

CLINICAL PRACTISE

Legionella spp. in dental unit waterlines

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OBJECTIVE: To determine the current presence of *Legionella* spp. in the output water of dental unit waterlines (DUWLs) and examine its mitigation by disinfection at the Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc.

MATERIAL AND METHODS: The first stage of our survey involved collecting samples of DUWL output water from 50 dental chair units (DCUs), and 2 samples of the incoming potable water. In October 2015, a one-time disinfection (1 % Stabimed) of DUWLs was conducted. This was followed by collecting 10 control samples (survey stage 2).

RESULTS: From the total of 50 samples (survey stage 1), 18 samples (36.0 %) tested positive for *Legionella* spp. Following the disinfection, nine of the ten samples no longer showed any presence of *Legionella*.

CONCLUSION: Based on culture results, the one-time disinfection (1 % Stabimed) was effective. We are unable to comment on the duration of positive effect of disinfection on the occurrence of *Legionella* spp. in the outlet water. It was a one-time survey (Tab. 2, Ref. 32). Text in PDF www.elis.sk.

KEY WORDS: *Legionella* spp., water of dental unit waterlines, disinfection.

Introduction

The issue of microbial water contamination and biofilm formation in dental unit waterlines (DUWLs) has been discussed since the moment the first dental chair units (DCUs) were built. The first researcher to report the microbial contamination of DUWL output water was Blake in 1963 (1). This was followed by numerous studies describing both mechanical (rinsing, filtration) and chemical (sodium hypochlorite, chlorhexidine gluconate, hydrogen peroxide) elimination of microorganisms or biofilm in DUWL water (2, 3, 4, 5). It was also pointed out that the use of selected biocides (sodium hypochlorite, glutaraldehyde, isopropanol) may lead to the accumulation of these substances in biofilm matrix and their uncontrolled release. The authors consider this fact an additional risk for patients (6). Risk factors responsible for the contamination of DUWL water include the microbiological, biological, physical, and chemical indicators of the incoming water. Some of the most

important factors deciding the microbial contamination or quality of water include a very small lumen size (0.5–2 mm), extremely long distribution tubing (up to 10 m), frequent irregular stagnation of the incoming water, and biofilm formation. Microorganisms found in such environments are conditionally pathogenic bacteria, microscopic filamentous fungi, and various protozoa essential to the survival of *Legionella* (7, 8, 9). Therefore, it is necessary to establish the microbial diversity of microorganisms in the incoming water, and accordingly select the best biocides (10, 11). The effectiveness of biocides is tested under laboratory conditions as well as in dental office (12, 13). Tuttlebee and his team tested two peroxide-based biocides and found that Sanosil was more suitable for repeated DUWL decontamination (14). A multicenter study held in seven EU countries yielded highly interesting results concerning the microbial contamination of DUWL water. Only 49% samples of the DUWL output water complied with the recommendations of the American Dental Association (ADA), i.e. < 200 cfu/ml. The tests showed *Legionella pneumophila* sg. 1 to be rare (15). A similar study was conducted in 2006, aimed at establishing the effects of eight biocides on reducing the biofilm and planktonic microorganisms. The tests confirmed that continuous application of biocides produces better results compared with occasional applications. The most effective biocides were Dentosept P and Oxygenal 6.

In general, microorganisms living in the output water enter the dental office in form of water droplets of varied size as “bio-aerosol”, which poses a risk of cross-transmission of infectious agent to patients and medical staff. The risks of dental bioaerosol containing *Legionella* is highlighted by Szymańska (10). To date, only one terminal case of Legionnaire’s disease acquired in con-

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Acknowledgement: Grand support – Studentská grantová soutěž Univerzity Palackého 2015 – IGA LF 2015 005.

nection with dental treatment (a female patient aged 82 years) has been documented (16).

Even such a comprehensive publication as “Legionella and the Prevention of Legionellosis” dedicates not more than a few lines to the DCU water system (17). After 12 years, the Centers for Disease Control and Prevention (CDC) revised and updated its guidelines aimed at preventing nosocomial infections and infection control in dental health-care settings. The guidelines have largely remained the same, recommending again regular quantitative monitoring of microorganisms in DUWLs and preserving the bacteria limit at <500 cfu/ml of water (18). The ADA set a limit of < 200 cfu/ml for water used in dental operations. Another recommendation suggested qualitative testing of DUWL water for *Legionella* (18, 19).

The present descriptive cross-sectional study was designed to establish the current incidence or absence of *Legionella* bacteria in the DUWL water at the Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc.

Its findings are discussed on the basis of data from available literature, in particular those dealing with the concern that output water contaminated with *Legionella* poses a risk to patients and medical staff.

Material and methods

In the first stage of our survey, held in the second half of 2015, a total of 50 samples of DUWL output water were collected, together with 2 samples of potable water supplied to the entire clinic. The clinic receives water from a single supply of potable water from the Olomouc water distribution system. The sampler used in our survey was a 50-ml disposable sterile plastic conical vial with a screw cap (DispoLab). Prior to sampling, the incoming water distributed from the clinic’s water system was left to run for 5 minutes. Water entering the DUWLs was declared to be potable. The prosthetic, preservative, periodontal, and paediatric-dental departments were all equipped with the KaVo Systematica 1060 DCUs, and the Department of Conservative Dentistry had one more type of the DCU (one set) in use, namely Diplomat DM 10 Chirana. In the case of the Diplomat, a sample of distilled water from a built-in reservoir was collected. The orthodontic department was equipped with the KaVo ORTHOcenter 1058 O orthodontic DCUs.

The DUWL output water was sampled from a total of three points: micromotor supply tube, turbine hand piece quick coupler supply tube, and water/air blow gun supply tube. This output water was left to run for 5 minutes prior to sampling. In October 2015, the waterlines of 10 DCUs were disinfected with 1% Stabimed solution. This concentration requires a 30-minute exposure, and has bactericidal, fungicidal, mycobactericidal, tuberculocidal, and virucidal effects. The active substance is cocopropylendiamine (20). This particular method of DUWL disinfection was recommended by a service technician. After the exposure time had elapsed, the DUWLs were rinsed with potable water for 15 minutes. Prior to testing, the output water was left to run from the sampling site for the duration of 5 minutes.

Ten DCUs were selected for the DUWL disinfection. Of these, eight tested positive for *Legionella* sp. and two tested negative for *Legionella* sp. in their output water in Stage 1. Control sampling of output water (survey stage 2) was carried out on the same days as the disinfection, following the same procedure as described above.

Immediately after collection, the samples of output water were delivered at temperatures less than 18°C to the Testing Laboratory No. 1393, accredited by the Czech Accreditation Institute according to CSN EN ISO/IEC 17025, of the Public Health Institute Ostrava, Centre of Hygienic Laboratories, Olomouc branch, for processing.

The *Legionella* strain was culture-confirmed using method under SOPOV 913 (specification ČSN ISO 11731, ČSN ISO11731-2). Colonies of presumptive *Legionella* organisms were confirmed by serotyping. In one case, *Legionella* was determined with the help of molecular biological typing at the Ostrava Health Institute, Department of Molecular Biology (*Legionella quateirensis*).

Note: The confirmed estimated number of *Legionella* bacteria is specified as cfu (colony forming units) of *Legionella* species per 100 ml of water sample.

Results

From the total of 50 samples of output water (survey stage 1), 18 samples (36.0 %) tested positive for *Legionella* bacteria, whose quantity was specified as cfu/100 ml of sample. The tests confirmed the presence of *L. pneumophila* and presumptive presence of other *Legionella* species. Serotyping identified the following species:

Tab. 1. Microbial contamination of dental unit waterlines (survey stage 1).

	Department and number of dental units (n)					Total n=50
	Conservative dentistry n=11	Prosthetic n= 12	Periodontology n=7	Pediatric dentistry n=10	Orthodontic n=10	
Positive samples (%)	4 (36 %)	6 (50 %)	4 (57 %)	4 (40 %)	0 %	18 (36 %)
Range (cfu/100ml)	2.8x10 ³ -2.9x10 ⁵	10-6.6x10 ³	60-5.8x10 ³	10-7.5x10 ⁴	0	10-2.9x10 ⁵
<i>L. pneumophila</i> sg. 4						
Positive samples (%)	4 (100 %)	2 (33 %)	4 (100 %)	4 (100 %)	–	14 (78 %)
<i>L. anisa</i>						
Positive samples (%)	–	3 (50 %)	1 (25 %)	–	–	4 (22 %)
<i>L. quateirensis</i>						
Positive samples (%)	1 (25 %)	4 (67 %)	2 (50 %)	–	–	7 (39 %)

cfu – colony forming units

Tab. 2. Qualitative and quantitative changes after disinfection of DUWLs.

Department	Samples before disinfection (cfu/100ml)	Microbiological indicator	Samples after disinfection (cfu/100ml)	Microbiological indicator
Conservative dentistry	3.4x10 ³	<i>L. pneumophila</i> sg. 4 <i>L. quateirensis</i>	0	–
	0	–	0	–
Prosthetic	2.8x10 ³	<i>L. pneumophila</i> sg. 4	0	–
	10	<i>L. pneumophila</i> sg. 4	0	–
Periodontology	3.6x10 ³	<i>L. pneumophila</i> sg. 4 <i>L. anisa</i>	0	–
	1.8x10 ³	<i>L. pneumophila</i> sg. 4 <i>L. quateirensis</i>	0	–
Pediatric dentistry	8x10 ²	<i>L. pneumophila</i> sg. 4 <i>L. anisa</i> <i>L. quateirensis</i>	4x10 ²	<i>L. pneumophila</i> sg. 4
	7.5x10 ⁴	<i>L. pneumophila</i> sg. 4	0	–
Orthodontic	1.4x10 ³	<i>L. pneumophila</i> sg. 4	0	–
	0	–	0	–
	0	–	0	–

cfu – colony forming units

Legionella pneumophila sg. 4 alone or mixed with other *Legionella* species was found in 14 (i.e. 77.78 %) of the total 18 positive samples of the output water.

Legionella anisa (4 samples, all mixed with another *Legionella* species)

Legionella quateirensis (8 samples, of which 6 were mixed with another *Legionella* species).

All 10 samples of output water in the orthodontic offices were culture-negative, free of any *Legionella*.

The two samples of the incoming potable water were culture-negative; no *Legionella* was detected. Table 1 shows the number of the output water samples collected at the clinic's departments and the results of the determination of *Legionella* bacteria (presence/absence) in Stage 1 of the survey.

In October 2015, the waterlines of 10 DCUs were disinfected with 1 % Stabimed solution. Of the eight samples of DCU output water that had been previously found to contain *Legionella*, seven tested negative for *Legionella* after disinfection. The eighth sample of output water, which showed the presence of *Legionella* in Stage 1 remained positive even after disinfection. The sample, however, underwent both qualitative and quantitative changes. Two samples of DCU output water that were found free of *Legionella* in Stage 1 of the survey, yielded the same results in Stage 2 of the survey.

Discussion

DUWLs are regularly contaminated with microorganisms ranging from 10² to 10⁸ cfu/ml of output water. The first mention of *L. pneumophila* sg. 1 in the DCU output water dates to 1986, Germany, where water from 42 dental units was surveyed and *Legionella* was identified in four of the units (21). The Czech Republic dental chair units are required to be connected to potable water mains. Its quality needs to conform to current legis-

lation (22). During dental procedures, dentists need a considerable amount of water. As scientists have long established the fact that output water is microbially contaminated to varied levels, dentists are recommended to use sterile water for surgical procedures (18). Microbial contamination of DUWL water occurs as a result of three vehicles: microbial quality of incoming water, bacteria present in the biological material of the treated patient (back flow), and proliferation of all these microorganisms, which may produce a biofilm on the DUWL's inside wall. Potable water is not sterile and contains planktonic aerobic Gram-negative and non-fermenting bacteria. Most of these organisms are non-pathogenic or conditionally pathogenic. The most accurate current information on the composition and dynamics of DUWL bacterial community was published by Costa et al. They worked with pyrosequencing data to identify changes in the DUWL bacterial community, and included *Legionella* spp. and *Pseudomonas* spp. among potential human pathogens. These two genera were detected both in the incoming water and output water following water stagnation, and subsequently in output water following the application of rotary instruments. The authors conclude that microbes in this community may infect patients or medical staff at any point of time and harm their health. These microbes are small droplets in an infectious bioaerosol, which develops during dental treatment (23, 24).

Our study aimed solely at detecting *Legionella* in DUWLs. From the total of 50 samples of output water (survey stage 1), 18 samples (36.0 %) tested positive for *Legionella* organisms, whose quantity was specified as CFU/100 ml of sample. Serotyping identified the following species: *Legionella pneumophila* sg. 4, *Legionella anisa* and *Legionella quateirensis*. The prevalence of microbial contamination of CDUs is established to be 36.0 % which corresponds to the values of contaminated DCUs reported by Szymańska in her review (10–78 %). Likewise, quantitative values such as cfu/100 ml of output water correspond to the data

reported by Szymańska based on multiple sources. The range of quantitative values established in our study appears near the bottom limits of the mentioned figures (10).

The first case of an infection caused by *Legionella pneumophila* sg. 4 was reported in 1978 in Los Angeles (25). Another case of an infection caused by *L. pneumophila* sg. 4 was also described in Los Angeles. This *Legionella* species was cultured from the articular tissue of a woman aged 80 years. The majority of extrapulmonary infections are associated with direct inoculation of the microorganism into a wound site during bathing or upon contact with contaminated water (26). In general, extrapulmonary *Legionella* infections are more prevalent in immunocompromised patients and patients with rheumatoid arthritis (27).

Legionella anisa was described as a new *Legionella* species by Gorman and his team (CDC, Atlanta) from potable water collected in hospitals in Chicago and Los Angeles during an outbreak of hospital-acquired Legionnaires' disease, and from cooling tower water (28). Bornstein and his team in France were the first to isolate this *Legionella* species from clinical material, particularly pleural fluid of a young man who had had two cytotoxic chemotherapy regimens (29). *Legionella anisa* currently ranks among non-*L. pneumophila* spp. that pose a threat to immunosuppressed patients. In 2003, Yamamoto and his team isolated *L. anisa* from multiple sites of a hospital water system, including shower heads in an obstetrics ward. This marked the first isolation of *L. anisa* in a hospital (30). As his *Legionella* species is more often isolated from environment than from clinical material, it is less pathogenic for humans compared with *L. pneumophila*. However, the authors stress the fact that *L. anisa* may cause infection in immunocompromised patients (31).

Legionella quateirensis was isolated as a separate *Legionella* species from water and was described on the basis of serological and biochemical properties of colonies cultured on buffered charcoal-yeast extract agar (BCYE) in 1993 (32).

Conclusions

Water quality was tested according to the above-mentioned SOP. The prevalence of microbially contaminated DCUs established in our study, namely 36.0 %, corresponds to the values of contaminated DCUs reported in foreign literature. The effectiveness of one-time disinfection was proven by the absence of *Legionella* and by quantitative and qualitative changes that took place in one sample of the output water. We are unable to comment on the duration of effects of the biocide used. We believe that based on foreign literature data on DUWL microbial contamination, regular decontamination of DCUs should be included in the operating rules of dental offices, and above all, carried out.

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Received January 12, 2017.

Accepted January 28, 2017.