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Legionella in the dental office

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Abstract: *Objective:* This study aimed to assess the microbiology of dental unit water and municipal water in terms of *Legionella* species and total bacteria levels. *Methods:* The presence of *Legionella* species was investigated using the culture method, direct fluorescent antibody and polymerase chain reaction techniques in collected dental unit water and municipal water samples from 71 dental offices in Ankara, Turkey. In addition, total bacterial counts were assessed using the culture method. *Results:* In 27% of the dental unit water samples and in 13% of municipal water samples, the number of colony-forming units (cfu ml^{-1}) significantly exceeded acceptable values for high-risk group patients. No *Legionella* spp. was found in the dental unit water samples. *Legionella* SG3 was found in only one municipal water sample. *Conclusion:* The dental unit water systems examined in this study did not include *Legionella* spp., but other bacteria at high numbers were determined. This is a potential threat, especially for elderly people, the medically compromised patients receiving regular dental treatment and the dental clinic staff.

Key words: dental unit water systems; *Legionella*; microbial contamination

Introduction

Water circulated in dental units by an 'in-line' system can be contaminated with micro-organisms (1, 2). The dental unit waterlines (DUWL) have been implicated as potential reservoirs of bacteria, including *Legionella pneumophia*, *Mycobacterium tuberculosis*, *Gram-positive rods*, *non-haemolytic streptococci* and *enterococci*. These organisms are known to be present in very low numbers in the public water supply, and are most likely acquired from that source. However, these micro-organisms, including *Legionella* spp. (3), are found in high numbers within the complex network of tubing of the dental units.

Surveillance studies show that the presence of *Legionella* in dental unit water systems does not lead to detectable incidences of human infection (4, 5). However, seroconversion of this micro-organism has been proven (6, 7).

Legionella spp. is an important cause of sporadic and epidemic pneumonia in developed countries (8). Although there is no epidemiological evidence of a widespread public health problem, the risk of exposure to contaminated water in the dental office still exists (9). Microbial biofilms form inside the dental unit waterlines delivering water to the dental equipment. The flushing out water delivered from the dental equipment always carries the risk of being inhaled or ingested, and inoculated to open wounds.

Challacombe and Fernandes (10) indicated that units contaminated with *Legionella pneumophila* also could contain high numbers of other bacteria (mostly *Pseudomonas*). Furthermore, dental units are suspected as an important source of *Legionella* spp. In one case, a dentist in Northern California died due to *Legionella* infection acquired from the dental unit (11).

The *Legionella* and bacterial infections seem to depend on several factors (12), including host susceptibility and aerosolization of the organism. There is greater risk to elderly or immunocompromised patients, and high-risk groups for influenza-related complications include diabetics, chronic pulmonary, renal, liver and cardiovascular diseases (10, 13).

The American Dental Association (ADA) addressed the standards of dental water quality and what should be considered safe for human consumption; the dental unit water should not exceed 200 CFU ml^{-1} (14).

The aim of this study was to determine the bacterial contamination of DUWL by examining the prevalence of *Legionella* species and total bacterial count.

Materials and methods

Samples were collected from an air–water syringe of 71 dental units in different dental offices' waterline connected to the public water source and to sink taps that represented office faucet water samples from the municipal water system of Ankara, Turkey, in spring.

Both types of water samples were examined for the presence of *Legionella* species and total bacterial count. Chlorine concentration was also determined.

A number of factors were evaluated for the total bacterial count and *Legionella* detection, including the chlorine levels of public and dental unit water: the bacterial status of municipal water and its relation with dental unit water counts; the age of the dental units; the heating system fittings of the clinics; and the number of floors in the buildings.

The water was run for 15 s from taps and air–water syringes before the sample were collected. Then, 500 ml water samples were collected into sterile glass containers. For the determination of chlorine concentration, 5 ml of water was pipetted from each glass container and added into test tubes. *ortho*-Toluidine was dropped into the test tubes, and the quantity of chlorine was measured via a chlorometer Permodid Comparator P 777 (Permodid, Istanbul, Turkey) that showed the chlorine concentration in p.p.m. The remaining water samples were used to identify *Legionella* species and to determine the total bacterial count.

Identical procedures were performed for the dental unit and the municipal water samples. Once samples were taken, they were brought immediately to the microbiology laboratory for processing.

Culture procedures, DFA assay and polymerase chain reaction (PCR) detection for *Legionella* spp. and total plate count definition were analysed with standard methods by the microbiologist of the National Legionella Reference Laboratory in Refik Saydam Hygiene Center.

Total plate count definition

To determine the total number of bacteria in 1 ml of water, the pour plate method was used. One millilitre of water sample was dropped directly onto the solid Plate Count Agar (PCA; Tryptone Glucose Yeast Agar, Oxoid, England) plates and spread over the plate. Plated cultures on PCA were incubated at 37°C for 24 h (15). Bacterial colonies were counted using a Quebec Darkfield colony counter (Reichert Products, New York, NY, USA) and all measurements were recorded.

Culture procedures for *Legionella*

Water samples of 100 ml were filtered through 0.2 μm pore cellulose filters (Millipore, Billerica, MA, USA). Filters were washed 10 min in an acid buffer (pH 2.2), then rinsed in a Ringer solution (Merck, Darmstadt, Germany), and were placed onto buffered charcoal yeast extract (BCYE) agar medium for incubation.

Buffered charcoal yeast extract agar medium, supplemented with Growth Supplement SR 110 A and the Selective GVPC Supplement SR 152 E (Oxoid, Basingstoke, England), was used for isolation of *Legionella*. Inoculated agar plates were incubated for 7 days at 37°C with a daily check for growth. Colonies of Gram-negative bacteria grown after 4–7 days were

isolated and examined for their ability to grow on media with and without cysteine. Strains unable to grow on media without cysteine were considered suspected *Legionella* strains. The isolates were identified to species and serogroup level with the use of the Legionella Latex Kit (Oxoid) that, on the basis of microcoagulation with latex particles sensitized with specific rabbit antibodies, enables a separate identification of *L. pneumophila* serogroups.

Cultures identified as *Legionella* (+) also were confirmed with DFA.

Direct fluorescent antibody assay

Twenty microlitres of the pellet suspensions from the concentrated water samples were pipetted onto fluorescent antibody slides. The slides were air-dried, heat-fixed and stained with fluorescent polyclonal antibody prepared against *L. pneumophila* and other *Legionella* species.

After rinsing in phosphate-buffered saline (pH 7.6) and deionized water, the slides were incubated at 37°C for 30 min and air-dried before being viewed under an Olympus CH 40 epifluorescent microscope (Olympus Optical Co. Ltd., Tokyo, Japan). The cells that fluoresced a brilliant green-yellow colour were qualified as *Legionella*.

PCR detection of *Legionella* spp.

A *Legionella* detection PCR kit (MPI Fermentas, St. Leon-Rot, Germany) was used as described in the manufacturer's instructions for molecular detection of *Legionella* species. A 100 ml aliquot of each sample was passed through a 0.22-μm bacteriological field monitor filter (Millipore Corp.) to trap bacterial cells. An aliquot of the sample was transferred to a reaction vessel for amplification of the diagnostic gene sequences. The MPI detection kit (MPI Fermentas) was used for PCR amplification and gene probe detection.

After the amplification process, each specimen was loaded to ×6 loading buffer and 1.5% aparoz gel. A DNA ladder 100 bp (MPI Fermentas) was used to accomplish helicoidal unity. Specimens were stained with 0.5 μg ml^{-1} Ethidium-Bromide before application of the electrophoresis process at 100 V for 40 min.

The specimens were evaluated using a UV transilluminator.

Statistical analysis

The bacterial counts were not distributed normally. Therefore, the relationship between chlorine levels and bacterial counts was evaluated by using Spearman's rho test and Kendall's Tau-b test. Mann–Whitney *U*-test, Kruskall–Wallis *H*-test and Median non-parametric tests were used to test the differences of means for factor levels.

Results

In 71 dental unit water samples, *Legionella* spp. was not isolated by culturing. Only in one of the tap water samples *Legionella* SG 3 was isolated by culturing. No *Legionella* spp. was found with the DFA and PCR methods.

Chlorine concentrations of tap water and DUWS are presented in Table 1. The results showed that total bacterial counts were decreased both in tap and dental unit waters when the chlorine level was raised. Correlation values between the chlorine level of municipal water dental unit water and total bacterial count of dental unit water were −0.264; $P < 0.05$ and −0.117; $P > 0.05$ respectively.

Twenty-seven per cent of the dental unit water samples were contaminated with bacteria above 200 cfu ml^{-1}. This rate was 13% for the municipal water samples.

There was a positive relationship between the total bacterial count of municipal water and the dental unit water (correlation value: 0.449). This relationship was statistically significant ($P < 0.01$).

Clinics with central heating systems had more contaminated water in tap water and dental unit water than locally heated clinics. The difference was statistically significant (Table 2).

There was a positive relationship between tap/dental unit water total bacterial quantity and the number of floors in buildings (Table 3).

Table 1. **Chlorine concentrations of tap waters and dental unit waterlines**

Chlorine concentration (p.p.m.)	Number of samples of dental unit water	Number of samples of tap water
0	63	3
0.1	0	5
0.2	1	15
0.3	3	23
0.4	2	9
0.5	2	16
$x > 0.5$	0	0

Table 2. **Differences between heating system of offices and mean of total bacterial counts of municipal and dental unit water (Mann–Whitney *U*-test)**

	Total bacterial count value of dental unit water		Total bacterial count value of municipal water	
	Central	Local	Central	Local
Mean of total bacterial count value	255.155	104.077	160.948	7.692E-02
Total	58	13	58	13
Mean of rank value	38.54	24.65	39.55	20.15
Z-value	−2.204		−3.287	
P-value	0.028		0.001	

Table 3. **Differences between number of floors in building and total bacterial counts level of municipal and dental unit water (Mann–Whitney *U*-test)**

	Total bacterial count value of dental unit water		Total bacterial count value of municipal water	
	$x < 4$	$x \geq 4$	$x < 4$	$x \geq 4$
Mean of total bacterial count value	85.625	285.508	541.125	79.476
Total	8	63	8	63
Mean of rank value	33.56	36.31	45.56	34.79
Z-value	−0.356		−1.493	
P-value	0.722		0.135	

Discussion

The main infection route during operative dental treatment is via aerosol droplets. The presence of *Legionella* in the aerosol is particularly dangerous to patients with compromised immunity (3, 5). Furthermore, *Legionella* spp. within dental lines may cause respiratory illnesses among dentists and the dental staff (16). A study by Atlas *et al.* (11) showed that the contribution of bacteria caused long-term sub-clinic infections.

In this study, *Legionella* spp. was not found in dental unit water samples. Many researchers, however, have observed *Legionella* in DUWL – both in the water flowing from dental handpieces and in biofilm (3, 17). In fact, higher capacity water systems provide a more attractive environment for the growth of the *Legionella* species (17). The main reason for this is that bacteria reside in the water system, and colonization occurs in the water system. In this study, there was a difference in urban waterline structures; therefore, increase in contamination may be due to the wide waterline systems of the buildings. Clinics that participated in this study were at maximum four- to five-flat apartment buildings.

The other factor may be the chlorine concentration of the region. The majority of the recorded chlorine levels were between 0.2 and 0.5 p.p.m. in tap water. However, studies have shown that *Legionella* is present in the municipal water source in spite of the current filtration and chlorination procedures (8). Once *Legionella* reaches the building water system, it settles down into a biofilm layer of stagnant water. By means of this layer, *Legionella* can protect itself from antimicrobial agents, and then multiply. According to a study by Costerton *et al.* (18), biofilms, once established, are notoriously difficult to remove because of their complex nature. Additionally, Walker *et al.* (19) claimed that chemicals, such as hydrogen peroxide and iodine could not totally remove the biofilm in DUWL. By contrast, Meiller *et al.* (20) assert that chlorhexidine and gluteraldehyde agents have reduced the volume of biofilm.

Another factor in bacterial colonization is the maintenance frequency of the building's water storage. This is through mechanical and chemical means to prevent biofilm formation and to prevent microbial colonization (18–20). In this study, no such maintenance activity was performed in the examined clinics.

There were positive relationships between the total bacterial count of municipal water and dental unit water counts of the clinics. All of the dental offices in this study were receiving their water from a municipal water source. There were no water storage and no antimicrobial apparatus in the dental units. Therefore, there were direct connections for the micro-organisms in the water that entered and left the pipeline system of the dental units. Although 27% of the dental unit water samples in this study were contaminated with bacteria, this figure was only 13% for the water samples collected from a faucet. This result illustrates the importance of colonization in the dental unit water system. In applied inquiry, it showed that clinicians had not considered the importance of contamination factors in dental unit water systems. This subject relates to the general lack of knowledge and concern.

In this study, clinics fitted with local heating systems had lower bacterial counts (in both municipal and dental unit water) than those with central heating systems. This finding demonstrates that the temperature of central heating systems was more suitable for bacterial growth.

In this study, *L. pneumophila* (SG 3) was detected with the culture method in tap water in one sample. Negative results were obtained for *Legionella* bacteria in DFA. A possible explanation may be that the DFA technique is a subjective method having less sensitivity, and the specificity of this tech-

nique may not be enough to detect this bacterium due to interference resulting from the presence of other bacteria. Environmental conditions that exist in water samples (e.g. bacteria, humic acid and rust in pipes) may change the precision of the PCR technique (21). For this reason, it is questionable whether the PCR technique should be used to detect *Legionella* bacteria in water, although some researchers have found that the sensitivity of the culture method was even lower than the sensitivity of the PCR technique for detecting of *Legionella* spp. On the other hand, it is possible that the higher total bacterial count in the water sample may mask the presence of the bacteria of concern and also produce cross-reactions between various bacteria during the analytical procedures. Thus, the PCR technique can no longer be used for the detection of *Legionella* in water.

Legionella bacteria create a greater risk to elderly people and immuno-compromised patients. Cases have been reported in which DUWL-originated bacteria caused infections (11). Especially for the high-risk group patients, it is important to ensure that the hygiene standards are kept high. The water quality in the dental units should be controlled to eliminate opportunistic pathogens and to provide water for dental treatment that meets public health standards for potable water. Clinicians can apply some preventive methods, as follows: (1) flushing through the chair for 3 min at the start of each day; (2) supplying the dental unit reservoir with sterile and good quality water; (3) autoclaving dental handpieces after each use; (4) regular maintenance of the dental unit system; and (5) regular chlorination of the water system of the dental clinic.

Conclusion

Dental unit waterlines may be contaminated with opportunistic bacteria. The DUWL examined in this study did not include *Legionella* spp., but other bacteria at high numbers were determined. This is a potential threat, particularly for elderly people, the medically compromised patients receiving regular dental treatment, and the dental clinic staff.

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