martin, 1987). Subsequent investigations revealed that *Pseudomonas* was a contaminant of the dental water, and that healthy individuals who received treatment in the same dental units had their oral cavity colonized by the same microorganisms. The contamination of DUW is also a cause of concern due to a recent report which indicates that consumption of water with high numbers of heterotrophic bacteria is associated with gastrointestinal maladies (Payment *et al.*, 1991).

Contamination of dental unit water by eukaryotes could also increase the health risks. Certain free living amoeba species have been identified a serious water borne agents of

disease (Ma *et al.*, 1990; and Martinez and Vivesvara, 1991) and isolates of DUW belong to this classification group (Michel and Borneff, 1989). Amoebae also serve as hosts for pathogenic organisms, like *Legionella* and coliforms, and are suspected to be the source of sensitizing allergens in Pontiac Fever (Newsome *et al.*, 1985; Barbaree *et al.*, 1986; Rowbotham, 1986; King *et al.*, 1988; and Fields *et al.*, 1993).

Potential health risks in dentistry:

Dental aerosols:

A contributor to the health risks associated with dental water contamination is the production of aerosols during dental procedures. High speed handpieces, specifically, produce aerosols contaminated with bacteria present in the water (Kazantzis, 1961; Madden and Hausler, 1963; Stevens, 1963; Belting *et al.*, 1964; Hausler and Madden, 1964; Larato *et al.*, 1966; Abel *et al.*, 1971; and Earnest and Laesche, 1991). Aerosols produced by dental instruments are made up of particles of an average size of 50 um or less, which form a colloid (Kazantzis, 1961; Belting *et al.*, 1964; Hausler and Madden, 1966; Micik *et al.*, 1969; and Abel *et al.*, 1971). The colloid permits aerosol particles to be suspended in air for long periods by Brownian motion and to be carried by air currents, such as those produced by air conditioners. The formation of aerosols also prevents desiccation of contaminating microorganisms, prolongs the infectivity of pathogenic organisms like *Legionella pneumophila* (Hambleton *et*

al., 1983), and exposes all areas of the dental office and all personnel to dental water (Belting *et al.*, 1964; Hausler and Madden, 1966; and Micik *et al.*, 1969). The danger of contaminated aerosols is that particles of 5 um or less in diameter can be inhaled and trapped in the alveoli of the lungs during respiration. Greater than 95% of the droplets produced by handpieces are less than 5 um in size (Micik *et al.*, 1969), and these could serve as a means of transmission of DUW contaminants known to be stable in aerosol particles (Hambleton *et al.*, 1983; Macfarlane, 1983; Zuravleff *et al.*, 1983; Muder *et al.*, 1986; and CDC, 1990b).

The production of contaminated aerosols by dental instruments was first demonstrated in 1963 by Kazantzis with the isolation of human oral microbiota from aerosols. Madden and Hauser (1964, 1966) also reported that aerosols produced during dental procedures could be contaminated by microorganisms present in the oral cavity of humans. These findings uncovered a potential health risk to dental professionals. The problem was highlighted further by the isolation from the air of *Mycobacterium tuberculosis* during dental treatment of patients suffering from tuberculosis, who had mycobacteria in their sputum (Belting *et al.*, 1964). The investigators determined that mycobacteria could be isolated from the air within a 4 foot semicircle in front of the patient, with the greater concentration of aerosol particles present in the dental health care workers' close working area. This obviously exposes dental professionals to the

majority of the aerosol particles produced by the instruments. Characterization of the composition of the contaminated aerosols produced by handpieces, air water syringes, and ultrasonic scalers identified the presence of bacteria like pneumococci, alpha and beta hemolytic streptococci, *Pseudomonas aeruginosa*, and various *Staphylococcus* species, with contaminating bacteria originating from the oral cavity of patients and contaminants of DUW (Kazantzis, 1961; Belting *et al.*, 1964; Hausler and Madden, 1964; Larato *et al.*, 1966; Holbrook *et al.*, 1978; and Earnest and Laesche, 1991).

Contaminated aerosols could be associated with the propensity of dental personnel to have respiratory problems (Carter and Seal, 1953; Burton and Miller, 1963; and Mandel, 1993). A survey of dentists in the US has determined that respiratory maladies are the leading afflictions suffered by dentists today (Mandel, 1993). Reports by Carter and Seal (1953) and Burton and Miller (1963), have indicated that dental personnel and dental students have a higher incidence of colds and upper respiratory tract infections than their counterparts in other health science fields. Further proof of the potential danger of contaminated aerosols is the altered nasal microbiota of dentists (Clark, 1974). Dentists working in units contaminated with bacteria of the genera *Pseudomonas* and *Proteus*, common contaminants of dental unit water and not normal members of nasal microbiota, have their nasal cavity colonized by them. Also, contaminated aerosols were suspected to be the infective vector for *Chlamydia trachomatis* in a

dentist that developed purulent conjunctivitis (Midulla *et al.*, 1987).

Further cause for concern about the association of aerosols and disease transmission is the finding that *Legionella pneumophila* is a common contaminant of dental unit water (Rheinthaller and Mascher, 1986; Oppenheim *et al.*, 1987; Pankhurst *et al.*, 1990; and Lück *et al.*, 1993). The etiological agent of Legionnaires' Disease and Pontiac Fever is transmitted by aerosols similar to those produced by dental instruments (Macfarlane, 1983; Zuravleff *et al.*, 1983; Muder *et al.*, 1986; and CDC, 1990), and remains viable for up to 2 hours in a mist colloid suspension (Hambleton *et al.*, 1983) . Studies of the exposure of dental personnel to *Legionella* in Austria and Czechoslovakia (Lück), Germany (Rheinthaler, 1987, 1988), and the United States (Fotos), have revealed the presence of increased anti-*Legionella* antibodies in dental professionals' sera when compared to other health professionals and members of the population (Fotos *et al.*, 1985; Rheinthaler *et al.*, 1987, 1988; and Lück *et al.*, 1993).

Increased antibody titers to *Legionella pneumophila* in dental personnel appear to be associated to their work experience (Fotos *et al.*, 1985; and Rheinthaler *et al.*, 1987 and 1988). Dentists and dental staff with greater work experience appear to have a greater exposure to *Legionella* and have higher antibody titers (Fotos *et al.*, 1985; and Rheinthaler *et al.*, 1987, 1988)). The recent death of a dentist in California as a result of *Legionella* pneumonia

raises questions about the presence of this bacteria in dental water and its potential as a serious health issue. An investigation of the dentist's death revealed that he most likely acquired his *Legionella* infection from his dental operatory (J.F. Williams *et al.*, submitted for publication).

Another health risk associated with the presence of *Legionella* in dental water and aerosols could be the presence of free living amoebae in dental water (Michel and Borneff, 1989). *Legionella* is known to infect free living amoebae, to survive in these organisms through exposure to environmental hazards, and to multiply in them (Newsome *et al.*, 1985; Barbaree *et al.*, 1986; Rowbotham, 1986; Fields *et al.*, 1993; and Kuchta *et al.*, 1993). The ability of *Legionella* to infect and multiply within protozoa, makes amoebae the perfect disease carrier for inoculation of high numbers of *Legionella* into the lungs by aspiration of aerosol particles (O'Brien and Bhopal, 1993).

Another problem with the bacterial contamination of dental water and the production of contaminated aerosols is the recent increase of tuberculosis cases in the general population (Faecher *et al.*, 1993). *Mycobacterium* has the ability to infect the oral mucosa of humans and to infect the tooth socket after extractions (Roderick Smith *et al.*, 1982; and Penderson and Reibel, 1989). The disease can produce ulcers with infective microorganisms in the oral cavity and tongue in the early stages of the disease and prior to the development of systemic infection, and in sputum in an

infection of the lungs (Prabhu *et al.*, 1978; Rauch *et al.*, 1978; and Dimitrakopoulos *et al.*, 1991). Thus, the presence of mycobacteria in the oral cavity can produce contaminated aerosols, as demonstrated in sputum positive individuals by Belting *et al.*, and this may expose dental professionals and patients to the bacteria.

Other infection routes:

Another caused for concern is the potential for cross contamination of patients by water retraction into the dental unit and the saliva ejector (Bagga *et al.*, 1984; Miller and Palenik, 1986; ADA, 1988b; Crawford and Broderius, 1988, 1990; Anonymous - Lancet, 1992; Lewis and Boe, 1992; Lewis *et al.*, 1992; and Watson and Whitehouse, 1993). Retraction of water during dental procedures contaminates the inside of dental instruments and the attached water line with microorganisms, bacteria and viruses, present in the oral cavity, blood, and debris (Bagga *et al.*, 1984; Miller and Palenik, 1986; ADA, 1988b; Crawford and Broderius, 1988, 1990; Lewis *et al.*, 1992; and Lewis and Boe, 1992). Thus, this could provide material for the production of contaminated aerosols and transfer it to another patient directly during a dental procedure.

The possibility of cross contamination caused the ADA/CDC to recommend the use of anti-retraction check valves and the sterilization of dental instruments and flushing of the water lines between patients (CDC, 1993). Although this recommendation has been in place for some time, the internal

contamination of dental instruments and water lines remains a problem. Part of the problem is that anti-retraction check valves are ineffective after prolonged use, and these devices are not monitored once installed. Also, dental instruments are not always sterilized between patients (Christensen, 1991; Anonymous - Lancet, 1992; and Dental Products Report, 1993).

A recent publication indicated that the common practice of creating a seal around the saliva ejector with the mouth, creates negative pressure which could also release retracted fluids derived from the mouth of a previous patient (Watson and Whitehouse, 1993). Thus any microorganisms present in dental instruments and the saliva ejector could be released into the oral cavity, injected into surgical sites, or aerosolized during dental procedures, exposing dental professionals and patients to potentially pathogenic bacteria.

Aerosol contamination is not the only health risk associated with the use of contaminated water during dental procedures. Microorganisms in dental water could cause bacteremias in patients, as a result of the injection of microorganisms directly into the bloodstream during invasive dental treatments and surgical procedures. Pathogens like *Legionella pneumophila*, *Mycobacterium*, and *Pseudomonas*, have the ability to infect a human host also through open wounds, making a dental procedure an excellent mode of infection by the direct inoculation route (Roderick Smith *et al.*, 1982; Brabender *et al.*, 1983; Martin, 1987; Lowry *et al.*, 1991; and Pendersen and Reibel, 1991).

A recent scientific investigation by Ely *et al.* associated contaminated air used during dental procedures with the development of pneumomediastinum, fatal descending necrotizing mediastinitis, and Lemierre's syndrome in four dental patients. However, the authors did not consider that the water lines could equally well have been the source of the infections they observed; they attributed the source of the infection to the air, even though there is little evidence that air delivered by the dental unit is contaminated.

BIOFILMS:Their nature and the extent of the problem in medical care.

The design of the dental unit, with its long and narrow tubes, and the stasis of water inside the unit, create ideal conditions for the colonization of the inner surface of dental unit water lines and subsequent biofilm formation by aquatic bacteria and human contaminants retracted inside the water lines. Studies have revealed that bacteria prefer to live in association; in alpine streams 1 out of every 1000 bacteria are found in a planktonic (motile) stage, and the others associate to produce a slimy biofilm on rock surfaces (Costerton *et al.*, 1978). This association appears to produce a selective advantage to the adherent bacteria (Costerton *et al.*, 1981, 1987; LeChevallier *et al.*, 1988). Microorganisms present in water distribution systems have an increased resistance to chlorine just by surface attachment

(LeChevallier *et al.*, 1988). Control experiments with a fastidious laboratory organism, like *Legionella*, have revealed that this organism is more resistant to biocides, like chlorine, when grown in association with other bacteria, than when grown in pure cultures (Cargill *et al.*, 1992).

Biofilm formation:

Biofilm development on any surface begins with the change of bacteria from a planktonic stage to a sessile one. Microorganisms adsorb to surfaces by hydrophobic forces in a reversible manner. With adsorption certain bacteria begin to secrete extracellular components, an "extracellular polymeric substance" (EPS) necessary for a more permanent adhesion by polar bonds. This begins the formation of a glycocalyx matrix and serves as the foundation of the biofilm. Bacterial adhesion by polar bonds causes attached bacteria to divide and to secrete more EPS. The increased production of EPS provides optimal conditions for the attachment of planktonic bacteria to the biofilm and the formation of heterogeneous microcolonies. This glycocalyx acts like an anion exchange resin which can trap nutrients according to ionic charge. "Maturation" of the biofilm creates a new ecosystem formed by a mucopolysaccharide glycocalyx matrix of varying layers and widths, with channels, embedded with microcolonies of heterogenous bacteria, filamentous fungi, and other protozoa. These organisms act as interacting communities with different survival requirements that are met within the biofilm.

Biofilm formation also creates the development of oxygen and nutrient gradients within the structures. Depending in their position in the biofilm or on the microcolonies, microorganisms could live in an aerobic environment located closer to the flow of water, in a microaerophilic environment found in the middle layers of the biofilm or inside of the microcolonies, or in a strictly anaerobic one, found in the inner layers of the biofilm or inside the microcolonies. Nutrients present in the water are readily used by organisms present in the aerobic and microaerophilic layers. Anaerobic organisms use byproducts of the other organisms as nourishment and process these nutrients by fermentation. Also, they respire by using electron acceptors other than oxygen. For a review of biofilms and their formation refer to Costerton *et al.* (1981, 1987), Characklis and Cooksey (1983), Marshall (1992) or Mayette (1992).

Maturation of the biofilm also provides its inhabitants with protection from the action of antibiotics and biocides. The formation of biofilms inhibits the access of antibiotics and biocides to constituent microorganisms, thus increasing their resistance to these compounds. Only microorganisms in contact with fluids are affected (Costerton *et al.*, 1981, 1987; LeChevallier *et al.*, 1988; Fackelmann, 1990; van der Wende and Characklis, 1990; Anwar and Costerton, 1992; Marshall, 1992; and Mayette, 1992).

The ability of bacteria to produce biofilms is expressed in biotic and abiotic conditions. Biofilm formation occurs in

industrial settings and is associated with corrosion, the blockage of pipelines and filters, fouling of products, and the production of harmful metabolites, like H_2S (Characklis and Cooksey, 1983; and Costerton, 1984). The formation of biofilms is not always harmful and it is exploited in the treatment of water and waste water, using the microorganisms' abilities to break down pollutants (Charaklis and Cooksey, 1983).

In animals, one of the best described biofilms in nature is the formation of dental plaque on teeth, a potentially important biofilm in humans (Costerton *et al.*, 1987). Biofilms are also formed in the gastrointestinal tract and genitourinary tract of mammals, protecting the organisms against pathogenic microorganisms (Costerton *et al.*, 1981, 1987; and Anonymous - ASM News, 1993). Any disturbances of these biofilms could result in the development of infections. Biofilm formation in humans is also the cause medical problems. Osteomyelitis results from the development of biofilm in the bones, and its treatment requires the elimination of biofilm structures. Prolonged treatment is needed with antibiotics at higher than normal concentrations due to the drugs' limited access to microorganisms in the biofilm (Anwar and Costerton, 1992; and Marshall, 1992).

Biofilms in biomedical devices:

The production of biofilms in biomedical devices is also associated with prolonged use of implanted catheters, and may

cause urinary tract infections, endocarditis, and catheter-related sepsis in patients with implants (Peters *et al.*, 1981; Kluge, 1982; Marrie *et al.*, 1982; Costerton *et al.*, 1987; Russell *et al.*, 1987; Nickel *et al.*, 1992). Biofilms found in implanted medical devices are produced by microbiota of the skin, *Staphylococcus aureus* and *Staphylococcus epidermidis*, and gram negative organisms, like *Pseudomonas aeruginosa* and *Alcaligenes calcoaceticus* (Peters *et al.*, 1981; Kluge, 1982; Marrie *et al.*, 1982; Gristina *et al.*, 1988; Anwar and Costerton, 1992; Marshall, 1992; Nickel *et al.*, 1992; and Passerini *et al.*, 1992). These bacteria can form biofilms along the catheters at a rate of 2-3 cm per hour, even against the flow of antibiotic containing fluids, by using fibronectin to attach to surfaces with polar bonds, and immediately beginning development of a glycocalyx matrix after the implant of the devices (Russell *et al.*, 1987; and Nickel *et al.*, 1992).

The development of endocarditis and septicemia in patients with implanted pacemakers has been determined to be caused by biofilm formation in pacemaker leads and appears to depend on the contamination of the device prior to surgery (Peters *et al.*, 1981; and Marrie *et al.*, 1982). Similar medical problems are observed in patients with implanted arterial grafts and prosthetic cardiac valves (Kluge, 1982). The implantation of prosthetic hip joints and artificial hearts leads to the formation of biofilms in the devices, necrosis of tissue surrounding the implanted device, and

finally rejection and removal of implants (Gristina and Costerton, 1984; Gristina *et al.*, 1988; and Marshall, 1992).

Dental unit water line biofilms:

The contamination of water in water distribution systems with high numbers of bacteria and fungi result of the formation of biofilms in the pipes of the water distribution system (Rigway and Olson, 1981; Rosenzweig *et al.*, 1986; and LeChevallier *et al.*, 1987). Something similar occurs in dental units where the production of biofilm is the result of the creation of optimal conditions for the formation of biofilms. The long and narrow tubes with static water are combined with known biofilm producers like *Staphylococcus*, *Pseudomonas*, *Legionella*, and fungi, all of which are common contaminants of DUW (Kelstrup *et al.*, 1977; Peters *et al.*, 1981; Kluge, 1982; Marrie *et al.*, 1982; Oppenheim *et al.*, 1987; Russell *et al.*, 1987; Gristina *et al.*, 1988; Mayo *et al.*, 1990; Pankhurst *et al.*, 1991; Anwar and Costerton, 1992; Marshall, 1992; Nickel *et al.*, 1992; Passerini *et al.*, 1992; Williams *et al.*, 1993; and Wireman *et al.*, 1993).

The microscopic examination of discolored, badly tasting, foul smelling water with flakes, by Kelstrup *et al.* (1977) revealed the presence of aggregated bacteria and fungi in the floccules present in DUW. Upon inspection of the dental water line by phase contrast and electron microscopy a similar arrangement was found in the inner surface of dental water lines. The inner walls of the dental unit water lines were

covered with different bacteria and fungal hyphae embedded in a matrix. This report established the presence of biofilms in dental unit water lines. Examination of biofilms formed in dental unit water lines has revealed the presence of complex microbial communities (Williams *et al.*, 1993). Microscopic examination of dental water line biofilms *in vivo* and *in situ* with the use of Nomarski optics and electron microscopy uncovered the presence of not only bacterial and fungi in the biofilm structures, but also the presence of amoebae and nematodes, which feed on the bacteria of the biofilm (Williams *et al.*, 1993).

Mayo *et al.* and Whitehouse *et al.* associated bacterial contamination found in dental water with the formation of biofilms in dental water lines, providing a constant source of contamination of the water. Water samples taken from a working operatory at different times demonstrated that bacterial contamination of the water may fluctuate during a working day (Abel *et al.*, 1971; Tippett *et al.*, 1988; and H.N. Williams *et al.*, 1994).

The biofilm in the dental lines also provides a form of protection to microbial constituents against biocides. The use of compounds like H_2O_2, sodium hypochlorite, chlorhexidine gluconate, Stericol, povidone iodine, was ineffective in eliminating bacterial contamination of dental water (Abel *et al.*, 1971; McEntegart and Clark, 1973; Kelstrup *et al.*, 1977, Mills *et al.*, 1986; and Pankhurst *et al.*, 1990). These compounds only decreased the number of bacteria in the water

for short periods of time and lead to the selection of biocide resistant organisms (Armstrong *et al.*, 1982; Murray *et al.*, 1984; LeChevallier *et al.*, 1988; and van der Wende and Characklis, 1990).

Preventive measures for the control of dental unit water contamination:

The health risks associated with the presence of microorganisms in dental water have caused some investigators to explore options for the elimination of bacteria from dental water. Some researchers have used biocides, sterile water reservoirs, flushing procedures for water lines, and depth and membrane filters, to control dental water contamination (Blake, 1963; Abel *et al.*, 1971; McEntegart and Clark, 1973; Gross and Devine, 1976; Gross *et al.*, 1976; Molinari and Crawford, 1976; Kelstrup *et al.*, 1977; Dayoub *et al.*, 1978; Scheid *et al.*, 1982; Mills *et al.*, 1986; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Pankhurst *et al.*, 1990; Whitehouse *et al.*, 1991; Bierle, 1993; H.M. Williams *et al.*, 1994; and H.N. Williams *et al.*, 1994) Unfortunately, they have been generally unsuccessful. This section will review different approaches used in attempts to reduce or eliminate microorganisms from dental water.

Biocide flushes of dental unit water lines:

The attempted elimination of bacteria from dental water by treatment of the lines with biocides - which include sodium hypochlorite, chlorhexidine gluconate, Stericol, H_2O_2, and

povidone iodine - has been unsuccessful. The treatments reduce planktonic organisms in the line, and cause a temporary drop in the bacterial contamination of water (Abel *et al.*, 1971; McEntegart and Clark, 1973; Kelstrup *et al.*, 1977; Mills *et al.*, 1986; and Pankhurst *et al.*, 1990). The use of 10% povidone iodine and sterile water at first seemed encouraging, because overnight exposure of the water lines to the biocide and the use of a sterile water reservoir, eliminated bacteria in dental water for 3 to 14 days, depending on the dental unit (Mills *et al.*, 1986). However, resistant forms soon appeared (Mills *et al.*, 1986).

The ineffectiveness of biocides is associated with the limited access of disinfecting compounds to the inner aspects of the biofilm, leaving a healthy bacterial community inside the slime layer (Costerton *et al.*, 1987; LeChevallier *et al.*, 1988; Fackelmann, 1990; Anwar and Costerton, 1992; Marshall, 1992; and Mayette, 1992). Even killing of all organisms in the biofilm is not successful in eliminating bacteria for prolonged periods of time, because the glycocalyx matrix is left behind as a nutrient layer and can be easily colonized (Fackelmann, 1990). The only way to eliminate biofilm bacterial contaminants from dental water is by the removal of the glycocalyx matrix and the prevention of bacterial adhesion to the tube surface (Costerton *et al.*, 1987; LeChevallier *et al.*, 1988; and Fackelmann, 1990).

Sterile water reservoirs:

The use of only sterile water reservoirs in dental units does not assure dentists the delivery of sterile water through their instruments (Blake, 1963; Molinari and Crawford, 1976; Mills *et al.*, 1986; Whitehouse *et al.*, 1991; J.F. Williams *et al.*, 1993; and H.N. Williams *et al.*, 1994); biofilms can form in the water lines of dental units receiving only sterile water, probably due to the retraction of patient and environmental bacteria during dental treatments (Williams *et al.*, 1993). Thus the presence of established biofilms in the DUW lines makes delivery of sterile water by dental instruments possible only for limited periods of time, without routine sterilization of the tubing, which is an impractical procedure for most offices.

Flushing of dental unit water lines:

The CDC recommends that "high-speed handpieces should be run to discharge water and air for a minimum of 20-30 seconds after the use on each patient...Additionally, there is evidence that overnight or weekend microbial accumulation of water lines can be reduced substantially by removing the handpiece and allowing water lines to run and discharge for several minutes at the beginning of each clinic day." (CDC, 1993). Similar procedures should be performed with all dental instruments which deliver water. Some investigators have presented data indicating the complete removal of bacteria from dental water by flushing of dental unit water lines for

2 minutes. (Tippett *et al.*, 1988; and Bierle, 1993). Investigators working with units with an average bacterial concentration in water of 1,720 cfu/mL, demonstrated that 2 minutes flushing of dental lines could eliminate bacteria in some dental units (Bierle, 1993); sometimes bacteria could not be eliminated even after 5 minutes of flushing of the lines (Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Bierle, 1993; and H.M Williams *et al.*, 1994). Moreover the literature suggests that bacterial contamination of dental unit water is generally much worse than 1,720 cfu/mL (Williams *et al.*, 1993).

Other investigators working on dental units with more representative bacterial contamination of water, have not found flushing as effective as Tippett *et al.* and Bierle in the removal of bacteria. Although flushing reduced contamination levels by greater than 90% in some studies (Abel *et al.*, 1971; Gross and Devine, 1976; Gross *et al.*, 1976; Scheid *et al.*, 1982; and Mayo *et al.*, 1990), the ineffectiveness of the procedure is highlighted by Mayo *et al.* They demonstrated that after a 6 minute flush, contamination was reduced by 99.9%, but the bacterial presence in the water was 1,300 cfu/mL, a contamination level unacceptable for human consumption. There is evidence that even when bacteria are eliminated from the water by flushing for 20-30 minutes, reappearance occurs within 30 minutes, and contamination climbs to previous levels in 24 hours (Whitehouse *et al.*, 1991).

Theoretically a flush of dental lines should be effective

in the elimination of planktonic bacteria, but this does not affect the bacterial biofilm. Overall, the unpredictable nature of bacterial removal by flushing in published studies is likely to be the result of laminar flow within the water line. The mechanisms of laminar flow permit the movement of water in the tube at different velocities (Cutnell and Johnson, 1989). Water velocities within the tubing decrease from the central lamina towards the inner surface of the tube, where the velocity is zero. This means that the flow of water over the surface of the biofilm will not affect its structure and microbial constituents. Flushing removes planktonic bacteria from the moving water laminae, and leaves the biofilm intact to act as a contaminating reservoir for the dental water. The concentration of bacteria in dental water is going to depend on the rate of microbial division within the biofilm, temperature, degree of stagnation, and nutrient content of the dental unit. Another drawback of flushing is that this procedure is only performed between patients, at best, and the water in the lines is only flowing for about 2 minutes in 3-5 second bursts during a typical dental procedure. The average dental procedure takes approximately 30 minutes, and water is therefore stagnant most of the time. This allows the biofilm ample time to recontaminate all the water laminae.

In line filters:

The use of prefilters and filters helps reduce or

eliminate the presence of bacteria in water. Three um pore size pleated membrane prefilters, attached between the water supply and the dental unit reduce bacteria contamination of dental water lines from a range of 48,100 cfu/mL - 1,360,000 cfu/mL to a range of 220 cfu/mL - 67,400 cfu/mL (Dayoub *et al.*, 1978). The addition of a cellulose acetate filter with a 0.45 um pore size in the line connected to the handpiece eliminated bacteria for up to 32 hours, if used with sterile dental instruments (Dayoub *et al.*, 1978; and Pankhurst *et al.*, 1990). The use of smaller pore size filters prevented adequate water flow by the unit. Pankhurst *et al.* installed charcoal depth filters in-line in an attempt to prevent *Legionella* contamination. The short term benefit was quickly overwhelmed by the colonization of the filter itself by *Legionella* within 7 days, and the resumption of high levels of contamination of the dental water.

In line checkvalves:

An efficient infection control procedure for dental unit water lines requires the use of anti-retraction checkvalves in dental instruments. Water retraction by the dental unit contaminates dental instruments and water lines with patient materials (Bagga *et al.*, 1984; Miller and Palenik, 1985; Crawford and Broderius, 1988, 1990; Anonymous - Lancet, 1992; and Lewis and Boe, 1992). Retraction of bacteria into dental water lines can be eliminated by placing a one way check valve in line in each unit (Bagga *et al.*, 1984; and Crawford and

Broderius, 1988, 1990). This decreases the number of bacteria which can contribute to the formation of a biofilm within the lines.

Conclusion:

The literature reviewed here, suggests that the dental profession will be compelled to adopt some kinds of preventive measures to avoid microbial contamination of dental water in the future. Engineering solutions may come about in the long run, and in the interim a variety of chemical and physical approaches are likely to appear. These will present dentists with some options in dealing with an area of infectious disease control that has been neglected by the profession for the last decade or more. The publicity given to dental office-acquired HIV infections has increased the awareness in both the public and organized dentistry of the risks of poor management of microbial contamination. It seems likely that this awareness will spill over into the improvement of procedures for the avoidance of water-borne infectious organisms in the dental operatory.

LIST OF REFERENCES

LITERATURE REVIEW

LIST OF REFERENCES:

Abel, L.C., Miller, R.L., Micik, R.E., and Ryge, G.
Studies of dental aerobiology: IV. Bacterial contamination of water delivered by dental units.
J. Dent. Res., 1971, 50:1567-1569.

American Dental Association.
Bureau of Economic and Behavioral Research.
1990 Survey of dental services rendered.
Chicago, Il:pubs. American Dental Association; 1992.

American Dental Association Reports.
Infection control in the dental office.
J. Am. Dent. Assoc., 1978, 97:673-677.

American Dental Association Reports.
Infection control recommendations for the dental office and the dental laboratory.
J. Am. Dent. Assoc., 1988a, 116:241-248.

American Dental Association Reports.
Dental units and water retraction.
J. Am. Dent. Assoc., 1988b, 116:417-420.

Anonymous.
Risk of HIV transmission during dental treatment.
The Lancet, 1992, 340:1259-1260.

Anonymous.
Natural biofilms and human health:Case studies.
Am. Soc. Microbiol. - News, 1993, 59:388-389.

Anwar, H. and Costerton, J.W.
Effective use of antibiotics in the treatment of biofilm-associated infections.
Am. Soc. Microbiol. - News, 1992, 58:665-668.

Armstrong, J.L., Calamoris, J.J., and Seidler, R.J.
Selection of antibiotic-resistant standard plate count bacteria during water treatment.
Appl. Environ. Microbiol., 1982, 44:308-316.

Bagga, B.S.R., Murphy, R.A., Anderson, A.W., and Punwami, I.
Contamination of dental unit cooling water with oral microorganisms and its prevention.
J. Am. Dent. Assoc., 1984, 109:712-716.

Barbaree, J.M., Fields, B.S., Feeley, J.C., Gorman, G.W., and Martin, W.T.
Isolation of protozoa from water associated with a legionellosis outbreak and demonstration of intracellular multiplication of *Legionella pneumophila*.
Appl. Environ. Microbiol., 1986, 51:422-424.

Bayliss, R., Clarke, C., Oakley, C., Somerville, W., and Whitfield, A.G.W.
The teeth and infective endocarditis.
Brit. Heart J., 1983, 50:506-512.

Belting, C.M., Haberfelde, G.C., and Juhl, L.K.
Spread of organisms from dental air rotor.
J. Am. Dent. Assoc., 1964, 68:648-651.

Bierle, J.W.
Dental operatory water lines.
J. Calif. Dent. Assoc., 1993, 21:13-15.

Blake, G.C.
The incidence and control of bacterial infection in dental spray reservoirs.
Brit. Dent. J., 1963, 115:413-416.

Brabender, W., Hinthorn, D.R., Asher, M., Lindsey, N.J., and Liu, C.
Legionella pneumophila wound infection.
J. Am. Med. Assoc., 1983, 250:3091-3092.

Burton, W.E. and Miller, R.L.
The role of aerobiology in dentistry.
Proc. First Int. Symp. Aerobiol., 1963, pp. 87-94.

Cargill, K.L., Pyle, B.H., Sauer, R.L., and McFeters, G.A.
Effects of culture conditions and biofilm formation on the iodine susceptibility of *Legionella pneumophila*.
Can. J. Microbiol., 1992, 38:423-429.

Carter, W.J. and Seal, J.R.
Upper respiratory infections in dental personnel and their patients.
Research project report NM 005 251 1403,
Naval Medical Research Unit no. 4.
Administrative Command,
US Naval Training Center, Great Lakes, Il.,
February 25,1953.

Centers for disease control.
Recommendations for protection against viral hepatitis.
Morb. Mortal. Weekly Rep., 1985, 34:313-335.

Centers for disease control.
Recommended infection-control practices for dentistry.
Morb. Mortal. Weekly Rep., 1986, 35:237-242.

Centers for disease control.
Possible transmission of human immunodeficiency virus to a patient during invasive dental procedure.
Morb. and Mortal. Weekly Rep., 1990a, 39:489-493.

Centers for disease control.
Legionnaire's disease outbreak associated with a grocery store mist machine - Louisiana 1989.
Morb. Mortal. Weekly Rep., 1990b, 39:108-110.

Centers for disease control.
Update:Transmission of HIV infection during an invasive dental procedure-Florida.
Morb. Mortal. Weekly Rep., 1991, 40:21-27,33.

Centers for disease control.
Recommended Infection Control Practices for Dentistry.
Morb. Mortal. Weekly Rep., 1993, 42:1-12.

Characklis, W.G. and Cooksey, K.E.
Biofilms and microbial fouling.
Adv. Appl. Microbiol., 1983, 29:93-138.

Christensen, G.J.
Infection control:Some significant loopholes.
J.Am. Dent. Assoc., 1991, 122:99-100.

Clark, A.
Bacterial colonization of dental units and the nasal flora of dental personnel.
Proc. Roy. Soc. Med., 1974, 67:1269-1270.

Costerton, J.W., Geesey, G.G., and Cheng, K.J.
How bacteria stick?
Scientific American, 1978, 238:86-95.

Costerton, J.W., Irvin, R.J., and Cheng, K.J.
The bacterial glycocalyx in nature and disease.
Ann. Rev. Microbiol., 1981, 35:299-324.

Costerton, J.W.
The formation of biocide-resistant biofilms in industrial, natural, and medical systems.
Dev. Ind. Microbiol., 1984, 25:363-372.

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M., and Marrie, T.J.
Bacterial biofilms in nature and disease.
Ann. Rev. Microbiol., 1987, 41:435-464.

Cottone, J.A. and Molinari, J.A.
State of the art infection control in dentistry.
J. Am. Dent. Assoc., 1991, 123:33-41.

Crawford, J.J. and Broderius, C.
Control of cross infection in the dental operatory:prevention of water retraction by bur cooling spray systems.
J. Am. Dent. Assoc., 1988, 116:685-687.

Crawford, J.J. and Broderius, C.
Evaluation of a dental unit design to prevent retraction of oral fluids.
Quintessence Int., 1990, 21:47-51.

Cutnell, J.D. and Johnson, K.W.
Fluid Dynamics. pp 285-287.
In:Physics.
New York:pubs. John Wiley; 1989

Dayoub, M.B., Rusilko, D.J., and Gross, A.
A method of decontamination of ultrasonic scalers and high speed handpieces.
J. Periodont., 1978, 49:261-265.

Dental Products Report.
Handpieces and media hype: Is the worst over?
Dental Products Report, 1993, pp.1,70,78.

Dimitrakopoulos, I., Zouloumis, L., Lazaridis, N., Karakasis, D., Trigonidis, G., and Sichletidis, L.
Primary tuberculosis of the oral cavity.
Oral Surg. Oral Med. Oral Pathol., 1991, 72:712-715.

Earnest, R., and Laesche, W.
Measuring harmful levels of bacteria in dental aerosols.
J. Am. Dent. Assoc., 1991, 122:55-57.

Ely, E.W., Stump, T.E., Hudspeth, A.S., and Haponik, E.F.
Thoracic complications of dental surgical procedures:Hazards of the dental drill.
Am. J. Med., 1993, 95:456-465.

Environmental Protection Agency.
Drinking water:national primary drinking water regulations; filtration, disinfection; turbidity, *Giardia lamblia*, virus, *Legionella*, and heterotrophic bacteria.
Fed. Reg., 1989, 54:27486-27540.

Epstein, J.B., Guiseppe, R., Sibau, L., and Sherlock, C.H.
Rotary instruments and the potential risks of transmission infection: herpes simplex virus.
J. Am. Dent. Assoc., 1993, 124:55-59.

Fackelmann, K.A.
Pipe-dwelling bacteria use slimy strategy.
Sci. News, 1990, 137:6.

Faecher, R.S., Thomas, J.E., and Bender, B.S.
Tuberculosis:A growing concern for dentistry.
J. Am. Dent. Assoc., 1993, 124:94-104.

Fields, B.S., Utley-Fields, S.R., Loy, J.N.C., White, E.H., Steffens, W.L., and Shotts, E.B.
Attachment and entry of *Legionella pneumophila* in *Hartmannella vermiformis*.
J. Infect. Dis., 1993, 167:1146-1150.

Fitzgibbon, E.J., Bartzokas, C.A., Martin, M.V., Gibson, M.F., and Graham, R.
The source frequency and extent of bacterial contamination of dental unit water systems.
Brit. Dent. J., 1984, 157:98-101.

Fotos, P.G., Westfall, H.N., Snyder, I.S., Miller, R.W., and Mutchler, B.M.
Prevalence of *Legionella* - specific IgG and IgM antibodies in a dental clinic population.
J. Dent. Res., 1985, 64:1382-1385.

Gainey, P.L. and Lord, T.H.
The number of bacteria in water. pp 80-87.
In:An introduction to the microbiology of water and sewage.
Minneapolis MN:pubs. Burgess; 1950.

Geldreich, E.E.
Potable water:New directions in microbial regulations.
Am. Soc. Microbiol. - News, 1986, 52:530-534.

Gristina, A.G. and Costerton, J.W.
Bacteria-laden biofilms - a hazard to orthopedic protheses.
Infect. Surg., 1984, 3:655-662.

Gristina, A.G., Dobbins, J.J., Giammara, B., Lewis, J.C., and DeVries, W.C.
Biofilm development in artificial heart and tissue damage.
J. Am. Med. Assoc., 1988, 259:870-874.

Gross, A. and Devine, J.D.
Microbial contamination of dental units and ultrasonic scalers.
J. Dent. Res., 1976, 55B:B276 - abstract # 856.

Gross, A., Devine, M., and Cutright, D.E.
Microbial contamination of dental units and ultrasonic scalers.
J. Periodont., 1976, 47:670-673.

Hadler, S.C., Sorley, D.L., Acree, K.H., Webster, H.M., Schable, C.A., Francis, D.P., and Maynard, J.E.
An outbreak of hepatitis B in a dental practice.
Ann. Intern. Med., 1981, 95:133-138.

Hambleton, P., Broster, M.G., Dennis, P.J., Henstridge, R., Fitzgeorge, R., and Conlan, J.W.
Survival of virulent *Legionella pneumophila* in aerosols.
J. Hyg., 1983, 90:451-460.

Hausler, W.J. Jr. and Madden, R.M.
Aerobiology of dental handpieces.
J. Dent. Res., 1964, 43:903 - abstract # 378.

Hausler, W.J. Jr. and Madden, R.M.
Microbiologic comparison of dental handpieces. 2. Aerosol decay and dispersion.
J. Dent. Res., 1966, 45:52-58.

Holbrook, W.P., Muir, K.F., Macphee, I.P., and Ross, P.W.
Bacteriological investigation of the aerosol from ultrasonic scaler.
Brit. Dent. J., 1978, 144:245-247.

Kazantzis, G.
Air contamination from high-speed dental drills.
Proc. Roy. Soc. Med., 1961, 54:242-244.

Kelstrup, J., Funder-Nielsen, T.D., and Theilade, J.
Microbial aggregate contamination of water lines in dental equipment and its control.
Acta Path. Microbiol. Scand., 1977, 85:177-183.

Kent, G.P., Brondum, J., Keenlyside, R.A., La Fazia, L.M., and Scott, H.D.
A large outbreak of acupuncture associated hepatitis B.
Am. J. Epidemiol., 1988, 127:591-598.

King, C.H., Shotts, E.B., Wooley, R.E., and Porter, K.G.
Survival of coliforms and bacterial pathogens within protozoa during chlorination.
Appl. Environ. Microbiol., 1988, 54:3023-3033.

Kluge, R.M.
Infection of prosthetic cardiac valves and arterial grafts.
Heart Lung, 1982, 11:146-151.

Kuchta, J.M., Navratil, J.S., Wadowsky, R.M., Dowling, J.N., States, S.J., and Yee, R.B.
Effect of chlorine on the survival and growth of *Legionella pneumophila* and *Hartmanella vermiformis*. pp. 242-245.
In:*Legionella* - Current status and emerging perspectives.
Barbaree, J.M., Breiman, R.F., and Dufour, A.D., eds.
Washington DC:pubs. American Society of Microbiology; 1993.

Larato, D.C., Ruskin, P.F., Martin, A., and Delanko, R.
Effect of a dental air turbine drill on the bacterial counts in air.
J. Pros. Dent., 1966, 16:758-764.

LeChevallier, M.W., Babcock, T.M., and Lee, R.G.
Examination and characterization of distribution system biofilms.
Appl. Environ. Microbiol., 1987, 53:2714-2724.

LeChevallier, M.W., Cawthon, C.D., and Lee, R.G.
Factors promoting survival of bacteria in chlorinated water supplies.
Appl. Environ. Microbiol., 1988, 54:649-654.

LeChevallier, M.W., Seidler, R.J., and Evans, T.M.
Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies.
Appl. Environ. Microbiol., 1990, 40:922-930.

Levin, M.L., Maddrey, W.C., Wands, J.R., and Mendeloff, A.I.
Hepatitis B transmission by a dentist.
J. Am. Med. Assoc., 1974, 228:1139-1140.

Lewis, D.L., Arens, M., Appleton, S.S., Nakshima, K., Ryu, J., Boe, R.K., Patrick, J.B., Watanabe, D.T., and Suzuki, M.
Cross-contamination potential with dental equipment.
Lancet, 1992, 340:1252-1254.

Lewis, D.L. and Boe, R.K.
Cross-infection risks associated with current procedures for using high speed dental handpieces.
J. Clin. Microbiol., 1992, 30:401-406.

Lowry, P.W., Blankenship, R.J., Gridley, W., Troup, N.J., and Tompkins, L.S.
A cluster of *Legionella* sternal wound infections due to postoperative topical exposure to contaminated tap water.
N. Engl. J. Med., 1991, 324:109-113.

Lück, P.C., Bender, L., Ott, M., Helbig, J.H., Witzleb, W., and Hacker, J.
Analysis of *Legionella pneumophila* serogroup 6 strains isolated from dental units. pp. 240-242.
In:*Legionella* - Current status and emerging perspectives.
Barbaree, J.M., Breiman, R.F., and Dufour, A.D., eds.
Washington DC:pubs. American Society of Microbiology; 1993.

Ma, P., Vivesvara, G.S., Martinez, A.J., Theodore, F.H., Dagget, P.M., and Swayer, T.K.
Naegleria and *Acanthamoeba* infections.
Rev. Inf. Dis., 1990, 12:490-514.

Macfarlane, J.T.
Legionnaire's disease:update.
Brit. Med. J., 1983, 287:443-444.

Madden, R.M. and Hausler W.J. Jr.
Microbiological comparison of dental handpieces. 1. Preliminary report.
J. Dent. Res., 1963, 42:1146-1151.

Mandel, I.D.
Occupational risks in dentistry:comforts and concerns.
J. Am. Dent. Assoc., 1993, 124:41-49.

Manzella, J.P., McConville, J.H., Valenti, W., Menegus, M.A., Swierkosz, E.M., and Arens, M.
An outbreak of herpes simplex virus type I gingivostomatitis in a dental hygiene practice.
J. Am. Med. Assoc., 1984, 252:2019-2022.

Marrie, T.J. and Costerton, J.W.
A scanning and electron microscopic study of an endocardial pacemaker lead.
Circulation, 1982, 66:1334-1343.

Marshall, K.C.
Biofilm:An overview of bacterial adhesion, activity, and control at surfaces.
Am. Soc. Microbiol. - News, 1992, 58:202-207.

Martin, M.W.
The significance of the bacterial contamination of dental unit water systems.
Brit. Dent. J., 1987, 163:152-154.

Martinez, A.J. and Vivesvara, G.S.
Laboratory diagnosis of pathogenic free-living amoebas: *Naegleria*, *Acanthamoeba*, and *Leptomyxid*.
Clin. Lab. Med., 1991, 11:861-872.

Mayette, D.C.
The existence and significance of biofilm in water.
Water Rev., 1992, 10:1-3.

Mayo, J.E., Oertling, K.M., and Andrieu, S.
Bacterial biofilm:A source of contamination in dental air-water syringes.
Clin. Prev. Dent., 1990, 12:13-20.

McEntegart, M.G. and Clark, A.
Colonization of dental units by water bacteria.
Brit. Dent. J., 1973, 134:140-142.

McGowan, D.A. and Hardie, J.M.
Production of bacterial endocarditis in prepared rabbits by oral manipulations.
Brit. Dent. J., 1974, 137:129-131.

Michel, R. and Borneff, M.
Significance of amoeba and other protozoa in water conduit systems of dental units.
Zbl. Bakt. Hyg., 1989, 187:312-323.

Micik, R.E., Miller, R.L., Manzzarella, H.A., and Ryge, G.
Studies in dental aerobiology:I. Bacterial aerosols generated during dental procedures.
J. Dent. Res., 1969, 48:49-56.

Micik, R.E., Miller, R.L., and Leong, A.C.
Studies in dental aerobiology:III. Efficacy of surgical masks in protecting dental personnel from airborne bacterial particles.
J. Dent. Res., 1971, 50:626-630.

Midulla, M., Sollecito, D., Feleppa, F., Assensio, A.M., and Ilari, S.
Infection by airborne *Chlamydya trachomatis* in a dentist cured with rifampicin after failures with tetracycline and doxycycline.
Brit. Med. J., 1987, 294:742.

Miller, C.H. and Palenik, C.J.
Microbial contamination of dental water lines.
Dent. Asepsis Rev., 1985, 5(1).

Miller, C.H.
Cleaning and disinfection: Basis of microbial killing for infection control.
J. Am. Dent. Assoc., 1993, 124:48-56.

Mills, S.E., Lauderdale, P.W., and Mayhew, R.B.
Reduction of microbial contamination in dental units with povidone-iodine 10%.
J. Am. Dent. Assoc., 1986, 113:280-284.

Mills, S.E., Kuehne, J.C., and Bradley, D.V. Jr.
Bacteriological analysis of high-speed handpiece turbines.
J. Am. Dent. Assoc., 1993, 124:59-62.

Molinary, J.D. and Crawford, J.J.
Evaluation of an independent water reservoir system for high speed instrumentation.
J. Dent. Res., 1976, 55B:B245, abst. #855.

Muder, R.R., Yu, V.L., and Woo, A.H.
Mode of transmission of *Legionella pneumophila*. A critical review.
Arch. Intern. Med., 1986, 146:1607-1612.

Murray, G.E., Tobin, R.S., Junkins, B., and Kushner, D.J.
Effect of chlorination on antibiotic resistance profiles of sewage-related bacteria.
Appl. Environ. Microbiol., 1984, 48:73-77.

Newsome, A.L., Baker, R.L., Miller, R.D., and Arnold, R.R.
Interactions between *Naegleria fowleri* and *Legionella pneumophila*.
Infect. Immun., 1985, 50:449-452.

Nickel, J.C., Downey, J., and Costerton, J.W.
Movement of *Pseudomonas aeruginosa* along catheter surfaces - a mechanism in pathogenesis of catheter-associated infection.
Urology, 1992, 39:93-98.

O'Brien, S.J. and Bhopal, R.J.
Legionnaires' disease:the infective dose paradox.
Lancet, 1993, 342:5-6.

Oppenheim, B.A., Sefton, A.M., Gill, O.N., Tyler, J.E., O'Mahony, M.C., Richards, J.M., Dennis, P.J.L., and Harrison, T.G.
Widespread *Legionella pneumophila* contamination of dental stations in a dental school without apparent human infection.
Epidem. Infect., 1987, 99:159-166.

Pankhurst, C.L., Philpott-Howard, J.N., Hewitt, J.H., and Casewell, M.W.
The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems.
J. Hosp. Infec., 1990, 16:9-18.

Pankhurst, C.L. and Philpott-Howard, J.N.
The microbiological quality of water in dental chair units.
J. Hosp. Infect., 1993, 23:167-174.

Passerini, L., Lam, K., Costerton, J.W., and Garner-King, E.
Biofilms on indwelling vascular catheters.
Critic. Care Med., 1992, 20:665-673.

Payment, P., Franco, E., Richardson, L., and Siemiatycki, J.
Gastrointestinal health effects associated with the consumption of drinking water produced by point-of-use domestic reverse-osmosis filtration unit.
Appl. Environ. Microbiol., 1991, 57:945-948.

Pendersen, A. and Reibel, J.
Intraoral infection with *Mycobacterium chelonae*.
Oral Surg. Oral Med. Oral Pathol., 1989, 67:262-265.

Peters, G., Locci, R., and Pulverer, G.
Microbial colonization of prosthetic devices, II. Scanning electron microscopy of naturally infected intravenous catheters.
Zbl. Bakt. Mikrobiol. Hyg., 1981, 173:293-299.

Polish, L.B., Shapiro, C.N., Bauer, F., Klots, P., Ginier, P. Roberto, R.R., Margolis, H.S., and Alter, M.J.
Nosocomial transmission of hepatitis B virus associated with the use of a spring-loaded finger-stick device.
N. Engl. J. Med., 1992, 326:721-725.

Prabhu, S.R., Daftary, D.K., and Dholakia, H.M.
Tubercular ulcer of the tongue: report of case.
J. Oral Surg., 1978, 36:384-386.

Rauch, D.M. and Friedman, E.
Systemic tuberculosis initially seen as an oral ulceration: report of case.
J. Oral Surg., 1978, 36:387-389.

Reingold, A.L., Kane, M.A., Murphy, B.L., Checko, P., Francis, D.P., and Maynard, J.E.
Transmission of hepatitis B by an oral surgeon.
J. Infect. Dis. 1982, 145:262-268.

Reinthaler, F. and Mascher, F.
Legionella pneumophila in dental units.
Zbl. Bakt. Hyg. B., 1986, 183:86-88.

Reinthaler, F., Mascher, F., and Stünzner, D.
Legionella pneumophila:Seroepidemiologic studies of dentists and dental personnel in Austria.
Zbl. Bakt. Hyg. B., 1987, 185:164-170.

Reinthaler, F., Mascher, F., and Stünzner, D.
Serologic examination for antibodies against *Legionella* species in dental personnel.
J. Dent. Res., 1988, 67:942-943.

Rheinheimer G.
Numbers and biomass of microorganisms in water and sediment.
In:Aquatic Microbiology. pp. 65-109.
New York:pubs. J. Wiley and Sons; 1991.

Ridgway, H.F. and Olson, B.H.
Scanning electron microscope evidence of bacterial colonization of a drinking-water distribution system.
Appl. Environ. Microbiol., 1981, 41:274-281.

Roderick Smith, W.H., Mason, K.D., Davies, D., and Onions, J.P.
Intraoral and pulmonary tuberculosis following dental treatment.
Lancet, 1982, i:842-844.

Rosenzweig, W.D., Minnigh, H., and Pipes, W.O.
Fungi in potable water distribution systems.
J. Am. Assoc. Waterworks, 1986, 78:53-55.

Rowbotham, T.J.
Current views on the relationships between amoeba, *Legionella*, and man.
Isr. J. Med. Sci., 1986, 22:678-689.

Russel, P.B., Kline, J., Yoder, M.C., and Polin, R.A.
Staphylococcal adherence to polyvinyl chlorine and heparin-bonded polyurethane catheters is species dependent and enhanced by fibronectin.
J. Clin. Microbiol., 1987, 25:1083-1087.

Scheid, R.C., Kim, C.K., Bright, J.S., Whitely, M.S., and Rosen, S.
Reduction of microbes in handpieces by flushing before use.
J. Am. Dent. Assoc., 1982, 105:658-660.

Shaw, F.E., Barrett, C.L., Lam, R., Peave, R.B., Coleman, P.J., Hadler, S.C., Fields, H.A., and Maynard, J.E.
Lethal outbreak of hepatitis B in a dental practice.
J. Am. Med. Assoc., 1986, 255:3260-3264.

Simmons, J.S. and Gentzkow, C.L. eds.
Medical and Public Health Laboratory Methods. p 800.
Philadelphia:pubs. Lea and Febiger;1955.

Slots, J., Rams, T.E., and Listgarten, M.A.
Yeast, enteric rods, and pseudomonades in the subgingival flora of severe adult periodontitis.
Oral Microbiol. Immun., 1988, 3:47-52.

Stevens, R.E.
Preliminary study - air contamination with microorganisms during use of air turbine handpieces.
J. Am. Dent. Assoc., 1963, 66:237-239.

Tippett, B.F., Edwards, J.L., and Jenkinson, H.F.
Bacterial contamination of dental unit water lines-a possible source of cross infection.
N. Z. Dent. J., 1988, 84:112-114.

van der Wende, E. and Characklis, W.G.
Biofilm in water distribution systems.
In:Drinking water microbiology. pp. 249-268.
McFeters, G.A. ed. New York: pubs. Springer-Verlag; 1990.

Watson, C.M. and Whitehouse, R.L.S.
Possibility of cross-contamination between dental patients by means of the saliva ejector.
J. Am. Dent. Assoc., 1993, 124:77-80.

Whitehouse, R.L.S., Peters, E., Lizotte, J., and Lilge, C.
Influence of biofilms on microbial contamination in dental unit water.
J. Dent., 1991, 19:290-295.

Williams, J.F., Johnston, A.M., Johnson, B., Huntington, M.K., MacKenzie, C.D.
Microbial contamination of dental unit water lines:prevalence, intensity, and microbiological characteristics.
J. Am. Dent. Assoc., 1993, 124:59-65

Williams, H.M., Quimby, H., and Romberg, E.
Evaluation and use of a low nutrient medium and reduced incubation temperature to study bacterial contamination in the water supply of dental units.
Can. J. Microbiol., 1994,40:127-131.

Williams, H.N., Kelley, J., Folineo, D., Williams, G.C., Hawley, C.L., and Sibiski, J.
Assessing microbial contamination of clean water dental units and compliance with disinfection protocol.
J. Am. Dent. Assoc., 1994, 125:1205-1211.

Wireman, J.W., Schmidt, A., Scavo, C.S., and Hutchins, D.T.
Biofilm formation by *Legionella pneumophila* in a model domestic hot water system. pp. 231-234.
In:*Legionella*:Current Status and Emerging Perspectives.
Barbaree, J., Breiman, R.F., and Dufour, A.P. eds.
Washington, D.C.:pubs. American Society of Microbiology;1993.

Zuravleff, J.J., Yu, V.L., Shonnard, J.W., Rihs, J.D., and Best, M.
Legionella pneumophila contamination of a hospital humidifier. Demonstration of aerosol transmission and subsequent subclinical infection in exposed guinea pigs.
Am. Rev. Respir. Dis., 1983, 128:657-661.

ARTICLE # 1

MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINES: SHORT AND LONG TERM EFFECTS OF FLUSHING

by

José Iván Santiago Santiago and Jeffrey F. Williams.

INTRODUCTION:

Microbial contamination of dental unit water lines (DUWL) has been recognized since the early work of Abel *et al.* (1971) in the United States. Reports from Europe (McEntegart and Clark, 1973; Clark, 1974; Kelstrup *et al.*, 1977, Fitzgibbon *et al.*, 1984; Martin, 1987; and Oppenheim *et al.*, 1987) and Canada (Whitehouse *et al.*, 1991) make it clear that the problem is widespread and associated with the use of lengthy cooling and irrigation water lines.

The phenomenon is now attributed to the formation of an extensive microbial 'biofilm', a term coined in 1978 by Costerton *et al.* The term biofilm describes the accumulation of stable, cooperative microbial populations embedded in a glycopeptide glycocalix matrix on virtually all surfaces over which fluids flow (Costerton *et al.*, 1981, 1987; Characklis and Cooksey, 1983; Fackelman, 1990; Marshall, 1992; and Mayette, 1992). Serious pathogens, like *Legionella pneumophila* (Oppenheim *et al.*, 1987; Pankhurst *et al.*, 1990; Cargill *et al.*, 1992; Marshall, 1992; and Williams *et al.*,1993), as well as common opportunists, like organisms of the genera *Pseudomonas*, and *Staphylococcus* (Kelstrup *et al.*, 1977; Fitzgibbon *et al.*, 1984; Russell *et al.*, 1987; Fackelman, 1990; Mayo *et al.*, 1990; Pankhurst *et al.*, 1991;

Whitehouse *et al.*, 1991; and Williams *et al.*, 1993) frequently flourish in these biofilm structures.

The biofilm provides the dental unit water lines with a resident population of microorganisms and a matrix onto which bacteria present in the water can attach and become a permanent member of the biofilm community. This problem is not limited to dental instruments, but plagues the use of many medical devices, such as catheters (Peters *et al.*, 1981; Russell *et al.*, 1987; Anwar and Costerton, 1992; Nickel *et al.*, 1992; and Passerini *et al.*, 1992), drainage tubes (Peters *et al.*, 1981), pacemakers (Marrie *et al.*, 1982), bioprosthetics and mechanical heart valves (Kluge, 1982), prosthetic joints (Gristina and Costerton, 1984; and Marshall, 1992), and artificial hearts (Gristina *et al.*, 1988).

Experimental solutions to the problem referenced in dental-medical literature have included the use of sterile water reservoirs, chemical disinfectant flushes in the lines, and filtration systems (Abel *et al.*, 1971; McEntegart and Clark, 1973; Molinari and Crawford, 1976; Kelstrup *et al.*, 1977; Dayoub *et al.*, 1978; Mills *et al.*, 1986; and Pankhurst *et al.*, 1990). Some of the chemicals used include: sodium hypochlorite (Abel *et al.*, 1971; McEntegart and Clark, 1973; and Pankhurst *et al.*, 1990), 10% povidone iodine plus sterile water (Mills *et al.*, 1986), H_2O_2 (Kelstrup *et al.*, 1977), 0.1% Stericol (McEntegart and Clark, 1973), and chlorhexidine gluconate (McEntegart and Clark, 1973), but no satisfactory method for routine use has yet been devised and evaluated.

Current American Dental Association (ADA) and Centers for Disease Control (CDC) recommendations concerning the microbial fouling of dental water suggest that:

> " High speed handpieces should be run to discharge water and air for a minimum of 20 - 30 seconds after use on each patient....Additionally, there is evidence that overnight and weekend microbial accumulation in water lines can be reduced substantially by removing the handpiece and allowing water lines to run and to discharge water for several minutes at the beginning of each clinic day" (CDC, 1993).

However, the beneficial effects of water flushing appear to be highly variable in different reports (Abel *et al.*, 1971; McEntegart and Clark,1973; Gross and Devine, 1976; Gross *et al.*, 1976; Scheid *et al.*, 1982; Mills *et al.*, 1986; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Bierle, 1993; and Williams *et al.*, 1994). In view of the inevitable occurrence of laminar flow through the narrow bore tubing during flushing (Cutnell and Johnson, 1989), there is reason *a priori* to believe that biofilm would not be seriously affected by this procedure. Restoration of high levels of contamination might therefore be expected as soon as there is stasis of water in the line. A typical pattern of water use during routine dental procedures is to flush the lines between patients, at best, and the water in the lines is only flowing for about 2 minutes in 3-5 second periods during a normal dental procedure of 30 minutes duration. This permits bacteria in the biofilm ample time to recontaminate the water in the line's lumen.

In this report we confirm and extend observations on the

occurrence of heavy microbial contamination of DUWL, comparing the bacterial concentrations to those present in a variety of other water sources readily available to the public. Additional observations are presented on the microbiota present in DUWL with the use of specialized microscopic and biochemical tests. We also evaluate the short and long term changes induced by the use of two minute water flushes, in particular examining the bacteriological consequences of undertaking routine procedures on patients. Finally, we provide a comparison of the bacterial contamination levels of water samples taken during a working day, and after overnight stasis of water in the lines.

MATERIALS AND METHODS:

Specimen Collection:

Dental unit water (DUW) samples were collected from the lines of 64 dental operatories, 54 air/water syringes, 22 high speed handpieces, and 13 scalers, from dental practices in the states of California, Oregon, and Washington. Sixty three of the operatories were supplied with municipal water; samples were also collected from one operatory that received only purchased sterile water. Specimens were collected from lines detached from their respective instruments in order to prevent the collection of any contaminants present in the instruments. Samples covered a wide range of possible water delivery circumstances: these included collection after an overnight stasis, at various times during the normal working day of

dental procedures, post 2 minute flush of the lines, at 30 minutes post line-flush, and immediately post patient treatment. All specimens were refrigerated until shipment, and sent by overnight mail carrier packed in blue ice to our laboratory in Michigan State University (MSU)(East Lansing), and evaluated microbiologically as soon as possible upon arrival.

Water samples were also obtained from different domestic and environmental sites in the mid-Michigan area (Delta Township, Lansing, East Lansing, and Okemos), according to The Standard Methods for the Examination of Water and Wastewater (APHA, 1992). Samples came from taps, water coolers, and commodes. Tap water and water cooler samples were collected aseptically after a 2 minute flush of the pipes and processed for water quality analysis within 1 hour.

Environmental samples from creeks, rivers, ponds, and lakes were collected in the states of Washington (Duwamish River, Lake Washington, and Green Lake), Illinois (Lake Michigan), and Michigan (Grand River, Red Cedar River, Meridian Creek, Lake Lansing, and various ponds). All specimens (40-50 mL) were collected aseptically from the edge of each body of water. The samples from Washington were sent on blue ice by overnight carrier, and analyzed upon arrival. The Lake Michigan sample was surface transported chilled, and processed the next day. All mid-Michigan samples were collected and plated for microbial contamination within 1 hour of collection.

Electron Microscopy of DUWL Biofilm:

Sections of two functioning dental unit water lines were cut, plugged with cotton, and sent in a cool and moist environment from dentists' offices in Washington by overnight mail carrier to MSU. Upon arrival, biofilm was scraped from the luminal surface of the water line, fixed with 10% formalin, and processed for transmission electron microscopy according to the following protocol: samples were rinsed several times in 0.1 M phosphate buffer with dextrose, postfixed for 1 hour in buffered 1% osmium tetroxide, rinsed several times, then *en bloc* stained with 2% uranyl acetate solution for 1 hour. After dehydration through a graded alcohol series, the scrapings were given several rinses in propylene oxide, infiltrated overnight in a 1:1 solution of propylene oxide and Polybed/Araldite resin, and then in pure Polybed/Araldite epoxy resin for eight hours.

Five mm cut sections of dental water lines were fixed at 4°C for 1-2 hours in 4% glutaraldehyde buffered with 0.1 M sodium phosphate at a pH of 7.4 in preparation for scanning electron microscopy. Following a brief rinse in buffer, the sections were dehydrated in an ethanol series (25%, 50%, 75%, 95%) for 10 - 15 minutes at each gradation and then with three 10 minute changes in 100% ethanol. They were then critical point dried in a Balzer's critical point dryer using liquid carbon dioxide as the transitional fluid, and mounted on aluminum stubs using adhesive tabs. Specimens were then coated with gold (20 nm thickness) in an Emscope Sputter

Coater model SC 500, purged with argon gas, and examined in a JEOL JSM-35CF scanning electron microscope (Japan Electron Optics Limited).

Evaluation of Water Quality:

Microbiological water quality was assessed by doing heterotrophic plate counts of aerobic bacteria and fungi present in the water (APHA, 1992). Serial log dilutions of samples were made with sterile water and plated onto trypticase soy agar (TSA) plates. Inoculated plates were incubated at room temperature (≈ 25°C) for 96 hours, and the number of colony forming units (cfu) counted in each. For statistical purposes any plates with less than 30 cfu or greater than 300 cfu were considered to have too few or too many to count, respectively. When necessary to reach definitive counts, additional dilutions were made from the refrigerated water samples.

Identification of DUWL Microbiota:

DUW from lines supplied with sterile water from a reservoir bottle were examined for the presence of specific bacterial populations. 200 uL of the water samples were plated on sheep blood agar (SBA) and incubated at 37°C for 72 hours. Isolates were subcultured onto SBA by streak plating and incubated as above in order to determine hemolysis type. Speciation of isolated colonies was performed according to the procedures described by the American Society of Microbiology (ASM), Clinical Microbiology Manual (1989). Organisms were gram stained, grown on differential media, and analyzed with

biochemical tests.

RESULTS:

Heterotrophic bacterial counts in DUW water samples are compared with those of 37 domestic and environmental sources in Figure 1 and Table 1. Water quality assessment revealed the presence of great numbers of microorganisms in the specimens from lines transporting water to air/water syringes, high speed handpieces, and ultrasonic scalers. The scatter plot demonstrates that only 9 of 89 (7 samples from air/water syringes and 2 from scalers) had contamination levels within the accepted potable water standards (EPA, 1989). Potable water is defined by the U.S. Army as any chemically treated water with 300 cfu/mL or less, or any untreated or raw water with a bacterial presence of 500 cfu/mL or less (Simmons and Gentzkow, 1955). New regulations by the Environmental Protection Agency limit heterotrophic bacteria counts to 500 cfu/mL to prevent masking the presence of coliforms in water, and forbids the presence of *Legionella*, *Giardia*, and viruses in potable water (Geldreich, 1986; and EPA, 1989).

There was a sharp contrast between these dental water samples and the domestic water samples from taps, water coolers, and commodes. All, but one, fell within drinking water standards. The exception was a contaminated well, declared unsafe for consumption and condemned by local authorities due to the presence of high numbers of bacteria in the water. The condemned well had 4,400 cfu/mL after flushing

of the pipes for at least 10 minutes. When compared to all dental water samples, only 15 of 89 samples had equal or lower bacterial levels of contamination.

Bacterial concentrations in environmental water varied markedly among samples from rivers, streams, lakes, and ponds. On the whole there were much lower numbers of bacteria present than in samples from dental instruments. The two sources were only comparable at the lower end of the contamination range found in dental unit water samples. Bacteria in rivers and streams ranged from 4,900 cfu/mL in the Grand River (Moore's Park, Lansing, MI) to 125,000 cfu/mL in a stagnant drainage ditch (East Lansing, MI). There was an average bacterial count of 28,000 cfu/mL. Only 30/89 dental water samples had lower numbers of bacteria than the average present in rivers and streams. Also, 44/89 dental samples had higher levels of contamination than the stagnant ditch. Similar results were observed with water specimens from lakes and ponds, Figure 1.

Contamination of dental unit water appeared to fluctuate unpredictably during the working day. Figure 2 demonstrates and compares the levels of contamination in water samples from air water syringe lines and high speed handpiece lines, taken throughout the working day and after an overnight stasis. Bacterial contamination of dental instrument water after overnight stasis, and during the working day appears to be very similar. Contamination of air/water syringes taken throughout the day ranged from less than 30 cfu/mL to 1,265,000 cfu/mL, with an arithmetic mean of 165,000 cfu/mL.

FIGURE 1

Scatter plot showing heterotrophic plate counts in water samples from dental unit water lines and domestic and environmental sources. Bacterial presence in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL. The horizontal marks of the high-low plot mark the value of the data in the following increasing order: mean - 2 standard deviations, mean - 2 standard error, mean, mean + 2 standard error, and mean + 2 standard deviations.

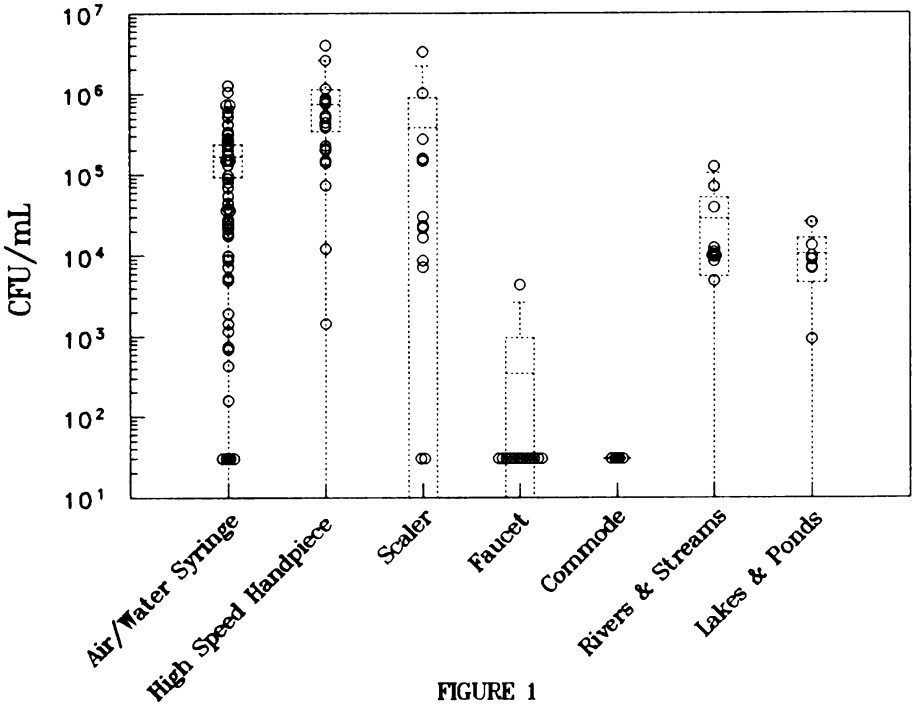
CFU/mL
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
High Speed Handpiece
Scaler
Faucet
Commode
Rivers & Streams
Lakes & Ponds

FIGURE 1

TABLE 1

Concentrations of heterotrophic bacteria in colony forming units per milliliter in water samples from dental unit lines and domestic and environmental sources.

TABLE 1

SOURCE	N	ARITHMETIC MEAN	RANGE
Air/Water Syringe	54	1.65×10^5	(< 30 - 1.26×10^6)
High Speed Handpiece	22	7.39×10^5	(1450 - 4.00×10^6)
Ultrasonic Scalers	13	3.84×10^5	(< 30 - 3.30×10^6)
Faucets	10	3.42×10^2	(< 30 - 4.40×10^3)
Water coolers	4	150	(0 - < 30)
Commode	5	150	(0 - < 30)
Lakes & ponds	7	1.04×10^4	(940 - 2.55×10^4)
Rivers & streams	11	2.82×10^4	(4900 - 1.25×10^5)

FIGURE 2

Scatter plot showing a comparison of heterotrophic plate counts of bacterial contamination of dental unit water samples collected from water lines after overnight stasis and during the working day. Concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL. The horizontal marks of the high-low plot mark the value of the data in the following increasing order: mean - 2 standard deviations, mean - 2 standard error, mean, mean + 2 standard error, and mean + 2 standard deviations.

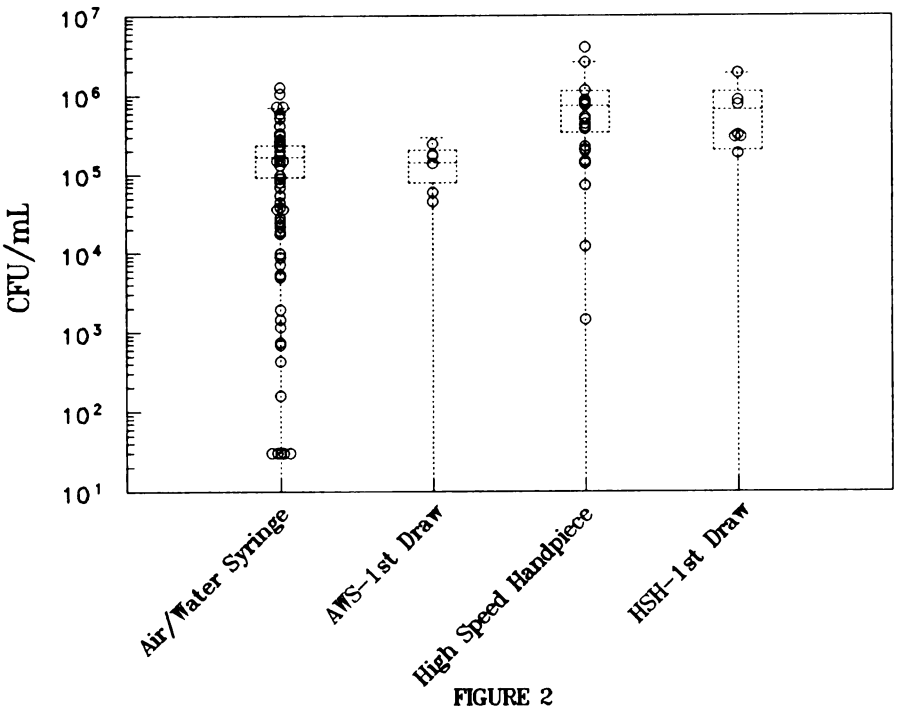
CFU/mL
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
AWS-1st Draw
High Speed Handpiece
HSH-1st Draw

FIGURE 2

After overnight stasis the range was 45,000 - 245,000 cfu/mL with a mean of 140,000 cfu/mL. Water samples from high speed handpieces had higher bacterial concentrations than those from air/water syringes, but the means were similar. Handpiece water samples taken throughout the day ranged from 1,450 - 4,000,000 cfu/mL, with a mean of 738,000 cfu/mL. Contamination of handpiece water after overnight stasis ranged from 185,000 - 1,900,000 cfu/mL with a mean of 662,000 cfu/mL.

Scanning electron micrographs of the luminal aspect of 2 working dental unit water lines revealed the presence of a biofilm with heterogenous microbial populations peculiar to each line embedded in a filamentous matrix. The heterogeneity of the microbiota is indicated by the presence of different sizes and shapes of bacteria observed. Figure 3 shows that the prevalent bacteria in the biofilm matrix of line #1 are cocci and bacilli, while spiral organisms are predominant in the biofilm found in line #2, Figure 4. Examination of water samples from line #2 by light microscopy revealed the presence of twirling spiral microorganisms.

Transmission electron micrographs of the biofilm (Figure 5 and Figure 6) revealed the presence of eukaryotic microorganisms. In Figure 5 an amoebic cyst with its thick cell wall protecting the amoeba inside can be seen. Figure 6 shows the presence of common metazoan nematode structures, like the cuticle and an alimentary tract, showing that nematodes form part of the DUWL biofilm.

Flushing of lines with water for at least 30 seconds is

recommended by CDC/ADA for the elimination of bacteria from the water (CDC, 1993). Figures 7 - 12 represent short and long term effects of 2 minute flushes with water of DUW lines and the presence of bacteria in dental water transmitted by air/water syringes lines and high speed handpieces lines. Figure 7 represents the effect of a 2 minute flush on the bacterial contamination of 20 air/water syringe water lines. Contamination ranged from less than 30 to 1,250,000 cfu/mL, with a mean of 159,000 cfu/mL, prior to flush. Flushing of the water line reduced the bacterial contamination in 16/20 samples to an average bacterial presence of 39,000 cfu/mL. In only, 13/20 samples did the contamination decrease to potable water standards; in contrast 4/20 samples the bacterial concentration in the water did not change or even increased as a result of flushing.

Two minute flushing was also effective in the short run in reducing the bacterial contamination of high speed handpieces, as demonstrated in Figure 8. Bacterial contamination of water delivered by high speed handpieces was reduced from an arithmetic mean of 1,100,000 cfu/mL in 14 samples to mean value of 485,000 cfu/mL, after the 2 minute flush. Although a reduction in the number of bacteria occurred in 13 of 14 samples, only in 4 of the samples did contamination decrease to potable water levels. Flushing caused an increase in the number of bacteria present in samples from 1 of the 14 handpiece lines.

FIGURE 3

Scanning electron micrograph of microbial biofilm on the inner aspect of a dental unit water line from office #1. This line had been in service immediately prior to sampling. The rich heterogeneity of the microbiota is apparent. (x7,800). Bar = 1 um.

FIGURE 4

Scanning electron micrograph of the inner aspect of a dental unit water line #2. This line had also been in service immediately prior to sampling. The microbiota of the biofilm is dominated by spirillar organisms of various sizes. (x11,180). Bar = 1 um.

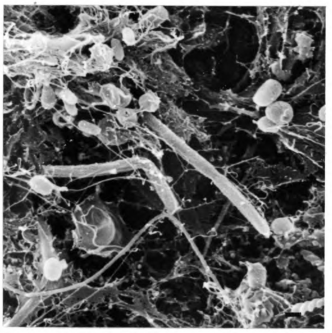

FIGURE 3

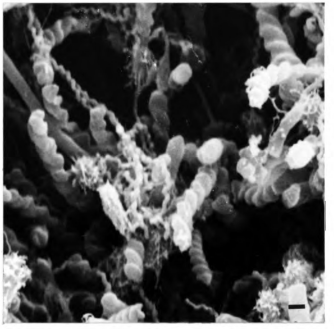

FIGURE 4

FIGURE 5

Transmission electron micrograph of microbial biofilm from a dental unit water line, showing an amoebic cyst. The thick cyst wall (arrowed) surrounds and protects the unicellular organism within. (x11,880). Bar = 1 um.

FIGURE 6

Transmission electron micrograph of microbial biofilm from a dental unit water line, illustrating a cross section of a nematode. The outer cuticle (small arrows) surrounds the body of the worm in which the alimentary tract can be seen (large arrow), with its angular lumen. (x9,690). Bar = 1 um.

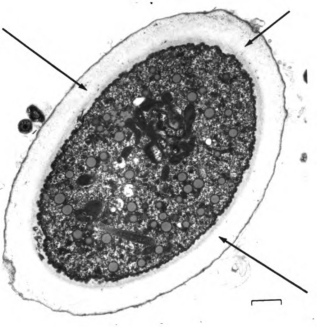

FIGURE 5

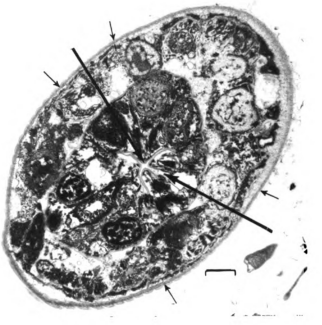

FIGURE 6

The long term effects of flushing were investigated by collecting dental water samples either after a stasis period of 30 minutes, or after a routine dental procedure with a patient. Figure 9 shows bacterial contamination of the water 30 minutes post flush compared to pre-flush levels of contamination. Bacterial concentrations in the water returned to equal or higher than pre-flush levels in 9/15 samples (8/9 air/water syringes and 1/5 handpieces), and were higher than or equal to post 2 minute flush concentrations in 13/15 samples, Figure 10. Contamination of samples taken immediately post-patient is represented in Figure 11. This figure shows that four of 13 samples had higher contamination levels than they had shown pre-flush. Also, in Figure 12 an increase in bacterial contamination levels is observed in 9/13 dental water samples when compared to post two minute flush contamination levels.

The problem of bacterial contamination of instrument water is not limited to units supplied with municipal water. Water samples obtained from a dental unit which has been supplied with sterile water only, revealed the presence of α-hemolytic and ß-hemolytic microorganisms, and the water delivered by the instruments had a bacterial contamination which ranged from 193,000 cfu/mL to 1,050,000 cfu/mL; even after flushes with 0.12% chlorhexidine gluconate. *Staphylococcus warnerii*, *Staphylococcus cohnei*, *Streptococcus mutans*, and *Micrococcus* species were isolated.

FIGURE 7

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected from 20 air/water syringe lines in dental operatories, pre-water flush of syringe line and post 2 minute water flush. Concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

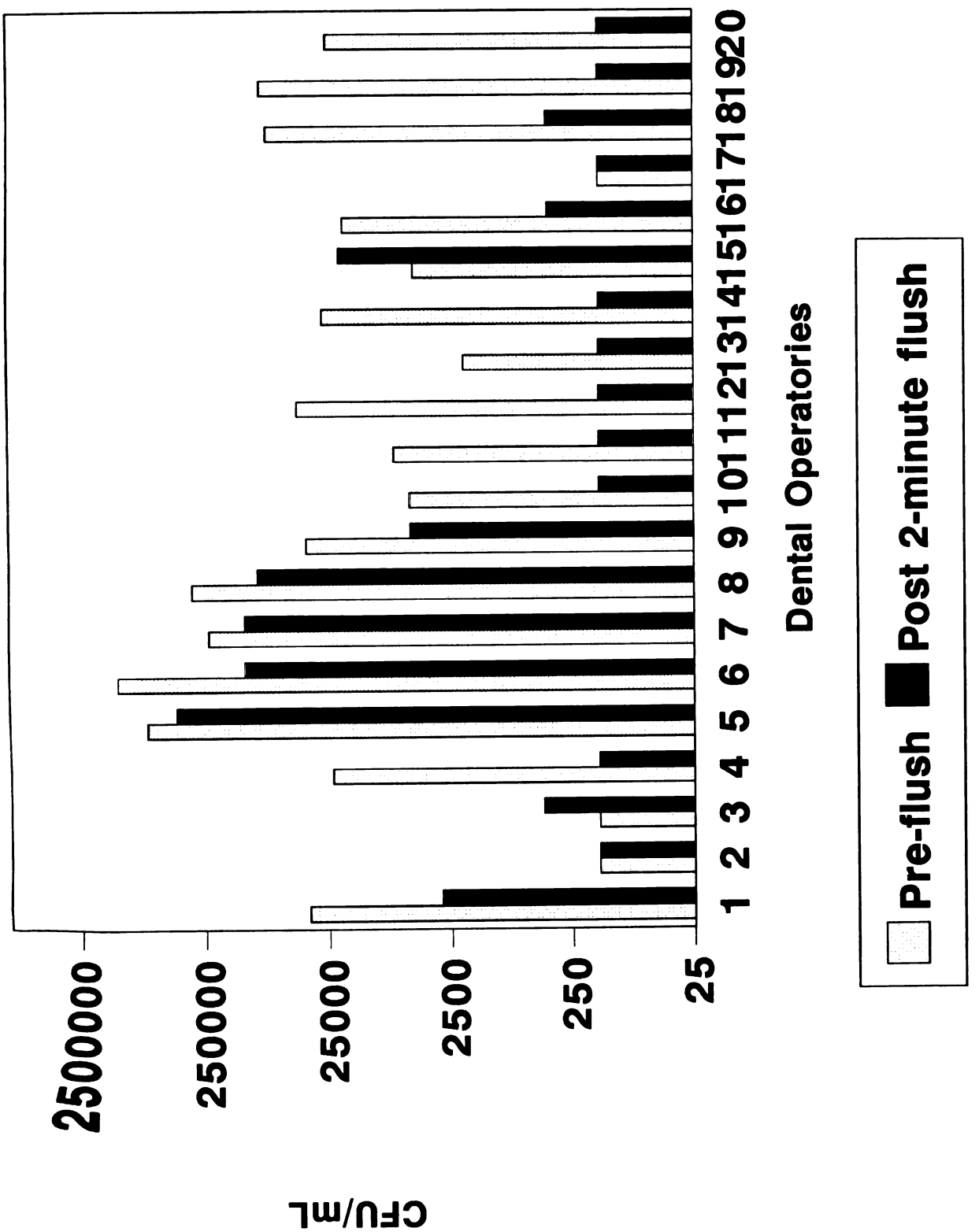
2500000
250000
25000
2500
250
25
CFU/mL
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Dental Operatories
Pre-flush
Post 2-minute flush

FIGURE 7

FIGURE 8

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected from 14 high speed handpiece lines in dental operatories, pre-flush of the handpiece line and post 2 minute water flush. Concentration in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

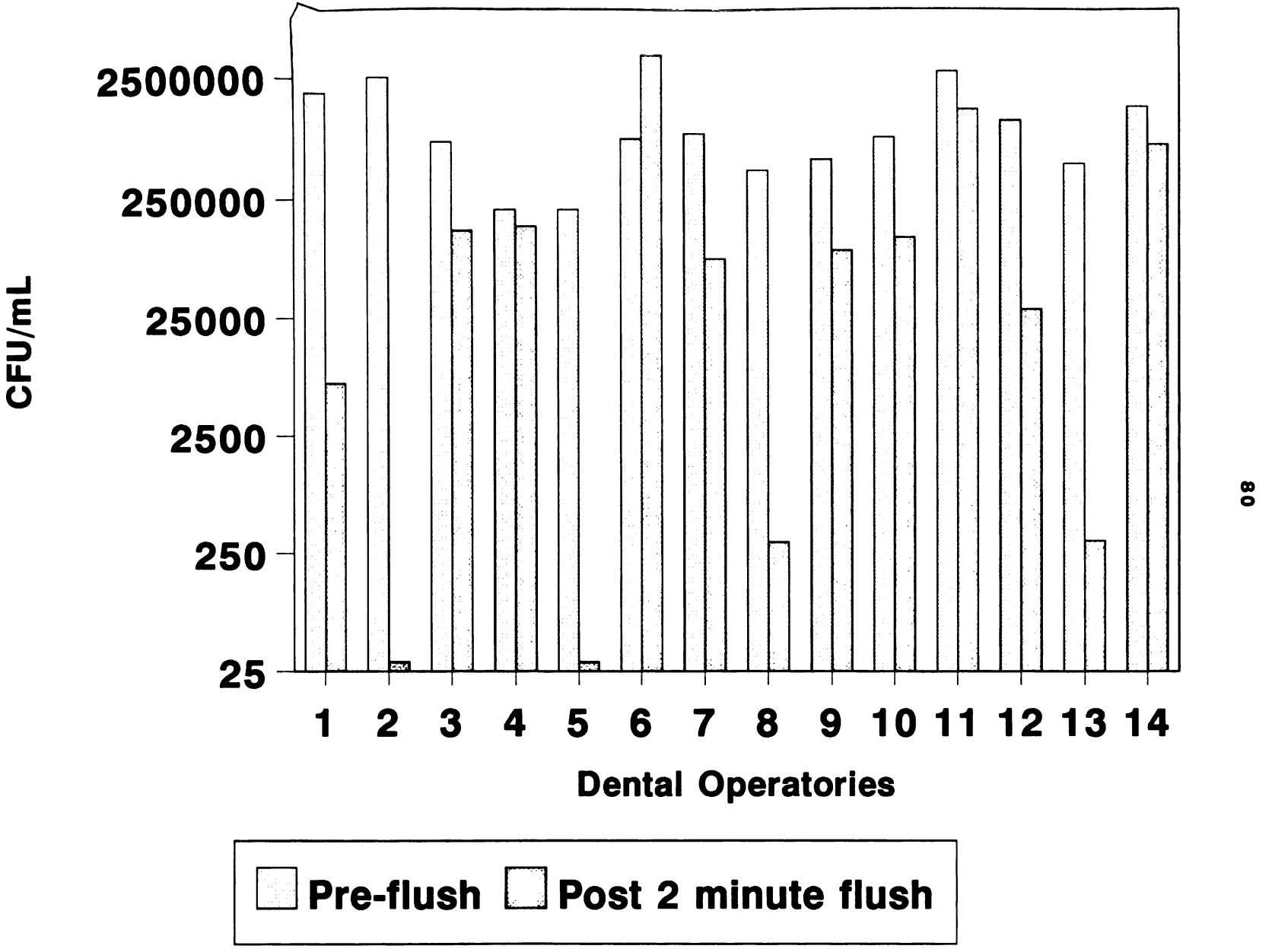
2500000
250000
25000
2500
250
25
CFU/mL
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Dental Operatories
Pre-flush
Post 2 minute flush

FIGURE 8

FIGURE 9

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected pre-flush from dental water lines and 30 minutes after the lines had the 2 minute water flush. Samples 1-9 were from air/water syringe lines and samples 10-15 were from high speed handpiece lines. Concentration in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

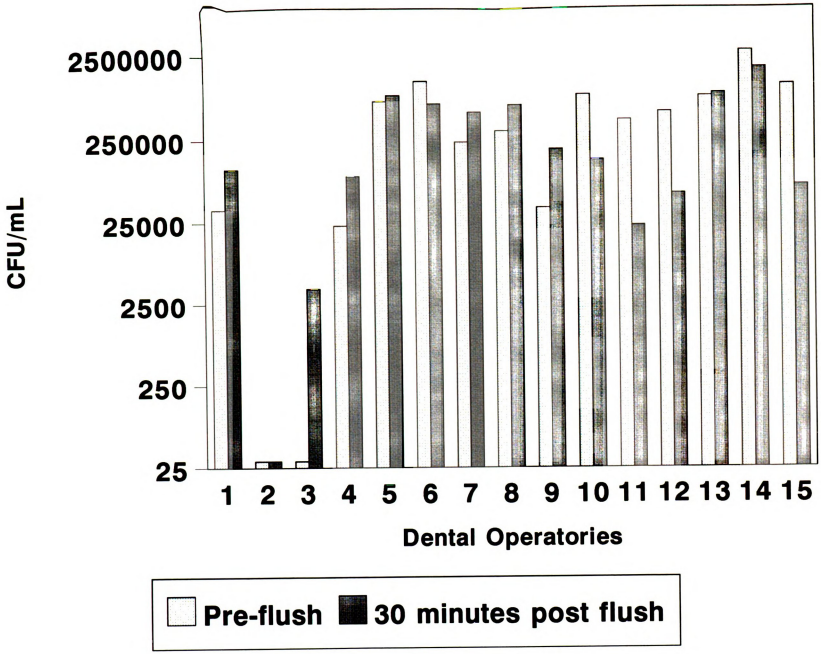
2500000
250000
25000
2500
250
25
CFU/mL
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Dental Operatories
Pre-flush
30 minutes post flush

FIGURE 9

FIGURE 10

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected post 2 minute flush of dental water lines and 30 minutes after flush. Samples 1-9 were from air/water syringe lines and samples 10-15 were from high speed handpiece lines. Bacterial presence in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

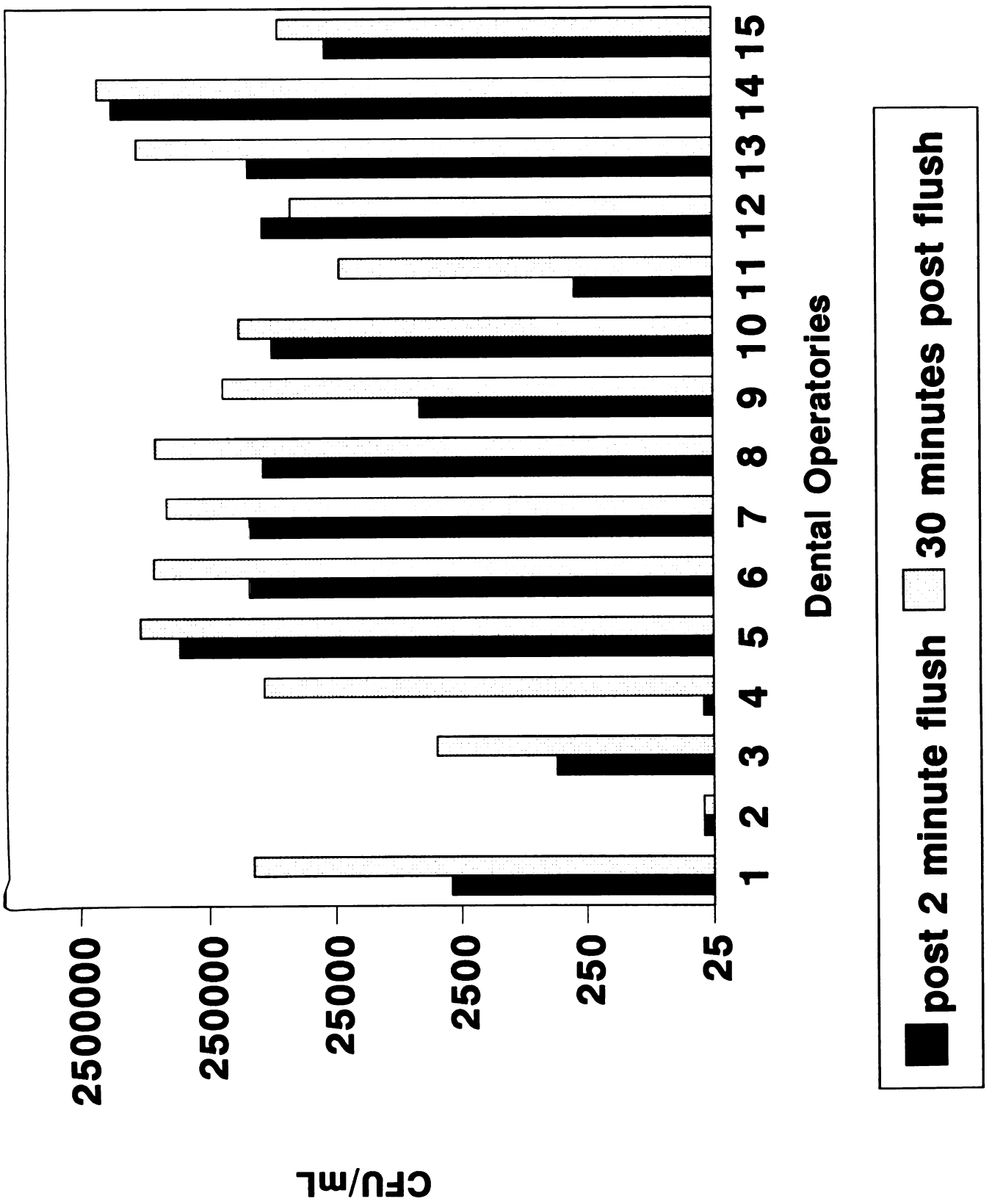
2500000
250000
25000
2500
250
25
CFU/mL
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Dental Operatories
post 2 minute flush
30 minutes post flush

FIGURE 10

FIGURE 11

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected pre-flush from dental water lines and immediately after completion of a routine procedure. Samples 1-7 were from air/water syringe lines and samples 8-13 from high speed handpiece lines. Bacterial presence in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

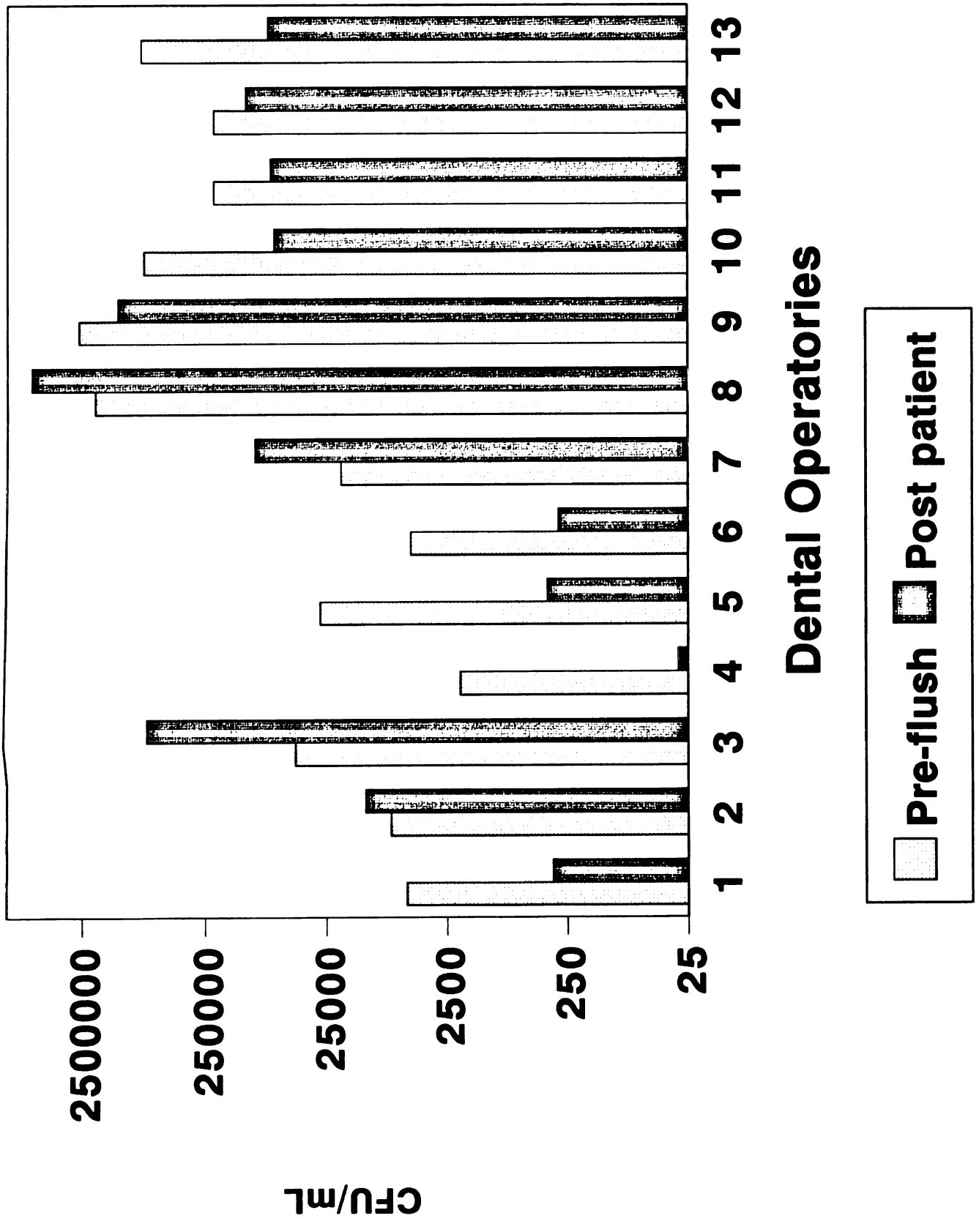
2500000
250000
25000
2500
250
25
CFU/mL
1
2
3
4
5
6
7
8
9
10
11
12
13
Dental Operatories
Pre-flush
Post patient

FIGURE 11

FIGURE 12

Histogram showing heterotrophic plate counts of bacterial contamination of dental unit water samples collected post 2 minute flush from dental water lines and immediately after completion of a routine procedure. Samples 1-7 were from air/water syringe lines and samples 8-13 from high speed handpiece lines. Bacterial presence in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

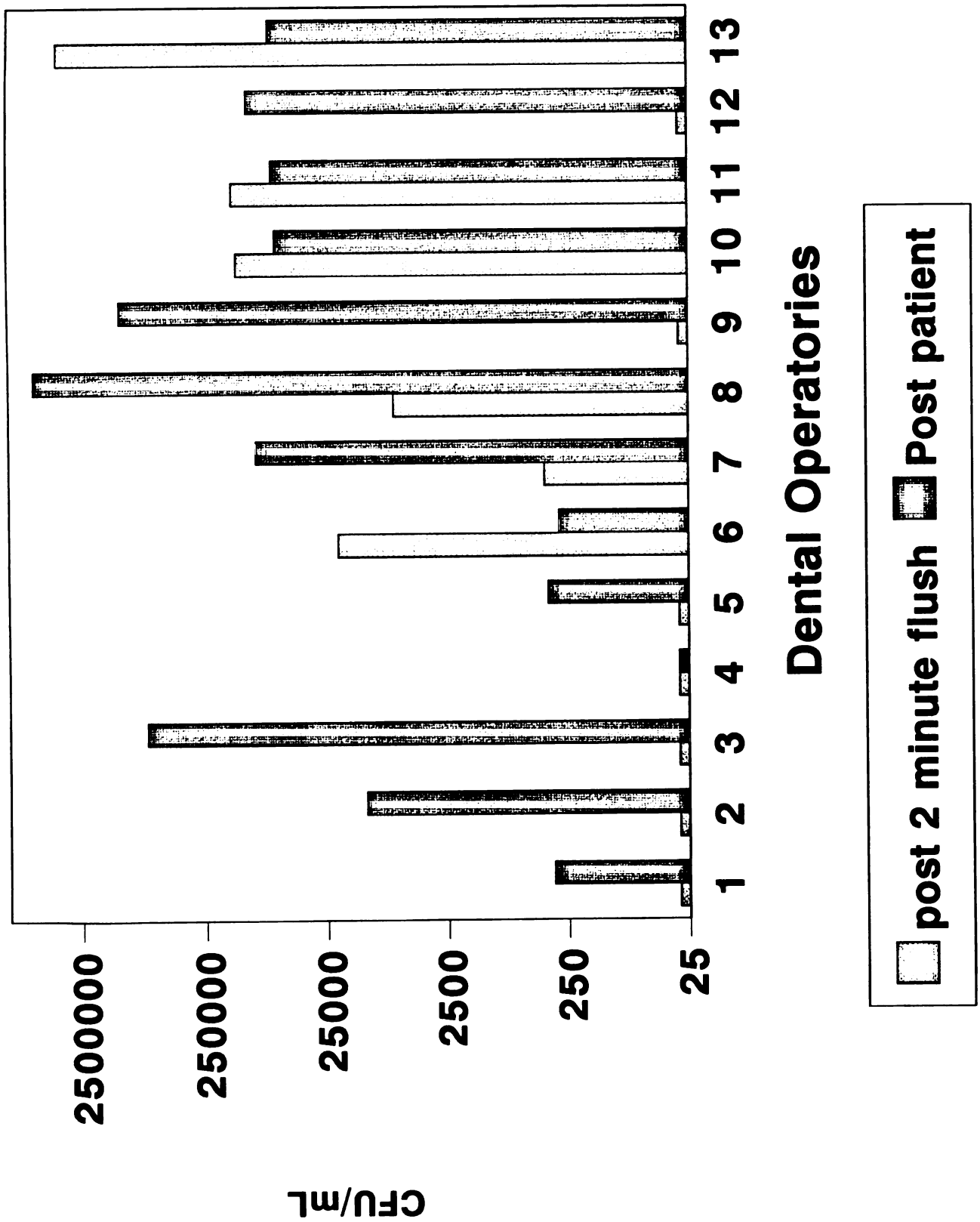
2500000
250000
25000
2500
250
25
CFU/mL
1 2 3 4 5 6 7 8 9 10 11 12 13
Dental Operatories
post 2 minute flush
Post patient

FIGURE 12

DISCUSSION:

The extent of bacterial contamination of dental unit water lines encountered in this study can not be considered unusual; instead, based on a series of recent reports (Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Pankhurst and Philpott-Howard, 1993; J.F. Williams *et al.*, 1993; and H.M. Williams *et al.*, 1994), it appears to be the normal profile to emerge from similar sample analyses. The literature reveals that contamination levels of dental unit water vary, but generally range from less than 30 cfu/mL to 10,000,000 cfu/mL (Able *et al.*, 1971; McEntegart and Clark, 1973; Clark, 1974; Dayoub *et al.*, 1978; Gross and Devine, 1976; Gross *et al.*, 1976; Scheid *et al.*, 1982; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; J.F. Williams *et al.*, 1993; and H.M. Williams *et al.*, 1994). Analysis of readily available domestic and environmental samples served to establish a basis for comparison and to validate dental water analysis methods. Bacterial levels in domestic water samples were consistent with the standards of the United States Safe Drinking Water Act of 1989 (EPA).

Heterotrophic bacterial counts in environmental samples varied widely among specimens, but were within the range for natural bodies of fresh water reported historically by Gainey and Lord (1950) and recently by Armstrong *et al.* (1982), LeChevallier *et al.* (1990) and Rheinheimer (1991). The contrast between contamination levels in dental unit water, and domestic and environmental samples is attributable to the

extraordinary productivity of microbial biofilms in dental unit water lines and the static nature of the of water in DUWL (Kelstrup *et al.*, 1977; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; and Williams *et al.*, 1993).

The biofilm produced in a dental unit is composed of microcolonies embedded in an anionic glycopeptide-glycocalyx matrix which stabilizes microbial communities, helps in the gathering of nutrients acting as a charged net, and protects participants from the effects of biocides and antibacterials by limiting their access to microorganisms embedded in the biofilm (Costerton *et al.*, 1981, 1987; LeChevallier *et al.*, 1988; Fackelman, 1990; van der Wende and Characklis, 1990; Anwar and Costerton, 1992; Cargill *et al.*, 1992; Marshall, 1992; and Mayette, 1992). The production of biofilm within DUWL is not surprising due to the presence of common dental unit water contaminants which are known biofilm "slime" formers, and include opportunistic pathogens like *Pseudomonas* sup. (Peters *et al.*, 1981; Fackelman, 1990; van der Wende and Characklis, 1990; and Nickel *et al.*, 1992) and *Staphylococcus* sup. (Peters *et al.*, 1981; Kluge, 1982; Marrie, *et al.*, 1982; Russell *et al.*, 1987; Anwar and Costerton, 1992; and Marshall, 1992) and pathogens like *Legionella pneumophila* (Cargill *et al.*, 1992; Marshall, 1992; and Wireman *et al.*, 1993).

The presence of pathogenic and opportunistic bacteria in dental water (Williams *et al.*, 1993) raises questions about the health risks to patients and dental personnel exposed to water of this quality, during the more than 200,000,000 dental

procedures which are performed annually (ADA, 1992). Common DUW contaminants are among the organisms which cause severe adult periodontitis (Slots *et al.*, 1988) and have the ability to colonize and cause abscesses in the naso/oral cavity of patients and dental health care professionals (Clark, 1974; and Martin, 1987). The health risk associated with contamination of DUW is increased by the production of contaminated aerosols by dental instruments (Kazantzis, 1961; Madden and Hausler, 1963; Stevens, 1963; Belting *et al.*, 1964; Hausler and Madden, 1964,1966; Micik *et al.*, 1969; Abel *et al.*, 1971; Holbrook *et al.*, 1978; and Earnest and Laesche, 1991) and the presence of bacteria which use aerosol as their infective vector (Hambleton *et al.*, 1983; Macfarlane, 1983; Zuravleff *et al.*, 1983; Midulla *et al.*, 1987; Muder *et al.*, 1988; and CDC, 1990). The danger of the production of aerosols by dental instruments is that the majority of aerosol particles produced fall in the range of 0.5 um to 5 um and these particles can be inhaled and trapped in the lung's alveoli (Hausler and Madden, 1964, 1966; and Micik *et al.*, 1969,1971). The production of contaminated aerosols could be related to the greater propensity of dental students and dental professionals to suffer from respiratory infections (Carter and Seal, 1953; Burton and Miller, 1963; and Mandel, 1993).

Another cause of great concern is the presence of *Legionella* sup. as a common DUW contaminant (Rheinthaler and Mascher, 1986; Oppenheim *et al.*, 1987; Pankhurst *et al.*, 1990,

and Lück *et al.*, 1993) and the high antibody titers to *Legionella* demonstrated in dental health care workers when compared to other health care workers (Fotos *et al.*, 1987; Rheinthaler *et al.*, 1987, 1988; and Lück *et al.*, 1993). The need to determine the hazards presented by pathogenic and opportunistic microorganisms has been stressed even further with the increase of immunocompromised individuals and tuberculosis carriers in the population (Faecher *et al.*, 1993).

Microbial biofilms, such as those revealed by our electron micrographs, are composed of a rich and diverse biota, which not only includes prokaryotic organisms, but also eukaryotes, such as amoebae and nematodes. The presence of protozoan and metazoan organisms in dental water lines has received little attention previously and could be a cause of concern. In a recent report German investigators revealed the presence of amoebae in most dental unit water lines (Michel and Borneff, 1989). Ninety-six of 100 water samples in their study were positive for the presence of amoebae, with almost all of the isolated organisms considered to be species of nonthermophilic *Naegleria* and *Acanthamoeba*; also, two of the water samples contained nematodes (Michel and Borneff, 1989). Even though the presence of amoebae and nematodes in dental water samples is not evidence that these organisms have a direct pathogenic potential, certain amoeba species have been identified as serious water borne agents of disease (Ma *et al.*, 1990; and Martinez *et al.*, 1991). Clarifying the roles

of amoebae as host cells for other pathogenic organisms such as *Legionella* and coliforms (Newsome *et al.*, 1985; Barbaree *et al.*, 1986; Rowbotham, 1986; King *et al.*, 1988; and Fields *et al.*, 1993) (20 of the operatories in the German study were *Legionella* positive (Michel and Borneff 1989), as a protective shell against biocides and antibacterials (King *et al.*, 1988; and Kuchta *et al.*, 1993) or as sources of sensitizing allergens, as suspected in the case of Pontiac Fever (Rowbotham, 1986), or as possible infective vectors for *Legionella* (O'Brien and Bhopal, 1993), requires much further work.

Our study on bacterial contamination of DUW after overnight stasis in the dental unit lines and during a normal working day has revealed that contamination levels between the samples are very similar. The belief that bacterial contamination of dental unit water decreases during a working day and increases during overnight stasis (CDC, 1993), is not supported by our data. The concentration of bacteria present in dental water fluctuates during the day in an unpredictable manner, with the number of bacteria in the water increasing or decreasing markedly; similar reports have been made by Tippett *et al.* in 1988 and H.M. Williams *et al.* in 1994. These fluctuations in the number of bacteria in dental water are in accord with the wide range of heterotrophic bacterial counts remarked upon in several previous reports (Abel *et al.*, 1971; McEntegart and Clark, 1973; Clark, 1974; Gross and Devine, 1976; Gross *et al.*, 1976; Dayoub *et al.*, 1978; Scheid *et al.*,

1982; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; J.F. Williams *et al.*, 1993; and H.M. Williams *et al.*, 1994), which suggest that the dynamics of bacterial contamination in water is a complex issue related to the number of bacteria present in the water supplied to the unit, the biofilm's productivity and release of microorganisms into the water, and the duration of periods of stasis during the day (during dental procedures) and night. The complexity of dental water contamination during a working day makes this area worthy of a more detailed study.

Certainly, the current popular belief in the effectiveness of flushing of the dental unit water lines as a suitable corrective measure to ensure lower levels of contamination in the water (CDC, 1993) is not sustained by our findings. The transient reductions in the number of bacteria after flushing seen here and in other reports (Abel *et al.*, 1971; McEntegart and Clark, 1973; Gross and Devine, 1976; Gross *et al.*, 1976; Scheid *et al.*, 1982; Mills *et al.*, 1986; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991) were sometimes not remarkable, with increases in bacterial numbers also observed as a result of flushing. The long term effect of flushing was inefficient in eliminating bacteria in the water, regardless of whether or not the line had remained inactive or the operatory had been put into routine use after flushing. In view of the transient reduction in bacterial number (Mayo *et al.*, 1990; and Whitehouse *et al.*, 1991), the inability to eliminate bacteria from water after extended periods of

flushing (Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Bierle, 1993; and Williams *et al.*, 1994), and the rapid recontamination of water afterward, reported by Mayo *et al.* (1990) and Whitehouse *et al.* (1991), our results should have been expected.

Increases in bacterial concentrations in water after flushing, rather than decreases, support the view that the flushing process has a variable effect on the presence of planktonic bacteria. Flushing does not take into account the ongoing release of bacteria into stagnant water by the biofilm, or the release of biofilm fragments with flushing; this may cause consequences opposite to those desired. Sudden increases in microbial numbers may come about during or after flushing as a result of sloughing of segments of biofilm into the lumen. Sloughing of biofilm in DUWL may be enhanced by stretching and contraction of the plastic tubing wall as pressure is applied to and removed from the water column. It may also be caused by the pulling and stretching motions of the tubing during routine use by the practitioner. Also, abrupt hydrodynamic changes may occur, especially at points where luminal diameters change, leading to turbulence and disruption of the biofilm layer, dislodging and dispensing it as slime fragments. Microscopic examination of visible floccules in water samples we received confirmed that these were heterogenous bacteria-rich clumps in an amorphous matrix, typical characteristics of biofilm.

The unpredictable nature of bacterial removal from water

by flushing should not be surprising due to physical properties of water flow in tubes. Flow occurs at different velocities due to the mechanism of laminar flow (Cutnell and Johnson, 1989). The velocities of water flowing in a tube are illustrated in Figure 13; water velocity decreases from the center water lamina of the tube towards the edge of the tube, where the velocity is zero. This results in the elimination of planktonic bacteria depending on their location in the water laminae, with the central core lamina being free of bacteria, while the other laminae show an increasing amount of bacteria with their proximity to the biofilm. Since the lamina of water above the surface of the biofilm is static and does not have any effect in the biofilm, the interface between water and biofilm can be heavily populated with bacteria and serve as a recontaminating reservoir to overlying water laminae in the tube.

The transient nature of reduced bacterial contamination levels after flushing and the rapid increase in bacterial numbers in water samples obtained after 30 minutes of stasis or after a patient procedure, raise questions about the nature of the bacterial populations present in the water in each case. If the populations are different this could reflect a contribution of the patients' oral biota to that present in the dental line, as a result of retrograde flow of fluids (Bagga *et al.*, 1984; Miller and Palenik, 1985; ADA, 1988; Crawford and Broderius, 1988, 1990; Anonymous - Lancet, 1992; Lewis and Boe, 1992; Lewis *et al.*, 1992; Faecher *et al.*, 1993;

and Mills *et al.*, 1993). Investigators have reported the contamination of dental water with oral bacteria (Abel *et al.*, 1971; Holbrook *et al.*, 1978; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Whitehouse *et al.*, 1991; and Williams *et al.*, 1993) and we isolated hemolytic *Staphylococcus*, *Streptococcus*, and *Micrococcus*, common inhabitants of dental plaque, the oral cavity, and skin of humans (Hardie, 1986; and Kloos and Heinz-Scheifer, 1986), from lines which had never received anything but commercial sterile water. Organisms of this type most likely arrived from a human source, and could be a consequence of inadequate control of retraction. This information, coupled with the ability of staphylococci to produce biofilms on other medical devices by adhering and spreading rapidly along cell walls of catheters or drainage tubes (Peters *et al.*, 1981; Russell *et al.*, 1987; Anwar and Costerton, 1992; Marshall, 1992; and Passerini *et al.*, 1992), permit us to conclude that oral bacteria can produce biofilms in dental units dispensing sterile water. Standard anti-retraction measures, even with fully functional valves, can result in the reflux of small volumes of fluid which could be heavily laden with bacteria, reportedly up to 54,100 bacteria in 900 uL (Bagga *et al.*, 1984). Recent publications have stressed the need to pay more attention to the phenomenon of fluid retraction of water in dental units (Christensen, 1991; Anonymous -Lancet, 1992; Lewis and Boe, 1992; Lewis *et al.*, 1992; and Faecher *et al.*, 1993).

How all these microbiological and hydrodynamic factors

FIGURE 13

Diagrammatic representation of laminar flow of water in a dental unit line. The velocity of the lamina over the biofilm is zero. Water in the lamina at the core of the flow moves at a higher rate.

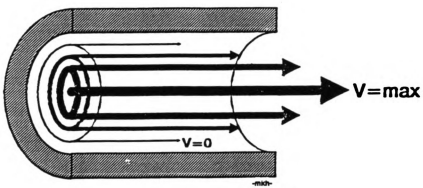

FIGURE 13

influence the establishment and turnover of bacterial and protozoan populations in DUWL is a complex question that deserves further experimental exploration. Alterations in delivery systems materials and design may eventually be introduced which discourage or limit the amount of biofilm accumulation in DUWL. In the meantime, barrier microfiltration of irrigation and coolant water at the point-of-use is the most appropriate means of ensuring the elimination of contaminants derived from biofilm. This principle is well established in other branches of medicine and health care. In combination with the introduction of disposable in-line checkvalves to overcome the problem of retraction of patient-derived microorganisms, it should be possible to maintain a constant flow of sterile water to dental handpieces and other devices through DUWL.

SUMMARY AND CONCLUSIONS:

The extensive microbial contamination of dental unit water seen in this study was consistent with previous reports. Comparisons with other potable water sources commonly available to the public emphasize the relatively high concentrations of microorganisms in dental unit water, and the low levels of bacteria in most domestic water samples. Microscopic evidence of the presence of bacteria, amoebae and nematodes in dental unit water points up the need for further qualitative and quantitative studies of these components of dental tubing biofilm, as well as the health risks they may

pose to dental personnel, patients, and immunocompromised individuals who visit a dental office.

Our data confirmed only the short term value of two minute flushes to diminish microbial dental unit water burdens. However, these findings were offset by several additional observations. First, increases in the bacterial concentrations, rather than decreases followed flushing on some occasions. Second, declines in bacterial numbers, when they occurred, were often trivial, and microbial contamination of water was frequently restored to pre-flush levels or higher after 30 minutes of stasis or after use of the water line in a routine dental procedure.

The long term ineffectiveness of flushing is understandable when the hydrodynamics of laminar flow of water in narrow bore tubing are taken into account. Increase of bacterial concentrations may also be attributable to sloughing of biofilm from the tubing wall as a result of stretching and movement of the line in routine manipulations. These two phenomena may undermine the utility of routine water flushes and result in the transience of any benefit from the procedure.

Variations in the bacterial contamination during the day overshadow the influence of overnight stasis, contrary to commonly held beliefs. The presence of hemolytic *Staphylococcus* and *Streptococcus* in water samples from lines that were supplied only from sterile water reservoirs, adds to the growing evidence that part of the microbiota in the dental

unit water line is derived from the oral cavity of patients. We conclude that additional prophylactic measures to limit bacterial contamination in dental unit water need to be implemented based on standard antimicrobial, physical, or chemical disinfection/sterilization principles.

Personnel:

José I. Santiago is a graduate assistant in Microbiology at Michigan State University (MSU). Jeffrey F. Williams is a Professor of Microbiology at MSU.

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LIST OF REFERENCES

ARTICLE # 1

LIST OF REFERENCES:

Abel, L.C., Miller, R.L., Micik, R.E., and Ryge, G.
Studies of dental aerobiology: IV. Bacterial contamination of water delivered by dental units.
J. Dent. Res., 1971, 50:1567-1569.

American Dental Association.
Bureau of Economic and Behavioral Research.
1990 Survey of dental services rendered.
Chicago, Il:pubs. American Dental Association; 1992.

American Dental Association Reports.
Dental units and water retraction.
J. Am. Dent. Assoc., 1988, 116:417-420.

American Public Health Association.
Standard Methods for the Examination of Water and Wastewater.
18th Edition.,
Washington, D.C.:pubs. APHA. 1992.

American Society of Microbiology.
Clinical Microbiology Manual.
Washington, D.C.:pubs. ASM Publications; 1989.

Anonymous.
Risk of HIV transmission during dental treatment.
Lancet, 1992, 340:1259-1260.

Anwar, H. and Costerton, J.W.
Effective use of antibiotics in the treatment of biofilm-associated infections.
Am. Soc. Microbiol. - News, 1992, 58:665-668.

Armstrong, J.L., Calamoris, J.J., and Seidler, R.J.
Selection of antibiotic-resistant standard plate count bacteria during water treatment.
Appl. Environ. Microbiol., 1982, 44:308-316.

Bagga, B.S.R., Murphy, R.A., Anderson, A.W., and Punwami, I.
Contamination of dental unit cooling water with oral microorganisms and its prevention.
J. Am. Dent. Assoc., 1984, 109:712-716.

Barbaree, J.M., Fields, B.S., Feeley, J.C., Gorman, G.W., and Martin, W.T.
Isolation of protozoa from water associated with a legionellosis outbreak and demonstration of intracellular multiplication of *Legionella pneumophila*.
Appl. Environ. Microbiol., 1986, 51:422-424.

Belting, C.M., Haberfelde, G.C., and Juhl, L.K.
Spread of organisms from a dental air rotor.
J. Am. Dent. Assoc., 1964, 68:648-651.

Bierle, J.W.
Dental operatory water lines.
J. Calif. Dent. Assoc., 1993, 21:13-15.

Burton, W.E. and Miller, R.L.
The role of aerobiology in dentistry.
Proc. First Int. Symp. Aerobiol., 1963, pp. 87-94.

Cargill, K.L., Pyle, B.H., Sauer, R.L., and McFeters, G.A.
Effects of culture conditions and biofilm formation on the iodine susceptibility of *Legionella pneumophila*.
Can. J. Microbiol., 1992, 38:423-429.

Carter, W.J. and Seal, J.R.
Upper respiratory infections in dental personnel and their patients.
Research project report NM 005 251 1403,
Naval Medical Research Unit no. 4.
Administrative Command,
US Naval Training Center, Great Lakes, IL.,
February 25, 1953.

Centers for disease control.
Legionnaire's disease outbreak associated with a grocery store mist machine - Louisiana 1989.
Morb. Mortal. Weekly Rep., 1990, 39:108-110.

Centers for disease control.
Recommended Infection Control Practices for Dentistry.
Morb. Mortal. Weekly Rep., 1993, 42:1-12.

Characklis, W.G. and Cooksey, K.E.
Biofilms and microbial fouling.
Adv. Appl. Microbiol., 1983, 29:93-138.

Christensen, G.J.
Infection control:Some significant loopholes.
J.Am. Dent. Assoc., 1991, 122:99-100.

Clark, A.
Bacterial colonization of dental units and the nasal flora of dental personnel.
Proc. Roy. Soc. Med., 1974, 67:1269-1270.

Costerton, J.W., Geesey, G.G., and Cheng, K.J.
How bacteria stick.
Scientific American, 1978, 238:86-95.

Costerton, J.W., Irvin, R.J., and Cheng, K.J.
The bacterial glycocalyx in nature and disease.
Ann. Rev. Microbiol., 1981, 35:299-324.

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M., and Marrie, T.J.
Bacterial biofilms in nature and disease.
Ann. Rev. Microbiol., 1987, 41:435-464.

Crawford, J.J. and Broderius, C.
Control of cross infection in the dental operatory:prevention of water retraction by bur cooling spray systems.
J. Am. Dent. Assoc., 1988, 116:685-687.

Crawford, J.J. and Broderius, C.
Evaluation of a dental unit design to prevent retraction of oral fluids.
Quintessence Int., 1990, 21:47-51.

Cutnell, J.D. and Johnson, K.W.
Fluid Dynamics. pp 285-287.
In:Physics.
New York:pubs. John Wiley; 1989

Dayoub, M.B., Rusilko, D.J., and Gross, A.
A method of decontamination of ultrasonic scalers and high speed handpieces.
J. Periodont., 1978, 49:261-265.

Earnest, R. and Laesche, W.
Measuring harmful levels of bacteria in aerosols.
J. Am. Dent. Assoc., 1991, 122:55-57.

Environmental Protection Agency.
Drinking water:national primary drinking water regulations; filtration, disinfection; turbidity, *Giardia lamblia*, virus, *Legionella*, and heterotrophic bacteria.
Fed. Reg., 1989, 54:27486-27540.

Fackelmann, K.A.
Pipe-dwelling bacteria use slimy strategy.
Sci. News, 1990, 137:6.

Faecher, R.S., Thomas, J.E., and Bender, B.S.
Tuberculosis: A growing concern for dentistry.
J. Am. Dent. Assoc., 1993, 124:94-104.

Fields, B.S., Utley-Fields, S.R., Loy, J.N.C., White, E.H., Steffens, W.L., and Shotts, E.B.
Attachment and entry of *Legionella pneumophila* in *Hartmannella vermiformis*.
J. Infect. Dis., 1993, 167:1146-1150.

Fitzgibbon, E.J., Bartzokas, C.A., Martin, M.V., Gibson, M.F., and Graham, R.
The source frequency and extent of bacterial contamination of dental unit water systems.
Brit. Dent. J., 1984, 157:98-101.

Fotos, P.G., Westfall, H.N., Snyder, I.S., Miller, R.W., and Mutchler, B.M.
Prevalence of *Legionella* - specific IgG and IgM antibodies in a dental clinic population.
J. Dent. Res., 1985, 64:1382-1385.

Gainey, P.L. and Lord, T.H.
The number of bacteria in water. pp 80-87.
In:An introduction to the microbiology of water and sewage.
Minneapolis MN:pubs. Burgess; 1950.

Geldreich, E.E.
Potable water:New directions in microbial regulations.
Am. Soc. Microbiol. - News, 1986, 52:530-534.

Gristina, A.G. and Costerton, J.W.
Bacteria-laden biofilms - a hazard to orthopedic protheses.
Infect. Surg., 1984, 3:655-662.

Gristina, A.G., Dobbins, J.J., Giammara, B., Lewis, J.C., and DeVries, W.C.
Biofilm development in artificial heart and tissue damage.
J. Am. Med. Assoc., 1988, 259:870-874.

Gross, A. and Devine, J.D.
Microbial contamination of dental units and ultrasonic scalers.
J. Dent. Res., 1976, 55B:B276 - abstract # 856.

Gross, A., Devine, M., and Cutright, D.E.
Microbial contamination of dental units and ultrasonic scalers.
J. Periodont., 1976, 47:670-673.

Hambleton, P., Broster, M.G., Dennis, P.J., Henstridge, R., Fitzgeorge, R., and Conlan, J.W.
Survival of virulent *Legionella pneumophila* in aerosols.
J. Hyg., 1983, 90:451-460.

Hardie, J.M.
Oral streptococci. pp. 1054-1063.
In: Bergey's Manual of Systemic Bacteriology. vol. 2.
Sneath, P.H.A., Mair, N.S., Sharpe, M.E., and Holt, J.G., eds.
Baltimore MD:pubs. Williams and Wilkins; 1986.

Hausler, W.J., Jr. and Madden, R.N.
Aerobiology of dental handpieces.
J. Dent. Res., 1964, 43:903 - abstract # 378.

Hausler, W.J., Jr. and Madden, R.N.
Microbiologic comparison of dental handpieces. 2. Aerosol decay and dispersion.
J. Dent. Res., 1966, 45:52-58.

Holbrook, W.P., Muir, K.F., Macphee, I.P., and Ross, P.W.
Bacteriological investigation of the aerosol from ultrasonic scalers.
Brit. Dent. J., 1978, 144:245-247.

Kazantzis, G.
Air contamination from high speed drills.
Proc. Roy. Soc. Med., 1961, 54:242-244.

Kelstrup, J., Funder-Nielsen, T.D., and Theilade, J.
Microbial aggregate contamination of water lines in dental equipment and its control.
Acta Path. Microbiol. Scand., 1977, 85:177-183.

King, C.H., Shotts, E.B., Wooley, R.E., and Porter, K.G.
Survival of coliforms and bacterial pathogens within protozoa during chlorination.
Appl. Environ. Microbiol., 1988, 54:3023-3033.

Kloos, W.E. and Heinz-Scheiffer, K.
Staphylococcus. pp. 1013-1035.
In:Bergey's Manual of Systemic Bacteriology. vol. 2.
Sneath, P.H.A., Mair, N.S., Sharpe, M.E., and Holt, J.G., eds.
Baltimore MD:pubs. Williams and Wilkins; 1986.

Kluge, R.M.
Infection of prosthetic cardiac valves and arterial grafts.
Heart Lung, 1982, 11:146-151.

Kuchta, J.M., Navratil, J.S., Wadowsky, R.M., Dowling, J.N., States, S.J., and Yee, R.B.
Effect of chlorine on the survival and growth of *Legionella pneumophila* and *Hartmanella vermiformis*. pp. 242-245.
In:*Legionella* - Current status and emerging perspectives.
Barbaree, J.M., Breiman, R.F., and Dufour, A.D., eds.
Washington DC:pubs. American Society of Microbiology; 1993.

LeChevalier, M.W., Cawthon, C.D., and Lee, R.G.
Factors promoting survival of bacteria in chlorinated water supplies.
Appl. Environ. Microbiol., 1988, 54:649-654.

LeChevalier, M.W., Seidler, R.J., and Evans, T.M.
Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies.
Appl. Environ. Microbiol., 1990, 40:922-930.

Lewis, D.L., Arens, M., Appleton, S.S., Nakshima, K., Ryu, J., Boe, R.K., Patrick, J.B., Watanabe, D.T., and Suzuki, M.
Cross-contamination potential with dental equipment.
Lancet, 1992, 340:1252-1254.

Lewis, D.L. and Boe, R.K.
Cross-infection risks associated with current procedures for using high speed dental handpieces.
J. Clin. Microbiol., 1992, 30:401-406.

Lück, P.C., Bender, L., Ott, M., Helbig, J.H., Witzleb, W., and Hacker, J.
Analysis of *Legionella pneumophila* serogroup 6 strains isolated from dental units. pp. 240-242.
In:*Legionella* - Current status and emerging perspectives.
Barbaree, J.M., Breiman, R.F., and Dufour, A.D., eds.
Washington DC:pubs. American Society of Microbiology; 1993.

Ma, P., Vivesvara, G.S., Martinez, A.J., Theodore, F.H., Dagget, P.M., and Swayer, T.K.
Naegleria and *Acanthamoeba* infections.
Rev. Inf. Dis., 1990, 12:490-514.

Macfarlane, J.T.
Legionnaire's disease:update.
Brit. Med. J., 1983, 287:443-444.

Madden, R.M. and Hausler, W.J., Jr.
Microbiological comparison of dental handpieces. 1. Preliminary report.
J. Dent. Res., 1963, 42:1146-1151.

Mandel, I.D.
Occupational risks in dentistry:comforts and concerns.
J. Am. Dent. Assoc., 1993, 124:41-49.

Marrie, T.J. and Costerton, J.W.
A scanning and electron microscopic study of an endocardial pacemaker lead.
Circulation, 1982, 66:1334-1343.

Marshall, K.C.
Biofilm:An overview of bacterial adhesion, activity, and control at surfaces.
Am. Soc. Microbiol. - News, 1992, 58:202-207.

Martin, M.W.
The significance of the bacterial contamination of dental unit water systems.
Brit. Dent. J., 1987, 163:152-154.

Martinez, A.J. and Vivesvara, G.S.
Laboratory diagnosis of pathogenic free-living amoebas: *Naegleria*, *Acanthamoeba*, and *Leptomyxid*.
Clin. Lab. Med., 1991, 11:861-872.

Mayette, D.C.
The existence and significance of biofilm in water.
Water Rev., 1992, 10:1-3.

Mayo, J.E., Oertling, K.M., and Andrieu, S.
Bacterial biofilm:A source of contamination in dental air-water syringes.
Clin. Prev. Dent., 1990, 12:13-20.

McEntegart, M.G. and Clark, A.
Colonization of dental units by water bacteria.
Brit. Dent. J., 1973, 134:140-142.

Michel, R. and Borneff, M.
Significance of amoeba and other protozoa in water conduit systems of dental units.
Zbl. Bakt. Hyg., 1989, 187:312-323.

Micik, R.E., Miller, R.L., Manzarella, H.A., and Ryge, G.
Studies in dental aerobiology:I. Bacterial aerosols generated during dental procedures.
J. Dent. Res., 1969, 48:49-56.

Micik, R.E., Miller, R.L., and Leong, A.C.
Studies in dental aerobiology:III. Efficacy of surgical masks in protecting dental personnel from airborne bacterial particles.
J. Dent. Res., 1971, 50:626-630.

Midulla, M., Sollecito, D., Feleppa, F., Assensio, A.M., and Ilari, S.
Infection by airborne *Chlamydya trachomatis* in a dentist cured with rifampin after failures with tetracycline and doxycycline.
Brit. Med. J., 1987, 294:742.

Miller, C.H. and Palenik, C.J.
Microbial contamination of dental water lines.
Dent. Aesepsis Rev., 1985, 5:1-2.

Mills, S.E., Lauderdale, P.W., and Mayhew, R.B.
Reduction of microbial contamination in dental units with povidone-iodine 10%.
J. Am. Dent. Assoc., 1986, 113:280-284.

Mills, S.E., Kuehne, J.C., and Bradley, D.V., Jr.
Bacteriological analysis of high-speed handpiece turbines.
J. Am. Dent. Assoc., 1993, 124:59-62.

Molinary, J.D. and Crawford, J.J.
Evaluation of an independent water reservoir system for high speed instrumentation.
J. Dent. Res., 1976, 55B:B245, abst. #855.

Muder, R.R., Yu, V.L., and Woo, A.H.
Mode of transmission of *Legionella pneumophila*. A critical review.
Arch. Intern. Med., 1986, 146:1607-1612.

Newsome, A.L., Baker, R.L., Miller, R.D., and Arnold, R.R.
Interactions between *Naegleria fowleri* and *Legionella pneumophila*.
Infect. Immun., 1985, 50:449-452.

Nickel, J.C., Downey, J., and Costerton, J.W.
Movement of *Pseudomonas aeruginosa* along catheter surfaces - a mechanism in pathogenesis of catheter-associated infection.
Urology, 1992, 39:93-98.

O'Brien, S.J. and Bhopal, R.J.
Legionnaires' disease:the infective dose paradox.
Lancet, 1993, 342:5-6.

Oppenheim, B.A., Sefton, A.M., Gill, O.N., Tyler, J.E., O'Mahony, M.C., Richards, J.M., Dennis, P.J.L., and Harrison, T.G.
Widespread *Legionella pneumophila* contamination of dental stations in a dental school without apparent human infection.
Epidem. Infect., 1987, 99:159-166.

Pankhurst, C.L., Philpott-Howard, J.N., Hewitt, J.H., and Casewell, M.W.
The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems.
J. Hosp. Infec., 1990, 16:9-18.

Pankhurst, C.L. and Philpott-Howard, J.N.
The microbiological quality of water in dental chair units.
J. Hosp. Infect., 1993, 23:167-174.

Passerini, L., Lam, K., Costerton, J.W., and Garner-King, E.
Biofilms on indwelling vascular catheters.
Critic. Care Med., 1992, 20:665-673.

Payment, P., Franco, E., Richardson, L., and Siemiatycki, J.
Gastrointestinal health effects associated with the consumption of drinking water produced by point-of-use domestic reverse-osmosis filtration units.
Appl. Environ. Microbiol., 1991, 57:945-948.

Peters, G., Locci, R., and Pulverer, G.
Microbial colonization of prosthetic devices, II. Scanning electron microscopy of naturally infected intravenous catheters.
Zbl. Bakt. Mikrobiol. Hyg., 1981, 173:293-299.

Reinthaler, F. and Mascher, F.
Legionella pneumophila in dental units.
Zbl. Bakt. Hyg. B., 1986, 183:86-88.

Reinthaler, F., Mascher, F., and Stünznerd, D.
Legionella pneumophila:Seroepidemiologic studies of dentists and dental personnel in Austria.
Zbl. Bakt. Hyg. B., 1987, 185:164-170.

Reinthaler, F., Mascher, F., and Stünzner, D.
Serologic examination for antibodies against *Legionella* species in dental personnel.
J. Dent. Res., 1988, 67:942-943.

Rheinheimer G.
Numbers and biomass of microorganisms in water and sediment.
In:Aquatic Microbiology. pp. 65-109.
New York:pubs. J. Wiley and Sons; 1991.

Rowbotham, T.J.
Current views on the relationships between amoeba, *Legionella*, and man.
Isr. J. Med. Sci., 1986, 22:678-689.

Russel, P.B., Kline, J., Yoder, M.C., and Polin, R.A.
Staphylococcal adherence to polyvinyl chlorine and heparin-bonded polyurethane catheters is species dependent and enhanced by fibronectin.
J. Clin. Microbiol., 1987, 25:1083-1087.

Scheid, R.C., Kim, C.K., Bright, J.S., Whitely, M.S., and Rosen, S.
Reduction of microbes in handpieces by flushing before use.
J. Am. Dent. Assoc., 1982, 105:658-660.

Simmons, J.S. and Gentzkow, C.L. eds.
Medical and Public Health Laboratory Methods. p 800.
Philadelphia:pubs. Lea and Febiger;1955.

Slots, J., Rams, T.E., and Listgarten, M.A.
Yeast, enteric rods, and pseudomonades in the subgingival flora of severe adult periodontitis.
Oral Microbiol. Immun., 1988, 3:47-52.

Stevens, R.E.
Preliminary study - air contamination with microorganisms during use of air turbine handpieces.
J. Am. Dent. Assoc., 1963, 66:237-239.

Tippett, B.F., Edwards, J.L., and Jenkinson, H.F.
Bacterial contamination of dental unit water lines - a possible source of cross infection.
N. Z. Dent. J., 1988, 84:112-114.

van der Wende, E. and Characklis, W.G.
Biofilm in water distribution systems.
In:Drinking water microbiology. pp. 249-268.
McFeters, G.A. ed. New York: pubs. Springer-Verlag; 1990.

Whitehouse, R.L.S., Peters, E., Lizotte, J., and Lilge, C.
Influence of biofilms on microbial contamination in dental unit water.
J. Dent., 1991, 19:290-295.

Williams, J.F., Johnston, A.M., Johnson, B., Huntington, M.K., MacKenzie, C.D.
Microbial contamination of dental unit water lines:prevalence, intensity, and microbiological characteristics.
J. Am. Dent. Assoc., 1993, 124:59-65

Wireman, J.W., Schmidt, A., Scavo, C.S., and Hutchins, D.T.
Biofilm formation by *Legionella pneumophila* in a model domestic hot water system. pp. 231-234.
In:*Legionella*:Current Status and Emerging Perspectives.
Barbaree, J., Breiman, R.F., and Dufour, A.P. eds.
Washington, D.C.:pubs. American Society of Microbiology;1993.

Williams, H.M., Quimby, H., and Romberg, E.
Evaluation and use of low nutrient medium and reduced incubation temperature to study bacterial contamination in the water supply of dental units.
Can. J. Microbiol., 1994, 40:127-131.

Zuravleff, J.J., Yu, V.L., Shonnard, J.W., Rihs, J.D., and Best, M.
Legionella pneumophila contamination of a hospital humidifier. Demonstration of aerosol transmission and subsequent subclinical infection in exposed guinea pigs.
Am. Rev. Respir. Dis., 1983, 128:657-661.

APPENDICES

APPENDIX A

Evaluation of Dental Unit Water Bacterial Contamination Control Methods

INTRODUCTION:

Heavy contamination of DUW with pathogenic and opportunistic bacteria has caused dental professionals to look for solutions to this problem. Investigators have tried to eliminate bacteria from DUWL by using chemical treatments in the building's water supply (Fiehn and Henriksen, 1988; and Liu *et al.*, 1994), by using chemical flushes of the lines with H_2O_2, $NaClO_4$, or povidine iodine (Abel *et al.*, 1971; McEntegart and Clark, 1973; Kelstrup *et al.*, 1977; Mills *et al.*, 1988; Pankhurst *et al.*, 1986; and H.N. Williams *et al.*, 1994), by flushing DUWL with water for extended periods of time (Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; and Bierle, 1993), or by changing from using the municipality's treated water to putting sterile water in pressurized reservoirs , or so called "Clean Water Systems" (CWS) (Blake, 1963; Molinary and Crawford, 1976; Mills *et al.*, 1986; Whitehouse *et al.*, 1991; and H.N. Williams *et al.*, 1994).

The results summarized below illustrate the ineffectiveness of: a) extended flushing of water lines,

b) the use of sterile water reservoirs and chemical line flushes, and c) the treatment of the water supply with antimicrobials, as approaches to the reduction of microorganism concentration in DUW.

MATERIALS AND METHODS:

All water samples were shipped and processed as described in the previous chapter.

a) Extended Water Flushes of DUWL:

Fifteen to fifty milliliter DUW samples from different instruments were collected after flushing the water lines for 30 seconds, 60 seconds, 2 minutes, 25 minutes, or 1 hour.

b) Clean Water Systems and Line Disinfection:

Fifty milliliter DUW samples were collected from air/water syringes attached either to CWS with reservoirs filled with sterile water, or from CWS using sterile water but in which the water lines were filled with a 1:6 dilution of household bleach over the weekend and flushed extensively every Monday morning.

c) Copper/Silver Ionization of the Building Water Supply:

Fifty Milliliter DUW samples were collected at 44 days and 86 days after installation of a copper/silver ionization system in the plumbing of a dental building water supply. The system in operation releases between

0.2 ppm to 0.3 ppm Cu^{+2} and Ag^{+} levels range between 10 and 30 parts per billion.

RESULTS:

a) Extended Water Flushes of DUWL:

Figure A-1 shows a comparison of contamination levels of DUW samples after different periods of flushing. Even after 60 minutes of flow at approximately 100 mL/min, contamination remained at unacceptably high levels.

b) Clean Water System and Line Disinfection:

Figure A-2 shows microbial contamination levels of samples from CWS units using sterile water reservoirs, and CWS connected to lines treated with a dilute bleach solution (1:6) and then flushed extensively with water. In both cases contamination continued at high levels.

c) Cooper/Silver Ionization of the Building Water Supply:

In Figure A-3 contamination of DUW is illustrated in samples taken at 44 days and 86 days after the installation of the Cu/Ag system. Again, levels of contamination in these water samples were well above potable water standards.

FIGURE A-1

Scatter plot showing a comparison of heterotrophic bacterial plate counts in water from dental unit water lines after flushing for 30 seconds, 60 seconds, 2 minutes, 20 minutes, or 1 hour. Bacterial contamination less or equal to 30 cfu/mL are expressed as 30 cfu/mL.

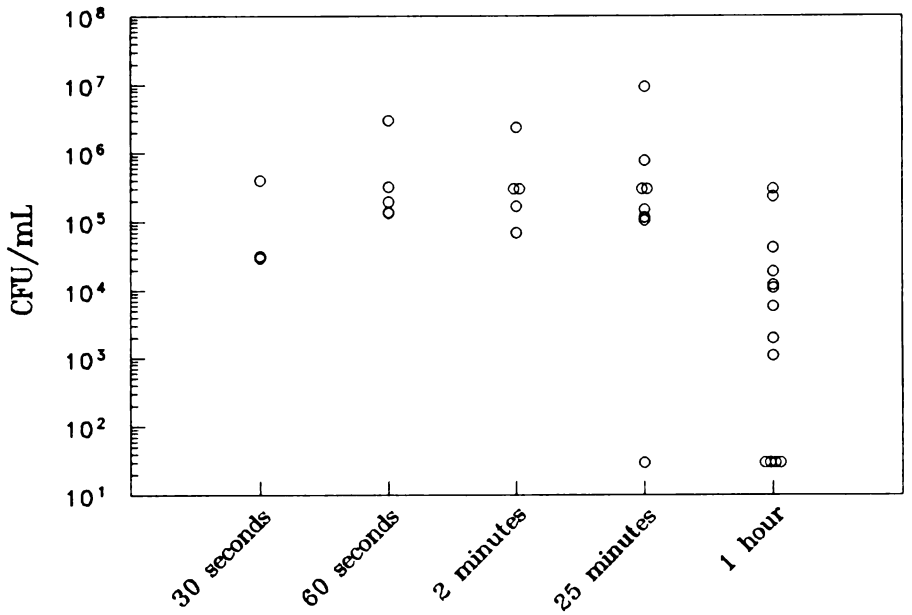
CFU/mL
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
30 seconds
60 seconds
2 minutes
25 minutes
1 hour

FIGURE A-1

FIGURE A-2

Scatter plot showing a comparison of heterotrophic bacterial plate counts in dental water from Clean Water Systems using reservoirs filled with sterile water and Clean Water Systems using sterile water reservoirs and which have the lines treated with a 1:6 dilution of household bleach over the weekend and are flushed extensively Monday morning. Bacterial contamination less or equal to 30 cfu/mL are expressed as 30 cfu/mL.

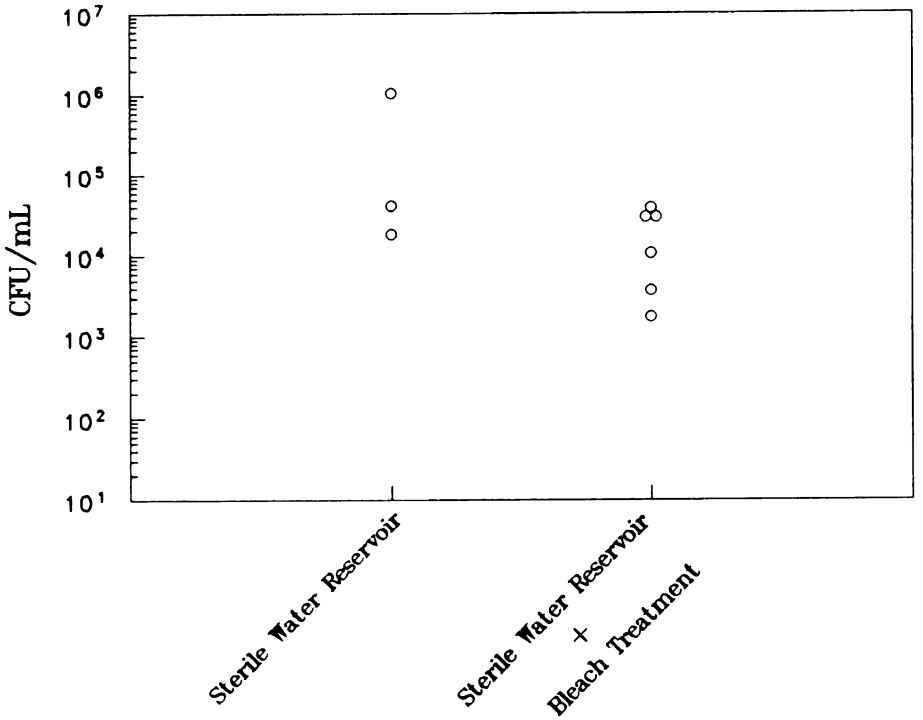
CFU/mL
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Sterile Water Reservoir
Sterile Water Reservoir + Bleach Treatment

FIGURE A–2

FIGURE A-3

Scatter plot showing heterotrophic bacteria contamination of dental unit water after the installation of a silver/copper water ionization system to a professional building. The ionization system was installed January 30, 1993. Bacterial contamination less or equal to 30 cfu/mL are expressed as 30 cfu/mL.

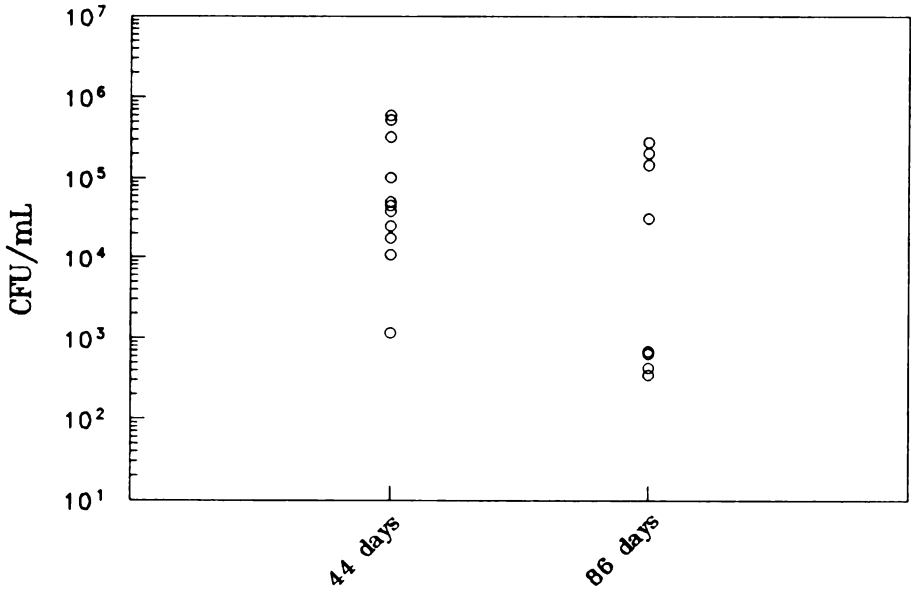

FIGURE A-3

DISCUSSION:

Although the CDC/ADA recommend flushing of DUWL for 2 minutes first thing in the morning and after each patient, and extended flushing after the stagnation in the lines over the weekend (CDC, 1993), the efficacy of this procedure in eliminating bacteria from DUW depends on the contamination levels of the DUW. It is true that flushing of water through DUW lines temporarily decreases the number of bacteria present in the water (Abel *et al.*, 1971; Gross and Devine, 1976; Gross *et al.*, 1976; Scheid *et al.*, 1982; Mills *et al.*, 1986; Tippett *et al.*, 1988; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Bierle, 1993; and H.M. Williams *et al.*, 1994). However, even with extended flushing, the water delivered by dental instruments may remain above potable water levels, or even much higher (Mayo *et al.*, 1990; and Whitehouse *et al.*, 1991). The results shown in Figure A-1 confirm this, and demonstrate how ineffective extended flushing of water lines is, even for 1 hour, in reducing bacteria. Only 4 of 12 units delivered water within potable water standards after flushing for one hour; 8 samples remained at contamination levels higher than 1×10^3 cfu/mL. Bacterial concentrations ranged up to 3.0×10^5 cfu/mL.

Use of sterile water reservoirs in CWS was also an ineffective approach to controlling bacterial contamination of dental water in previous work reported by Blake (1963), Molinari and Crawford, Mills *et al.* (1986), Whitehouse *et al.* (1991), and recently, H.N. Williams *et al.* (1994). The

results shown in Figure A-2 reveal that contamination of CWS water was similar to that recently reported by H.N. Williams *et al.* (1994). Figure A-2 also illustrates that bleach line flushes were not effective in controlling DUW microbial contamination. Our results are unlike the findings of H.N. Williams *et al.*, but this could be due to the selection of bleach tolerant bacteria by repeated bleach treatments of water lines (lines were treated weekly as stated for 3 years).

Although there was a reduction in the number of bacteria in DUW 86 days after the installation of the Cu/Ag ionization system, water delivered by dental instruments still remained heavily contaminated. Six of 8 samples were above the potable water contamination levels on 4-26-93 and ranged from 3.1 x 10^4 cfu/mL to 2.81 x 10^5 cfu/mL. These findings contrast with the success reported by Liu *et al.* (1994) in eliminating bacteria from hospital hot water plumbing systems. This could be associated with greater biofilm formation and longer periods of stagnation in DUWL.

SUMMARY AND CONCLUSIONS:

New methods of disinfection are needed to bring about the elimination of bacteria in DUW. This goal is important because of the recent increase in the population of immunocompromised individuals, who could suffer life-threatening infections by organisms contaminating DUW.

LIST OF REFERENCES

APPENDIX A

LIST OF REFERENCES:

Abel, L.C., Miller, R.L., Micik, R.E., and Ryge, G.
Studies of dental aerobiology: IV. Bacterial contamination of water delivered by dental units.
J. Dent. Res., 1971, 50:1567-1569.

Bierle, J.W.
Dental operatory water lines.
J. Calif. Dent. Assoc., 1993, 21:13-15.

Blake, G.C.
The incidence and control of bacterial infection in dental spray reservoirs.
Brit. Dent. J., 1963, 115:413-416.

Centers for disease control.
Recommended Infection Control Practices for Dentistry.
Morb. Mortal. Weekly Rep., 1993, 42:1-12.

Fiehn, N.E. and Henriksen, K.
Methods of disinfection of the water system of dental units by water chlorination.
J. Dent. Res., 1988, 67:1499-1504.

Gross, A. and Devine, J.D.
Microbial contamination of dental units and ultrasonic scalers.
J. Dent. Res., 1976, 55B:B276 - abstract # 856.

Gross, A., Devine, M., and Cutright, D.E.
Microbial contamination of dental units and ultrasonic scalers.
J. Periodont., 1976, 47:670-673.

Kelstrup, J., Funder-Nielsen, T.D., and Theilade, J.
Microbial aggregate contamination of water lines in dental equipment and its control.
Acta Path. Microbiol. Scand., 1977, 85:177-183.

Liu, Z., Stout, J.E., Tedesco, L., Boldin, M., Hwang, C., Diven, W.F., and Yu, V.L.
Controlled evaluation of copper-silver ionization in eradicating *Legionella pneumophila* from a hospital water distribution system.
J. Infect. Dis., 1994, 169:919-922.

Mayo, J.E., Oertling, K.M., and Andrieu, S.
Bacterial biofilm:A source of contamination in dental air-water syringes.
Clin. Prev. Dent., 1990, 12:13-20.

McEntegart, M.G. and Clark, A.
Colonization of dental units by water bacteria.
Brit. Dent. J., 1973, 134:140-142.

Mills, S.E., Lauderdale, P.W., and Mayhew, R.B.
Reduction of microbial contamination in dental units with povidone-iodine 10%.
J. Am. Dent. Assoc., 1986, 113:280-284.

Molinary, J.D. and Crawford, J.J.
Evaluation of an independent water reservoir system for high speed instrumentation.
J. Dent. Res., 1976, 55B:B245, abst. #855.

Pankhurst, C.L., Philpott-Howard, J.N., Hewitt, J.H., and Casewell, M.W.
The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems.
J. Hosp. Infec., 1990, 16:9-18.

Scheid, R.C., Kim, C.K., Bright, J.S., Whitely, M.S., and Rosen, S.
Reduction of microbes in handpieces by flushing before use.
J. Am. Dent. Assoc., 1982, 105:658-660.

Tippett, B.F., Edwards, J.L., and Jenkinson, H.F.
Bacterial contamination of dental unit water lines-a possible source of cross infection.
N. Z. Dent. J., 1988, 84:112-114.

Whitehouse, R.L.S., Peters, E., Lizotte, J., and Lilge, C.
Influence of biofilms on microbial contamination in dental unit water.
J. Dent., 1991, 19:290-295.

Williams, H.M., Quimby, H., and Romberg, E.
Evaluation and use of a low nutrient medium and reduced incubation temperature to study bacterial contamination in the water supply of dental units.
Can. J. Microbiol., 1994,40:127-131.

Williams, H.N., Kelley, J., Folineo, D., Williams, G.C., Hawley, C.L., and Sibiski, J.
Assessing microbial contamination of clean water dental units and compliance with disinfection protocol.
J. Am. Dent. Assoc., 1994, 125:1205-1211.

APPENDIX B

Institutional Profiles of Dental Unit Water Bacterial Contamination

INTRODUCTION:

Microbiological profiles in a number of reports from Europe and North America of dental unit water lines (DUWL) of institutional facilities indicate the presence of opportunistic and pathogenic bacteria, such as *Staphylococcus* sup., *Pseudomonas* sup., and *Legionella* sup., among others. Contamination levels were generally much higher than potable water standards (McEntegart and Clark, 1973; Gross and Devine, 1976; Kelstrup *et al.*, 1977; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Mills *et al.*, 1986; Oppenheim *et al.*, 1987; Fiehn and Henriksen, 1988; Pankhurst *et al.*, 1990; and H.N. Williams *et al.*, 1994).

Reports of exposure to contaminated water in dental educational institutions is surprising. Institutions might be expected to have structured infection control programs, such as routine flushing of water lines with water and germicides, and to follow these protocols better than private practitioners. However, institutions are often located in old buildings and face the problem of declining water quality due to ageing of the plumbing system. This can result in the chronic contamination of incoming water supplies with

bacteria, and the occurrence of nosocomial water borne infections, particularly legionellosis (Stout *et al.*, 1982).

In the following section data are presented that illustrate contamination of DUW in dental institutions throughout the United States.

MATERIALS AND METHODS:

Dental unit water samples of 50 mL were obtained from a total of 93 dental instrument lines in two dental clinics, one university affiliated dental school, and two dental hygiene schools in the states of California, Massachusetts, Michigan, and Minnesota. All water specimens were shipped and processed as described previously in Article #1.

RESULTS:

Figure B-1 is a scatter plot of data points on microbial contamination levels in water delivered by different dental instruments at all the institutions combined. Contamination ranged from < 30 cfu/mL to 26,500,000 cfu/mL.

Details of the results of contamination levels found in water delivered by air/water syringes, high speed handpieces, ultrasonic scalers, and faucet samples are shown in Table B-1. Figures B-2 - B-6 and Table B-2 represent details of the DUW contamination profile for each institution separately. Regardless of site and instrument type, contamination of water samples from dental units far exceeded that seen in faucet water.

FIGURE B-1

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at all institutions surveyed. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL. The horizontal lines of the hi-lo plot mark the value of data in the following increasing order: mean - 2 standard deviations, mean - 2 standard error, mean, mean + 2 standard error, and mean + 2 standard deviation.

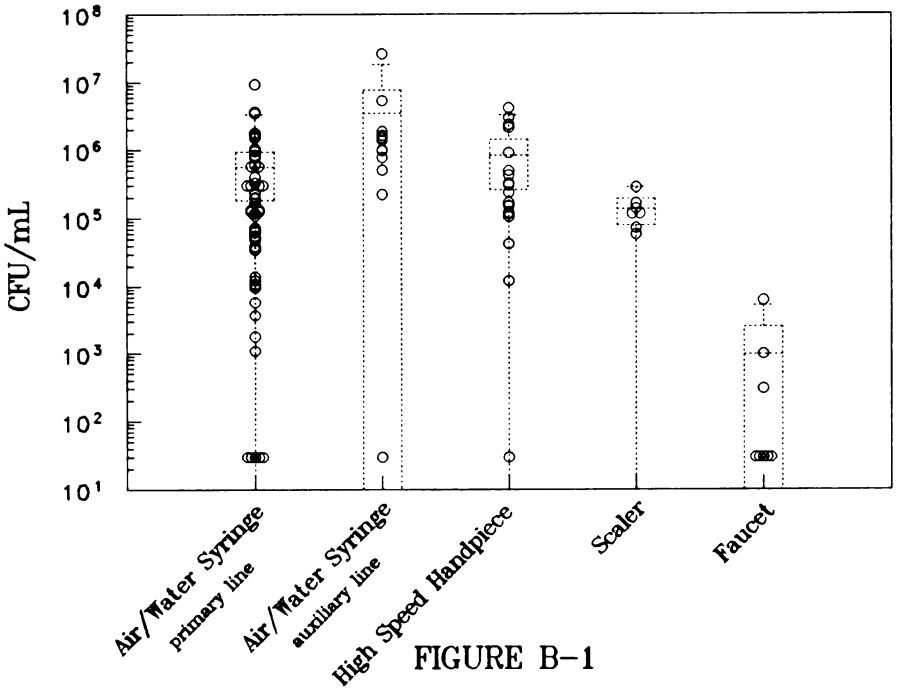
CFU/mL
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe primary line
Air/Water Syringe auxiliary line
High Speed Handpiece
Scaler
Faucet

FIGURE B-1

TABLE B-1

Heterotrophic bacterial contamination of dental unit water in colony forming units per milliliter delivered by dental instruments and faucets at different institutional sites throughout the United States. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

TABLE B-1

INSTRUMENT	N	ARITHMETIC MEAN	RANGE
Air/Water Syringe	56	5.56×10^5	< 30 - 9.35×10^6
AWS/auxiliary line	12	3.47×10^6	< 30 - 2.65×10^7
High Speed Handpiece	18	8.32×10^5	< 30 - 4.15×10^6
Ultrasonic Scaler	7	1.35×10^5	< 30 - 2.80×10^5
Faucet	8	9.50×10^2	< 30 - 6.20×10^3

FIGURE B-2

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at the School of Dental Hygiene A. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

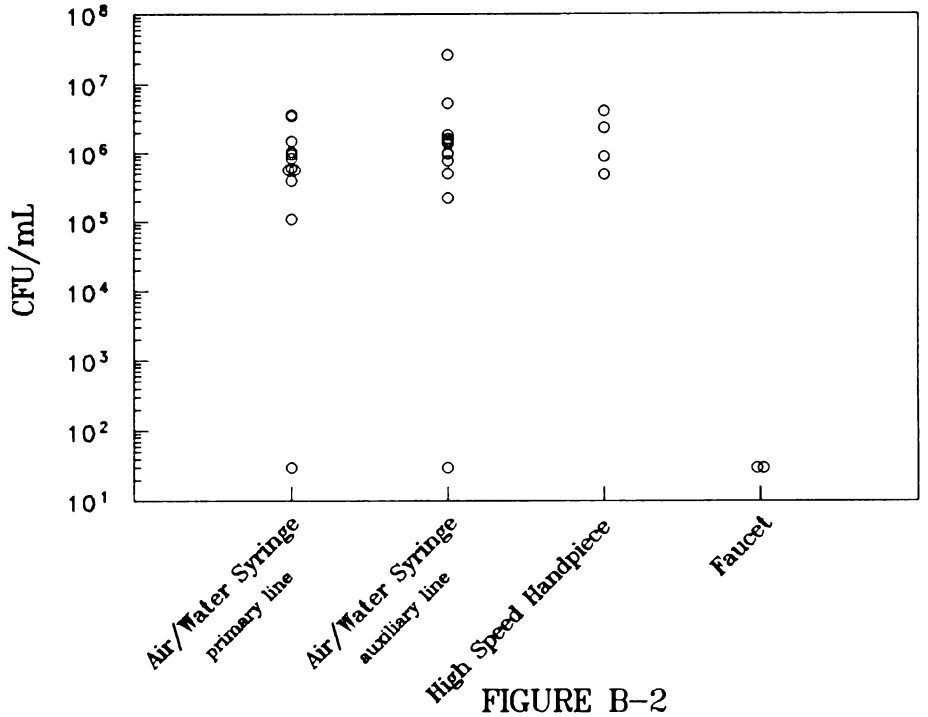
CFU/mL
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe primary line
Air/Water Syringe auxiliary line
High Speed Handpiece
Faucet

FIGURE B-2

FIGURE B-3

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at the School of Dental Hygiene B. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

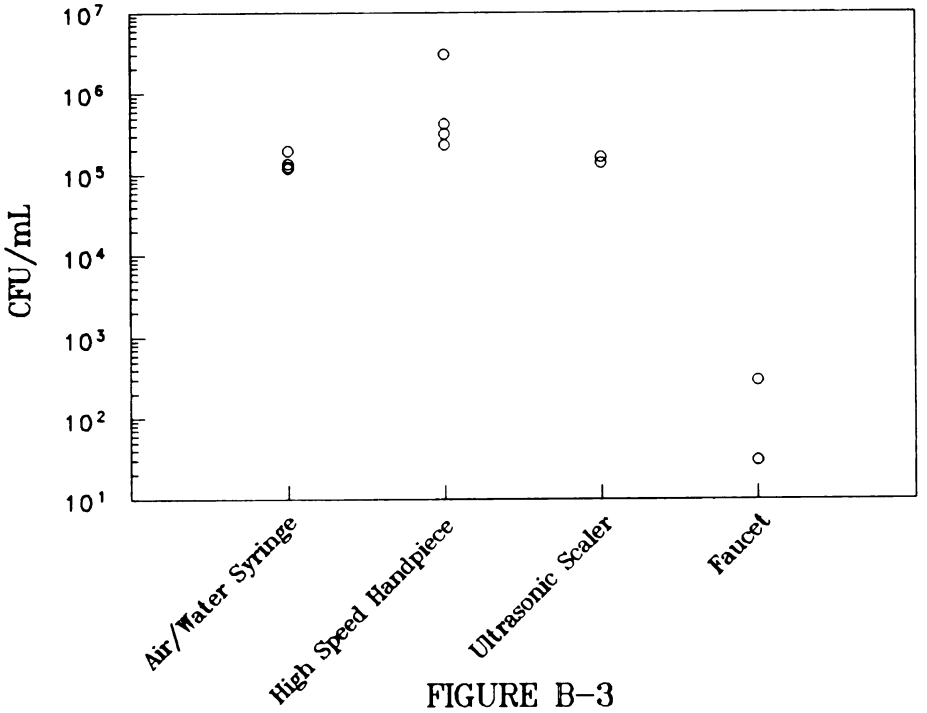
CFU/mL
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
High Speed Handpiece
Ultrasonic Scaler
Faucet

FIGURE B-3

FIGURE B-4

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at Dental Clinic A. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

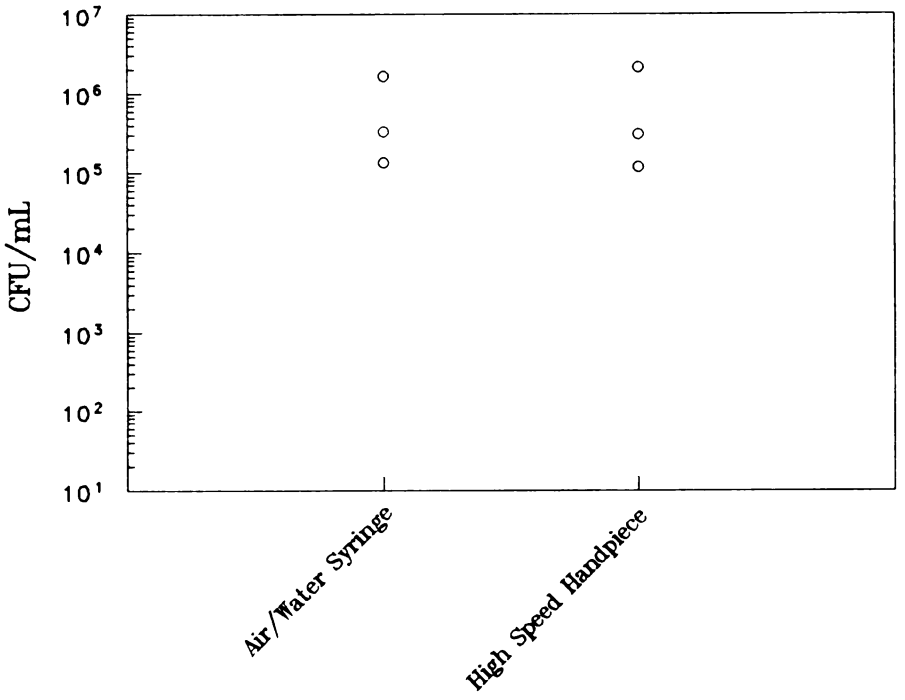
CFU/mL
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
High Speed Handpiece

FIGURE B–4

FIGURE B-5

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at Dental Clinic B. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

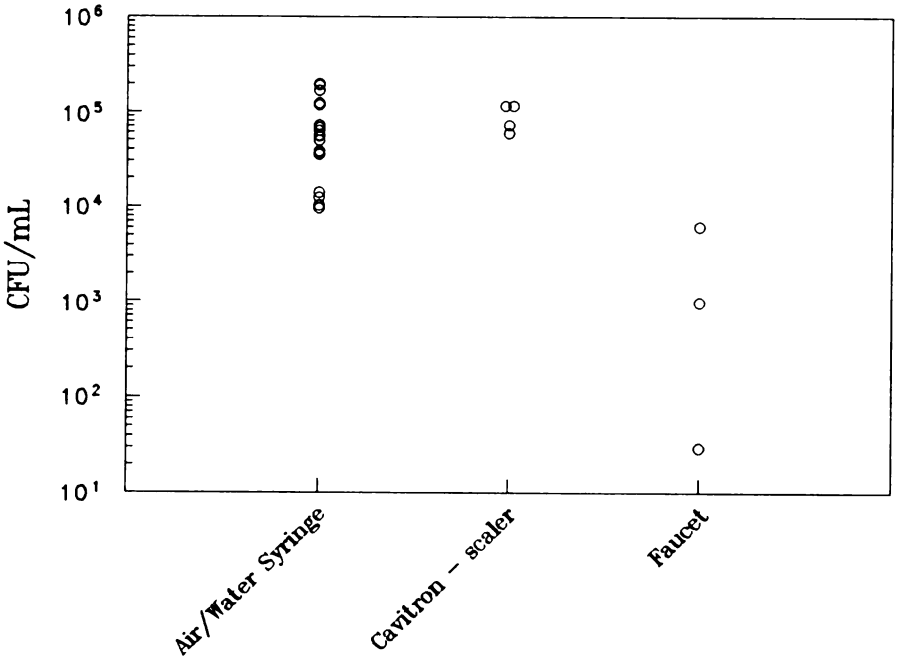
CFU/mL
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
Cavitron - scaler
Faucet

FIGURE B-5

FIGURE B-6

Scatter plot showing heterotrophic plate counts of microbial contamination from dental unit water at Dental School A. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

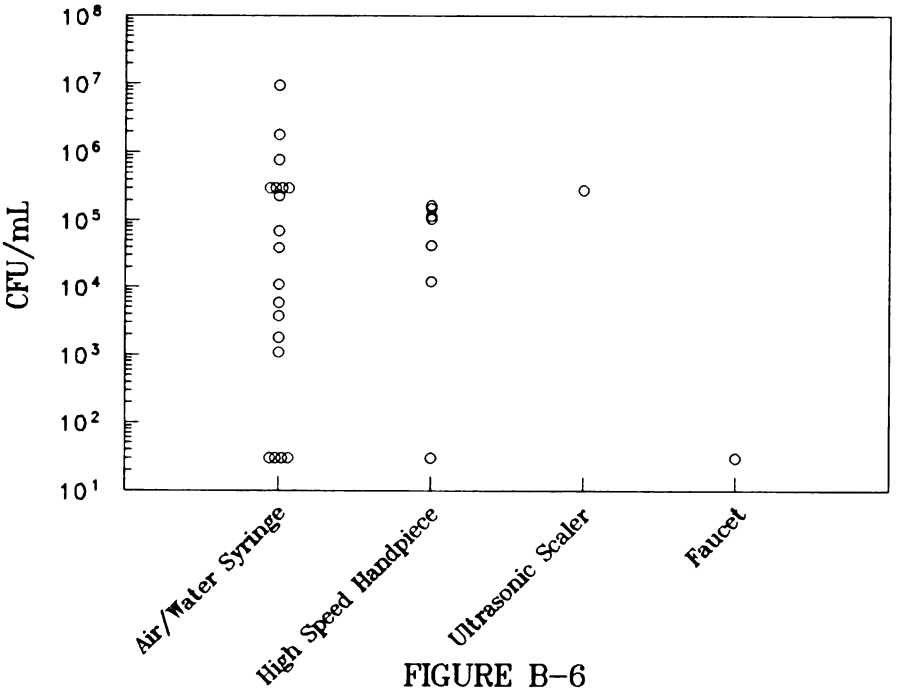
CFU/mL
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Air/Water Syringe
High Speed Handpiece
Ultrasonic Scaler
Faucet

FIGURE B–6

TABLE B-2

Average heterotrophic bacterial contamination of dental unit water, in colony forming units per milliliter, delivered by dental instruments at institutional sites throughout the United States. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL.

TABLE B-2

INSTITUTION	AWS	AUX	HSH	US
Institution A =	1.14×10^6	3.47×10^6	1.97×10^6	ND
Institution B =	1.43×10^5	ND	9.93×10^5	1.51×10^5
Institution C =	6.98×10^5	ND	8.48×10^5	ND
Institution D =	7.33×10^5	ND	ND	9.07×10^4
Institution E =	7.09×10^5	ND	8.44×10^4	2.80×10^5

DISCUSSION:

The results demonstrate that in large institutional clinics the problem of contamination of dental unit water appears to be similar to that seen in private dental offices (see Article #1). Dental unit water contamination at the institutions studied was comparable to that reported in the literature (McEntegart and Clark, 1973; Gross and Devine, 1976; Scheid *et al.*, 1982; Mills *et al.*, 1986; Fiehn and Henriksen, 1988; and H.N. Williams *et al.*, 1994). Exceptionally high contamination levels were present in auxiliary lines of air/water syringes; auxiliary lines averaged 3,500,000 cfu/mL, and the highest contamination exceeded 26,500,000 cfu/mL. Overall, only six samples out 93 from institutions were within potable water standards.

Only one institution had established protocols to combat contamination of water used in the treatment of AIDS patients. There dental unit water lines were filled with a 1:6 dilution of household bleach on Fridays and the lines flushed Monday mornings. They also used sterile water reservoirs, and flushed water through dental instruments lines for extended periods each morning in order to "control" bacterial contamination. Unfortunately, this process is not efficient in the removal of bacteria from dental unit water. Figure B-7 is a composite that illustrates the contamination profiles found in institutional and dental clinic DUW samples. It demonstrates that the results for institutions are similar to those from private dental offices.

FIGURE B-7

Scatter plot showing a comparison of heterotrophic plate counts of microbial contamination from dental unit water at institution and private clinics. Bacterial concentrations in the samples of 30 cfu/mL or less are expressed as 30 cfu/mL. The horizontal lines of the hi-lo plot mark the value of data in the following increasing order: mean - 2 standard deviations, mean - 2 standard error, mean, mean + 2 standard error, and mean + 2 standard deviation.

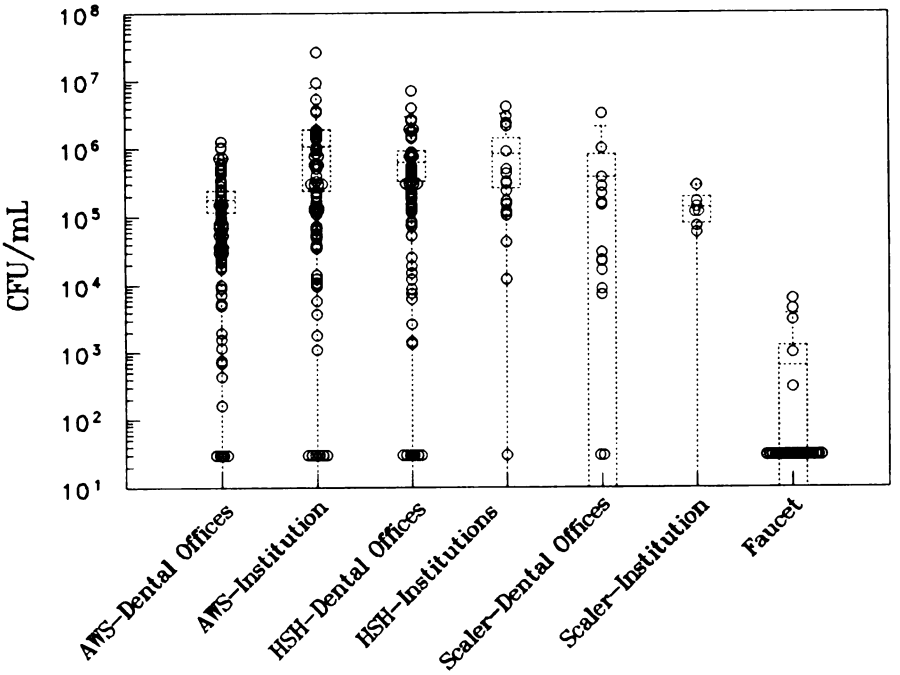
CFU/mL
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
AWS-Dental Offices
AWS-Institution
HSH-Dental Offices
HSH-Institutions
Scaler-Dental Offices
Scaler-Institution
Faucet

FIGURE B-7

Contamination levels of faucet water samples taken from the same rooms as DUW samples at the institutions and clinics studied indicated that 22 of 25 samples were within EPA standards for heterotrophic bacteria (EPA, 1989). The highest concentration in a faucet sample, 6,200 cfu/mL, was still lower than 213 of the 247 DUW samples. This makes it clear that the heavy contamination of DUW is a function of the DU, and is not due to failures in the municipal water supply.

SUMMARY AND CONCLUSIONS:

DUW contamination is widespread in institutional dentistry. Education of professionals in institutional leadership positions will be necessary for new solutions to this problem to be identified.

LIST OF REFERENCES

APPENDIX B

LIST OF REFERENCES:

Environmental Protection Agency
Drinking water: national primary drinking water regulations; filtration, disinfection, turbidity, *Giardia lamblia*, virus, *Legionella*, and heterotrophic bacteria.
Fed. Reg., 1989, 54:27486-27540.

Fiehn, N.E. and Henriksen, K.
Methods of disinfection of the water system of dental units by water chlorination.
J. Dent. Res., 1988, 67:1499-1504.

Fitzgibbon, E.J., Bartzokas, C.A., Martin, M.V., Gibson, M.F., and Graham, R.
The source frequency and extent of bacterial contamination of dental unit water systems.
Brit. Dent. J., 1984, 157:98-101.

Gross, A. and Devine, J.D.
Microbial contamination of dental units and ultrasonic scalers.
J. Dent. Res., 1976, 55B:B276 - abstract # 856.

Kelstrup, J., Funder-Nielsen, T.D., and Theilade, J.
Microbial aggregate contamination of water lines in dental equipment and its control.
Acta Path. Microbiol. Scand., 1977, 85:177-183.

McEntegart, M.G. and Clark, A.
Colonization of dental units by water bacteria.
Brit. Dent. J., 1973, 134:140-142.

Mills, S.E., Lauderdale, P.W., and Mayhew, R.B.
Reduction of microbial contamination in dental units with povidone-iodine 10%.
J. Am. Dent. Assoc., 1986, 113:280-284.

Oppenheim, B.A., Sefton, A.M., Gill, O.N., Tyler, J.E., O'Mahony, M.C., Richards, J.M., Dennis, P.J.L., and Harrison, T.G.
Widespread *Legionella pneumophila* contamination of dental stations in a dental school without apparent human infection.
Epidem. Infect., 1987, 99:159-166.

Pankhurst, C.L., Philpott-Howard, J.N., Hewitt, J.H., and Casewell, M.W.
The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems.
J. Hosp. Infec., 1990, 16:9-18.

Scheid, R.C., Kim, C.K., Bright, J.S., Whitely, M.S., and Rosen, S.
Reduction of microbes in handpieces by flushing before use.
J. Am. Dent. Assoc., 1982, 105:658-660.

Stout, J., Yu, V.L., Vickers, R.M., and Shonnard, J.
Potable water supply as the hospital reservoir for Pittsburgh Pneumonia Agent.
Lancet, 1982, i:471-472.

Williams, H.N., Kelley, J., Folineo, D., Williams, G.C., Hawley, C.L., and Sibiski, J.
Assessing microbial contamination of clean water dental units and compliance with disinfection protocol.
J. Am. Dent. Assoc., 1994, 125:1205-1211.