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Legionella pneumophila contamination of a dental unit water line system in a dental teaching centre

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Ma'ayeh SY, Al-Hiyasat AS, Hindiyeh MY, Khader YS. *Legionella pneumophila* contamination of a dental unit water line system in a dental teaching centre.

Abstract: *Objective:* This study aimed to evaluate the extent of *Legionella pneumophila* contamination in a dental unit water line (DUWL) at a Dental Teaching Centre in Jordan. *Methods:* Ten dental units were sampled from each teaching clinic, namely conservative dentistry, periodontology and prosthodontics. Samples were collected from the air/water syringe, high-speed hand piece and water cup filler. Sampling time was at the beginning of the working day (before the dental unit was used), after 2 min of flushing, and at midday. *Results: Legionella pneumophila* counts ranged between 0 and 8.35×10^3 (CFU ml^{-1}). *Legionella pneumophila* was detected in 86.7% of the dental units at the beginning of the working day, 40% after 2 min flushing and 53.3% at midday. The highest *L. pneumophila* counts were found at the beginning of the working day which were reduced by flushing the waterlines. The conservative dentistry clinic had the highest contamination level followed by the periodontology and prosthodontics clinics ($P < 0.05$). The rate of contamination can be ascribed to the dental procedures performed in the clinics, the degree of using the hand pieces, and water softening and heating. *Conclusions:* The difficulty of completely eliminating micro-organism contaminating water used for dental treatment and the resulting biofilm suggest that flushing of DUWL can be a first solution in reducing *L. pneumophila* counts, while the incorporation of a disinfection method is highly recommended. Water heating and softening should be considered in practicing dentistry as factors that may aid in *L. pneumophila* proliferation inside the DUWL.

Key words: dental unit water line; *Legionella pneumophila*; teaching clinics

Introduction

Legionella species may be present in a variety of water systems, including cooling towers, spas, water storage tanks and shower heads (1, 2). Following their isolation from such water systems, stagnant water in dental units has also been identified as a possible source for this micro-organism and its infection (3, 4). *Legionella* species are pathogenic micro-organisms which can be spread via aerosols (1) and result in two forms of disease: a pneumonic form called Legionnaires' disease (LD) and a non-pneumonic form called Pontiac fever which can even pass undiagnosed (1). *Legionella pneumophila* is the most common cause of *Legionella* pneumonia, with serogroup 1–6 being the most frequently implicated in infections of the respiratory tract (5).

Several studies have indicated that dentists and dental staff have higher rates of respiratory infections than the general public (6–8). Moreover, higher rates of seropositivity for *Legionella* antibodies have been found among dental personnel than among the general public (9–11). The aerosols generated by high-speed drills and sprays are means of spreading *Legionella* to the dental team or even to the patients (10, 12). Despite these facts, Oppenheim et al. (13) found no evidence that the presence of *Legionella* in dental units caused infection and there have been no cases that identify dental units as a source of LD.

Indeed, whatever the case, *Legionella* species are regularly isolated from dental units' waterlines (DUWLs), where they can reach concentrations of 10^2–10^4 CFU ml^{-1} (14, 15). *Legionella* survive within biofilm in water systems. The bacteria are more easily detected from swab samples of biofilm than from flowing water, suggesting that the majority of the Legionellae are biofilm associated (16).

The hypothesis of this study was that the dental procedures performed at different speciality clinic may have an influence on the level of contamination and colonization of *Legionella* in the DUWLs system. The use of municipal water in dental treatment, water softening and heating will be evaluated as factors that aid in the amplification of *L. pneumophila* inside the DUWL which could be applied elsewhere in the world as the clinics of different countries may operate under similar conditions. Therefore, this study was conducted to evaluate the extent of *L. pneumophila* contamination in the dental units' water (DUW) in the various clinics at a Dental Teaching Center. Moreover, this study will evaluate the effect of DUWLs flushing on the rate of contamination by *L. pneumophila*.

Materials and methods

Experimental design

Thirty dental units were selected for this study that were used most often for the dental treatments in the Dental Teaching Center of Jordan University of Science and Technology. Water samples were collected from 10 dental units from each of three groups of teaching clinics, namely conservative and pedodontics as a first group and was referred to as conservative dentistry clinic, the second group was the periodontology clinic and the third was the prosthodontics clinic. The water samples were collected from the outlet of air/water syringe, high-speed hand piece and water cup filler. Samples were taken at the beginning of the working day (before use), after 2 min of waterline flushing, and at midday when the dental units were being used (after 20 s of DUWLs flushing).

Sample collection and plating

Before sample collection, the end of each hand piece was disinfected with 70% alcohol to avoid other sources of contamination. Water splashing was minimized when filling the sample container and any contact between the hand piece and the container was avoided. A volume of 100 ml of water was collected in sterile containers, containing 0.1 gm 100 ml^{-1} of sodium thiosulphate ($Na_2S_2O_3{\cdot}5H_2O$) to remove residual chlorine. Samples were kept refrigerated at 4–8°C during transfer and processed directly after arriving at the laboratory in a period of less than 2 h. Water samples (100 ml) were filtrated using a 0.2-μm-pore size porosity polycarbonate filter (Nuclepore Corp., Pleasanton, CA, USA). The filter was then removed aseptically with sterile forceps and placed in a tube containing 10 ml of sterile phosphate-buffered saline. The tubes were vortexed for 1 min to detach bacteria from the membrane filter surface. The suspension was then placed into sterile tubes (aliquots of 1 ml). The aliquots of the concentrated samples were pretreated with hydrochloric acid–potassium chloride (pH 2.2) to eliminate non-*Legionella* organisms (1). A sterile 1 ml of the acid pretreatment reagent (pH 2.0, 0.2 M KCl/HCl) was added to the aliquots for 15 min at room temperature and then neutralized using 1 ml of alkaline neutralizing reagent (0.1 N KOH). A volume of 0.1 ml of the acid-treated aliquots was inoculated into buffered charcoal yeast extract-alpha (BCYE-α) agar medium supplemented with growth supplement SR 110 A and the selective GVPC supplement SR 152 E (Oxoid Ltd, Basingstoke, Hampshire, UK) (1).

The inoculum was spread with a sterile glass rod and incubated at 35°C humidified atmosphere for up to 10 days (1). Plating was performed in duplicates. *Legionella pneumophila* presumptive colonies were white–blue–grey in colour and up to 2 mm in diameter with a ground glass appearance. In addition, the water source supplying the dental units at the centre was investigated (two water tanks and two respective softeners). A volume of 100 ml of water sample was taken weekly over the sampling period (3 months) from the water tanks before the entrance of water to the softener filter, and after being softened through an attached faucet, the samples were treated as described previously.

Verification of *Legionella* spp. colonies

The American Type Culture Collection of *L. pneumophila* serogroup 1 (ATCC 33152) was used as a reference in the entire confirmatory testing. Presumptive colonies of *L. pneumophila* were isolated and streaked on the surface of BCYE-α agar plates supplemented with L-cysteine. The plates were incubated for 5 days at 35°C and then tested for Gram stain, catalase and oxidase reaction, motility test, and gelatin hydrolysis: (17). For the identification of *L. pneumophila* at the species level, the hippurate hydrolysis test was performed, in addition to the latex agglutination test (Oxoid Ltd, Basingstoke, Hampshire, UK) which was performed according to the manufacturer's protocol, and was based on clumping observation to determine the test species.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, version 11.5: SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2003 were used for data processing and data analysis. Medians and frequencies of bacterial counts over all combinations of time, source and clinic were presented. Because 30 dental units were sampled repeatedly, general linear model (GLM) repeated measures was used to analyse data. No data were missing. Results of evaluation of assumptions led to logarithmic transformation of *L. pneumophila* count to improve normality. The model comprises the explanatory variables sampling time (at the beginning of the working day, after 2 min of flushing and at midday), source of sample (water syringe, high-speed hand piece and the water cup filler) and clinic (conservative dentistry, prosthodontics and periodontology). Bonferroni test was used to perform pairwise comparisons between group means on the log scale. A value of $P < 0.05$ was considered statistically significant.

Results

Legionella pneumophila was not detected in any of the samples obtained from the first water tank (located on the ground floor) and its respective softener in the Dental Teaching Center of JUST. However, *L. pneumophila* was detected in the second water tank samples (located on the roof of the building) with an average of 2 ± 0 CFU ml^{-1}, and its respective softener water outlet with an average of 14 ± 2 CFU ml^{-1}. Statistically significant differences were found between the second water tank and its softened water samples (paired t-test, $P = 0.005$).

All of *L. pneumophila* isolates belong to serogroup 1 including those isolates of the second water tank and its respective softener. The counts of *L. pneumophila* obtained from the DUW samples ranged between 0 and 8.3×10^3 CFU ml^{-1}. *Legionella pneumophila* was detected in 86.7% (26/30) of the dental units at the beginning of the working day, in 40% (12/30) after 2 min of flushing and 53.3% (16/30) at midday. At the beginning of the working day, all dental units in conservative dentistry and periodontology clinics were contaminated by this micro-organism. The distribution of *L. pneumophila* counts for the dental units investigated by type of clinic and sampling time is depicted in Tables 1–3.

The medians of bacterial counts in DUW samples according to the type of clinic, source of the sample and sampling time are presented in Table 4. *Legionella pneumophila* was unevenly distributed among the three different clinics at the Dental Teaching Center, with the conservative dentistry showing the

Table 1. ***Legionella pneumophila*** **mean plate counts obtained from the air/water syringe in the conservative dentistry, prosthodontics and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		L. pneumophila mean plate counts (CFU ml^{-1})*				
Clinic	No. of units	0	10^0–10^1	11–10^2	101–10^3	>10^3
Before use						
Conservative	10	0	0	1	3	6
Prosthodontics	10	7	3	0	0	0
Periodontology	10	2	0	8	0	0
After 2 min of flushing						
Conservative	10	0	0	3	4	3
Prosthodontics	10	10	0	0	0	0
Periodontology	10	8	2	0	0	0
At midday						
Conservative	10	0	0	3	4	3
Prosthodontics	10	10	0	0	0	0
Periodontology	10	8	2	0	0	0

*Values represent the number of units that were contaminated within the range of the column title.

Table 2. ***Legionella pneumophila*** **mean plate counts obtained from the high-speed hand piece in the conservative dentistry, prosthodontics and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		L. pneumophila mean plate counts (CFU ml^{-1})*				
Clinic	No. of units	0	10^0–10^1	11–10^2	101–10^3	$>10^3$
Before use						
Conservative	10	0	0	1	4	5
Prosthodontics	10	4	5	1	0	0
Periodontology	10	0	0	8	2	0
After 2 min of flushing						
Conservative	10	0	0	4	3	3
Prosthodontics	10	10	0	0	0	0
Periodontology	10	8	0	2	0	0
At midday						
Conservative	10	0	0	4	3	3
Prosthodontics	10	10	0	0	0	0
Periodontology	10	4	4	2	0	0

*Values represent the number of units that were contaminated within the range of the column title.

Table 3. ***Legionella pneumophila*** **mean plate counts obtained from the water cup waterline in the conservative dentistry, prosthodontics and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		L. pneumophila mean plate counts (CFU ml^{-1})*				
Clinic	No. of units	0	10^0–10^1	11–10^2	101–10^3	$>10^3$
Before use						
Conservative	10	0	0	3	4	3
Prosthodontics	10	6	4	0	0	0
Periodontology	10	1	0	7	2	0
After 2 min of flushing						
Conservative	10	1	1	3	3	2
Prosthodontics	10	10	0	0	0	0
Periodontology	10	8	0	2	0	0
At midday						
Conservative	10	1	0	3	5	1
Prosthodontics	10	10	0	0	0	0
Periodontology	10	5	3	2	0	0

*Values represent the number of units that were contaminated within the range of the column.

highest median at the three sampling times, followed by periodontology and prosthodontics clinics. Moreover, the air/water syringe demonstrated the highest count for the clinic of conservative dentistry followed by the high-speed hand piece and the water cup filler. Overall, the highest *L. pneumophila* counts were obtained at the beginning of the working day.

The results of GLM repeated measures analysis for the log scale-transformed counts of the data showed a statistically significant clinic by sampling time interaction ($P < 0.001$). All other two- and three-way interactions were not statistically significant. Furthermore, there was no significant difference in the average log counts between the three sources ($P = 0.321$). Figure 1 plots the profiles for the three clinics over the three sampling times. For all clinics, the log count was the highest at the beginning of the day (before use). Purging the water lines for 2 min reduced the log count significantly in all clinics. At midday, log count increased for conservative dentistry and periodontology but remained lower than that obtained at the beginning of the working day. However, the log counts for prosthodontics clinic did not change.

Bonferroni test showed statistically significant differences in the log count at the beginning of the day between the three clinics being the highest between conservative dentistry and prosthodontics clinic. However, the log counts after flushing for 2 min and at midday was significantly different between the conservative dentistry and the other two clinics but not between prosthodontics and periodontology clinics.

Discussion

The main source of water used in the Dental Teaching Centre is coming from the municipal authority in Irbid Province. This water is treated by a softening system mounted at the main water pipeline supplying the centre to remove particles that may damage and corrode the dental units and water pipelines, then distributed to the clinics in the centre. In this study, the water source supplying the dental units was examined for the presence of *L. pneumophila*. The absence of *L. pneumophila* from the first water tank and its respective softener can be ascribed to its probable presence in lower counts which requires larger water volumes to be taken as a sample (18, 19). Similarly, Challacombe and Fernandes reported that the quantity of water will affect the chances isolating bacteria (20). However, the presence of *L. pneumophila* in the second water tank (third floor) despite the presence of free residual chlorine in a concentration of 0.1 mg l^{-1} (DPD colorimetric method) be ascribed to the higher distance from the distribution point (first water tank) which is present on the ground floor. Indeed, Legionellae survive within building water system (21). In a study that evaluated *Legionella* infection risk from domestic hot water, Borella *et al.* (22) found that residing in higher floors of large buildings increased the risk of *Legionella* contamination. Moreover, Borella *et al.* (22) found that *Legionella* was found in both chlorinated and untreated water, confirming the low efficacy of this disinfecting system on microbe eradication (23). Furthermore, it is worth mentioning that the water pipelines used in the dental centre are made of polyvinyl chloride

Table 4. **The medians of bacterial counts (CFU ml^{-1}) in dental units' waterline samples according to the type of clinic, source of the sample and sampling time**

Clinic	Source of sample	Sampling time		
		Beginning of the working day	Two minutes of flushing	Midday
Conservative	Air/water syringe	1615.0	679.0	723.0
	High-speed hand piece	1030.0	428.5	480.0
	Water cup filler	424.0	141.0	174.0
	Total	938.5	268.0	292.5
Prosthodontics	Air/water syringe	0.0	0.0	0.0
	High-speed hand piece	2.0	0.0	0.0
	Water cup filler	0.0	0.0	0.0
	Total	0.0	0.0	0.0
Periodontology	Air/water syringe	13.0	0.0	0.0
	High-speed hand piece	34.0	0.0	1.0
	Water cup filler	35.0	0.0	0.5
	Total	30.5	0.0	0.0

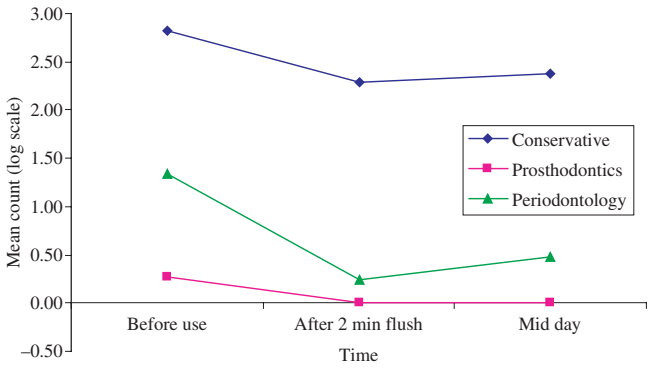

Fig. 1. The contamination profiles of *Legionella pneumophila* in the conservative dentistry, prosthodontics and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday).

(PVC) plastic (refurbished in year 2000) which may have an effect on supporting biofilm formation and the consequent amplification of *L. pneumophila* originating from the municipal water source. However, this could be based on postulations that require further investigation. The presence of *Legionella* in the second water softener and in higher numbers (six times more) can lead to the presumptive conclusion that water softener may provide an environment suitable for amplifying *L. pneumophila* within it by utilizing the accumulated minerals and nutrients in the softener system to grow.

In fact, higher temperatures were found for the DUW samples of the conservative dentistry and periodontology clinics (34 ± 1°C) in comparison with the water source, the softened water samples, and those of the prosthodontics clinic (24 ± 1°C). Such an elevation in DUW samples temperature is due to the fact that the Sirona C6 dental units (Sirona Dental Systems GmbH, Bensheim, Germany) which are used in the two previously mentioned clinics since 2001 posses a heating system that warm the water in the dental units. Such heating systems can comprise a favourable condition for microbial amplification inside the tubes of dental units. In comparison, the dental units that are in use in the prosthodontics clinic were from the A-dec (A-dec International, Newberg, OR, USA) and the Castellini type (Castellini S.P.A, Bologna, Italy) which are in use since 1995 and lack such water heating systems.

Legionella species are regularly isolated from dental unit waterlines (14, 20, 24). In this study, the isolates of *L. pneumophila* were found to be serogroup 1. In a recent study on contaminated dental units, Zanetti *et al.* recovered *L. pneumophila* serogroup 1 in nearly all sites positive for *Legionella* species (25). In these conditions, the possibility of contaminated aerosol inhalation might be more frequent for *L. pneumophila* serogroup 1. The counts of the isolated *L. pneumophila* from all DUW samples ranged between 0 and 8.35×10^3 CFU ml^{-1}. Barbeau *et al.* (14) found that *Legionella* spp. that are isolated from DUWLs can reach concentrations of 10^2–10^4 CFU ml^{-1}. However, in this study, *L. pneumophila* counts obtained from the water source and the softener outlet were much less than those obtained from the DUWLs, suggesting that *L. pneumophila* is amplified within the DUWLs' biofilm and shed to the water as it flows and forces pressure on the biofilm. Formation of biofilm can provide means for survival and dissemination of *L. pneumophila* (26, 27) and interfering with the efforts to eradicate bacteria from water systems (28).

In the present study, *L. pneumophila* was detected in 86.7% of the dental units. Luck *et al.* (29) isolated *L. pneumophila* from 58% of the dental offices they tested. However, other previous studies reported the isolation of *L. pneumophila* from 33.3% (30), 33% (31), 25% (32) and 21.8% (25) of the dental units that were investigated. On the other hand, Atlas *et al.* (33) were able to

detect *Legionella* spp. in 68% of the dental units using PCR gene probe. Montagna *et al.* (30) demonstrated that 43.5% of the tested DUW samples harboured *Legionella* in concentrations of 10^3–10^4 CFU l^{-1}, while 30.4% of the DUW samples exhibited counts of more than 10^4 CFU l^{-1}.

The conservative dentistry had the highest rate of colonization by *L. pneumophila* among the three clinics and the three sampling times, followed by the periodontology and prosthodontics clinics. This high contamination rate in the conservative dentistry can be ascribed primarily to the type of dental treatment performed in each clinic which provides *L. pneumophila* with the highly rich nutrients (saliva, blood, pus, etc.) (34–36) that can support its growth, especially when the dental units' anti-retraction valves cannot prevent the back flow of water from the patients' mouth into the DUWLs when they are damaged or not working properly. In addition, the (10 ± 1°C) increase in temperature of the DUW samples of the conservative and periodontology clinics can greatly enhance replication of *L. pneumophila* in DUW system as previously reported by Rogers *et al.* (16) The air/water syringe of the conservative dentistry demonstrated the highest counts of *L. pneumophila* followed by the high-speed hand piece and the water cup filler. Similar results have been reported by Challacombe and Fernandes (20) who found that the air/water syringe was more susceptible to contamination with *Legionella*, even the high-speed drill could have been affected with high rates. The flow rate and quantity of water that come out of the hand piece may have an influence on the contamination level due to the capacity for washing and removal of biofilm within the waterline (37).

The significant effect of the clinic can also be ascribed to a number of collective factors that may influence the bacterial levels in dental units: the type of materials used in the tubing, the bore size of the tubing and the frequency of use (38–41). Indeed, due to the fact that mainly the removable prosthodontics are performed in the prosthodontics clinic, the hand pieces will not be used frequently; therefore, the *L. pneumophila* was found to be present in much lower counts in the prosthodontics clinic than in the conservative dentistry and periodontology clinics, as a result of less utilization rate, no water heating and consequently, less favourable conditions for bacterial amplification. Challacombe and Fernandes (20) reported that dental units that had been unused for relatively long periods did not yield high levels of *L. pneumophila*. On the other hand, the authors (20) also stated that the colonization and detection of *L. pneumophila* depended more on the unit model than the amount of use. Indeed, in the present study the nature of the dental treatment performed in the clinic appeared to be the major factor that has an influence in the contamination level. Flushing the DUWLs for 2 min have reduced the counts in all clinics. Many previous reports dealing with DUWLs contamination with heterotrophic bacteria have shown that draining the waterlines for several minutes reduced the heterotrophic plate counts (HPC) significantly (41–44). Similar conclusion can be drawn here for the effect of flushing on reducing *L. pneumophila* counts in the DUWLs.

The engineering design and the nature of materials used in the devices may influence the human health (45, 46). O'Brien and Bhopal (47) suggest that inhaling a few *Legionella*-laden amoebae might cause LD. Thus, only few unfortunate people inhale enough amoebas, whereas others escape by not inhaling the dose required for illness. They also suggest that inhaling bacteria alone may cause subclinical infection (47). However, attack rates in outbreaks of LD are usually low (0.1–5% of exposed persons develop clinical symptoms), and older people, men, smokers and patients with chronic lung disease or systemic immunosuppression are more susceptible (48). Guidelines must be issued to circumvent such problems and protect both patients and dentists, while practicing dentistry. The presence of a pathogen, such as *L. pneumophila*, in water used for dental treatments may pose a threat to both patients and the dental team by inhaling contaminated aerosols. Heating the water must be reconsidered by dental units' manufacturers to overcome problems associated with temperature-enhanced microbial amplification. Moreover, water softeners must be checked and maintained. Anti-retraction valves must be maintained regularly to overcome problems associated with the flow back of bacteria and oral fluids from patient mouth into the DUWLs. In addition, a disinfection method must be applied to eliminate biofilm harbouring *L. pneumophila* organisms (49–53). Furthermore, the materials used in the manufacturing of tubing system of the dental units must be tested against the formation of biofilm and when possible replaced by ones that do not support biofilm formation or with antimicrobial properties (54). Overall, all these factors need to be further studied all together while monitoring the water source, pipelines and examining the presumptively present biofilms inside the waterlines to particularly identify the factors that play the major role in the contamination with *L. pneumophila*. The use of microbial filters fitted into the hand pieces may be a good solution to prevent microbial access into the atmosphere surrounding the dental workers and patients, open wounds and oral lesions of the patients' mouth.

Conclusion

Under the conditions of the present study the following was concluded:

1. Of 30 dental units investigated, the water of 26 units (86.7%) were contaminated with *L. pneumophila* at the beginning of the working day and before use.

2. Flushing the DUWL for 2 min significantly reduced *L. pneumophila* counts and the number of the units contaminated was reduced from 86.7% to 40%.

3. The conservative dentistry units significantly had higher counts of *L. pneumophila* compared with periodontology and prosthodontics clinics.

4. Raising the temperature of water must be considered as a factor that aid in microbial amplification in the DUW systems.

5. The softener filter, if used, must be maintained and checked periodically to overcome any problems that may appear from being an environment suitable for amplifying pathogens.

6. Disinfection methods of DUWL system should be considered and applied to eliminate biofilm harbouring *L. pneumophila* organisms.

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