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Three key factors influencing the bacterial contamination of dental unit waterlines: a 6-year survey from 2012 to 2017

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Background: The contaminated output water from dental unit waterlines (DUWLs) is a potential risk to both patients and dental personnel who are frequently exposed to this water or aerosols. **Aim:** The purpose was to evaluate the contamination level and prevalence of bacteria in the output water of DUWLs, and to identify key factors to provide technical support for formulating relevant policies. **Methods:** We developed a special sampling connector designed for collecting dental handpiece output water and a measurement device to assess retraction of a dental chair unit (DCU). Output water from dental handpieces and air/water syringes were collected as representative of DUWLs. Water samples were tested with reference to China's national standard. **Findings:** From 2012 to 2017, 318 DCUs were randomly selected from 64 hospitals in Tianjin, China. Of these DCUs, 78.93% had no disinfection to prevent DUWL contamination. Three-hundred and forty-three (56.14%) samples complied with the guidelines on DUWL output water. The highest concentration of bacteria was 1.8×10^6 colony-forming units (CFUs)/mL. The three key factors of influence were as follows: daily or weekly disinfection of DUWLs; water supply source being hospital self-made purified water or purchased purified bottled water; and DCU with a valid anti-retraction valve. Potential infectious agents, including *Bacillus cereus*, *Burkholderia cepacia* and *Pseudomonas aeruginosa*, were isolated. **Conclusion:** There was a high rate of contamination in DUWLs. This highlights the need to develop national standards. There is a need to disinfect the DUWLs periodically and use a cleaner source of water; more attention should be paid to the efficacy of DCU anti-retraction valves.

Key words: Dental unit waterlines, retraction, bacteria, water microbiology, dental handpiece, air/water syringe

INTRODUCTION

The dental chair unit (DCU), classified as a medical device according to the EU Medical Devices Directive, is one of the most essential pieces of equipment in the routine practice of dentistry[1]. The DCU uses water to cool and irrigate dental instruments and tooth surfaces and provides rinse water during dental treatments. A complex network of interconnected narrow-bore plastic dental unit waterlines (DUWLs) supply water to dental instruments. DUWLs are considered as reservoirs for potential pathogens of human or environmental origin[2–4], including *Legionella*, because the narrow-bore tubing offers an optimal environment for biofilm development[5]. Many studies have reported that the concentrations of bacteria in DUWLs can reach as high as 10^4–10^6 colony-forming units (CFUs)/mL[3,4,6], which is a potential risk for dental patients and staff, especially for patients with compromised immunity[7]. There are many factors that contribute to the contamination of DUWLs, including anti-retraction valve failure, the presence of water heaters, a piped water supply or an impure water supply.[4,7–13]. How to identify the key factors from many influencing factors is an important topic in infection control research. In 2017, Schonning *et al.*[14] reported the case of an elderly immunocompromised man who died from legionellosis at a hospital in Uppsala, Sweden. This report highlighted the risks that are associated with *Legionella* in the output water of DUWLs. The link was confirmed by pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS). The previously reported Italian case, to the authors' knowledge, was the first reported verified case of legionellosis acquired through a DCU[15]. With the application of molecular biology technology, such as PFGE, WGS and core genome multi-locus sequence typing (MLST), it may be possible to detect more such cases. According to the US Centers for Disease Control and Prevention (CDC), 68.2% of people in the USA made dental visits in 2015[16]. Assuming that this frequency is the same in North America, Europe, Japan, South Korea and Australia – highly developed countries with similar high-quality infectious disease surveillance systems – almost 1 billion people make dental visits annually in these countries[17]. Together with the rapid economic development in China, the Chinese people are increasingly seeking dental care[18]. A third National Oral Health Epidemiological Survey found that 63.41% of middle-aged adults and 60.32% of older adults were seeking dental visits[19]. Tianjin, one of the four municipalities of China, has provincial-level status (i.e. is situated directly below the central government). China has been late in monitoring bacterial contamination of DUWL output water. There were reports by other countries, as early as the 1960s, on the contamination of DUWLs[20]; in contrast, there were no published papers in Chinese on this subject until 2002. Tianjin CDC, a public institution that performs government functions using government finance, began to monitor contamination of DUWLs from 2012 onwards. To the best of our knowledge, no other Chinese investigators have reported on DUWL output water contamination in non-Chinese journals. The purpose of this study was to evaluate the degree of contamination and prevalence of bacteria in output water of DUWLs and to identify the key factors influencing this contamination in order to provide technical support for formulating relevant policies.

MATERIALS AND METHODS

Dental chair units

A total of 318 DCUs, from 64 hospitals in Tianjin, were selected from the DCU database by simple random sampling. At the time of this writing, there were about 991 DCUs in Tianjin, distributed across 16 districts, of which 10 are agricultural. Of the 318 DCUs, the average age was 5.72 ± 4.82 (range: 0.5–20) years, 14.15% were derived from stomatological hospitals, 66.98% were domestic brands, 33.33% were supplied with bottled water from independent water reservoirs, 16.98% were supplied directly by non-purified municipal water and for 78.93% no disinfection measures were taken to prevent DUWL contamination before our analysis. Of the 67 DCUs with DUWL control measures, 83.58% used chlorine-containing disinfectants, 13.43% applied electrochemically activated solutions and 2.98% used glutaraldehyde.

Collection of water samples

At each sampling, one of the authors randomly selected DCUs according to the sampling plan, and collected two water samples (one from the air/water syringe and the other from the dental handpiece) from each DCU, aseptically, using sterile gloves, single-use masks, and gowns at the beginning of the workday. In 2012, the first year of monitoring, only the air/water syringe output water was monitored because there was no suitable sampling method for collecting output water from the dental handpiece. For this purpose, a special sampling connector (*Figure 1*), designed for collecting dental handpiece output water, was developed. When this connector is connected to the DUWL, it replaces the dental handpiece; it only allows water to pass through and shields the air flow. Before taking the samples, we removed the dental handpiece and pressed the foot control to flush the waterline for 2 minutes, then installed the sampling connector to collect the water samples. Water samples (20 mL) from air/water syringes were collected, using a conventional approach[21], into sterilised test tubes, which were then placed at 4 °C in a sampling box and shipped to the laboratory within 2 hours of sample collection[22].

Processing of water samples

Water samples were tested and analysed with reference to China's national standard 'the standard examination methods for drinking water - microbiological parameters (GB/T 5750.12-2006)'[23]. One millilitre of well-mixed water was aseptically pipetted into a sterilised petri dish, then 15 mL of nutrient agar medium that had melted and cooled to about 45 °C was poured into the dish and the dish was immediately shaken to thoroughly mix the water sample with medium. Thereafter, the dishes were incubated at 37 °C for 48 hours. Heterotrophic plate counts (HPCs) were calculated as CFUs/mL. The threshold values established from American Dental Association (ADA) recommendations and US CDC guidelines (i.e. 500 CFUs/mL)[24] were used as criteria. The VITEK 2 (Vitek2 compack30; Biomerieux, Marcyl'Etoile, France) analyser was used to identify aerobic bacteria.

Testing the efficacy of DCU anti-retraction valve

In 2014, we developed a measurement device to assess retraction of a DCU[21] in accordance with ISO 7494-2:2015(E). If the DCU anti-retraction valve is valid, the volume of water retracted should not exceed

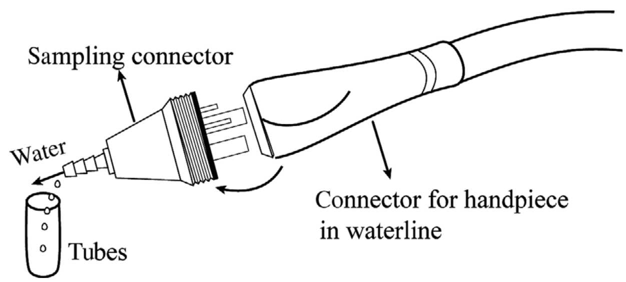

Figure 1. Sampling connector. The dimensions and thread characteristics of the sampling connector were identical to the hose connectors of air-driven dental handpieces (ISO 9168-2009). Water samples (20 mL) were obtained from a high-speed handpiece with the sterilised sampling connector.

40 μL, according to the American Dental Association/American National Standard (ADA/ANSI) specification #47 or ISO 7494-2:2015(E).

Collection of variables

A questionnaire of more than 10 variables was designed and administered, after each sampling, to a staff member. The variables were recorded and coded as follows: location of hospital (1 = Downtown area, 2 = Rural area); category of hospital (1 = Stomatological hospital, 2 = General hospital); level of hospital (1 = Level 1, 2 = Level 2, 3 = Level 3); type of DCU (1 = Imported, 2 = Domestic); DCU supply water (1 = Municipal water, 2 = Hospital self-made purified water, 3 = Purchased bottled water); DUWLs disinfection (1 = Not disinfected, 2 = Chlorine disinfectant, 3 = Other disinfectant); frequency of DUWLs disinfection (1 = Not disinfected, 2 = Once a day, 3 = Once a week, 4 = Once a month); bottle disinfection (1 = Not disinfected, 2 = Chlorine disinfectant, 3 = Other disinfectant); frequency of bottle disinfection (1 = Not disinfected, 2 = Once a day, 3 = Once a week, 4 = Once a month); and water monitoring (1 = Yes, 2 = No), using hot water (1 = Yes, 2 = No). DCU ID and sampling date were recorded for each water sample.

Statistical analysis

The microbial loads were converted into log10 to normalise the non-normal distributions for comparing the results over years. Chi-square tests were applied for comparing the differences of each value between groups and selecting variables for logistic regression analysis. We conducted a binary logistic regression analysis to correlate the variables with the quality of DUWL output water (0: ≤500 CFU/mL, 1: >500 CFU/mL). A significance level of 0.05 (two-sided) was used. Statistical Package for the Social Sciences Version 24.0 (IBM, Armonk, NY, USA) was used for data analysis.

Ethics approval

This study was independently reviewed and approved by the Institutional Review Board of Tianjin Centers for Disease Control and Prevention.

RESULTS

Microbial culture of water samples

A total of 611 DUWL output samples were collected from 2012 to 2017, comprising 318 samples from air/water syringes and 293 samples from dental handpieces. The microbial contamination values of water samples are shown in *Table 1*. The highest HPC values of output water samples were 1.8×10^6 CFUs/mL for handpieces and 1.7×10^6 CFUs/mL for air/water syringes. Taken together, 343 (56.14%) of 611 samples complied with the guidelines of the CDC on DUWL output water. Separately, the percent of output water from the dental handpiece and air/water syringes below the threshold were 53.58% and 58.49%, respectively. There were no significant differences between the two groups according to chi-square analysis ($\chi^2 = 1.491$, $P = 0.222$).

According to the annual statistics, except for 2012, the median of the bacterial concentrations of DUWL output water in all other years was lower than 500 CFUs/mL (*Figure 2*). From 2012 to 2017, the percent of DUWL and handpiece output water below the threshold was the same; from the low in 2012 the values began to rise yearly and after the peak in 2014 began to decline; in 2017, the percent was higher than in 2016 (*Figure 3*). By contrast, the percent of the air/water syringe output water below the threshold showed a decline from the peak reached in 2015.

Testing the efficacy of DCU anti-retraction

Two-hundred and ninety-three DCUs were tested for anti-retraction, of which 167 (57%) complied with ISO 7494-2:2015(E). *Table 2* shows that 334 (57%) of the 586 output water samples from DUWLs were below the threshold for the DCU anti-retraction test. Among these 334 samples, 224 (67.07%) complied with the guidelines of the CDC for DUWL output water (≤500 CFUs/mL). The percent of DUWL output water samples collected from DCUs using a valid anti-retraction valve was higher than that with an invalid anti-retraction valve, and the difference was statistically significant ($P < 0.05$).

Factor analysis

Table 2 presents the results of chi-square analysis for bacterial concentration (above or below the threshold of 500 CFU/mL) in DUWL output water, according to various underlying factors.

A logistic regression analysis was conducted to assess whether the model varied significantly. The Hosmer–Lemeshow goodness-of-fit test indicated that the model was well calibrated ($P = 0.731$). Only three factors were statistically significant after being brought into the model (*Table 3*). These were DUWL disinfection frequency, DCU supply water and DCU anti-retraction.

For DUWL disinfection frequency, daily disinfection [odds ratio (OR) = 0.246; 95% CI: 0.065–0.929] and

Table 1 Bacterial contamination of water samples from air/water syringes and dental handpieces [colony-forming units (CFUs)/mL]

Year	Air/water syringes					Dental handpieces					Total				
	n	Range	Median (P25–P75)	≤500 *n* (%)	≤100 *n* (%)	*n*	Range	Median (P25–P75)	≤500 *n* (%)	≤100 *n* (%)	*n*	Range	Median (P25–P75)	≤500 *n* (%)	≤100 *n* (%)
2012	24	2–30,000	970 (71.5–2,562.5)	11 (45.83)	10 (41.67)	0					24	2–30,000	970 (71.5–2,562.5)	11 (45.83)	10 (41.67)
2013	99	0–267,120	158 (33–1,360)	53 (53.54)	52 (52.53)	99	1–718,680	406 (100–11,200)	46 (46.46)	43 (43.43)	198	0–718,680	249.5 (55.25–3,000)	99 (50)	95 (47.98)
2014	58	0–1,700,000	125 (8.5–2,775)	38 (65.52)	31 (53.45)	58	0–1,800,000	390 (35.5–9,375)	36 (62.07)	32 (55.17)	116	0–1,800,000	235 (14.25–5,975)	74 (63.79)	63 (54.31)
2015	42	0–620,000	350 (73.5–1,375)	28 (66.67)	27 (64.29)	41	0–550,000	430 (140–2,500)	23 (56.10)	20 (48.78)	83	0–620,000	400 (98–1,600)	51 (61.45)	47 (56.63)
2016	50	0–66,000	180 (4–1,975)	26 (52.00)	24 (48.00)	50	0–64,000	370 (20.75–3,650)	27 (54.00)	19 (38.00)	100	0–66,000	290 (14.25–2,200)	53 (53)	43 (43.00)
2017	45	0–32,000	120 (26–1,600)	30 (66.67)	21 (46.67)	45	0–64,000	450 (91.5–2,950)	25 (55.56)	12 (26.67)	90	0–64,000	230 (35–1,925)	55 (61.11)	33 (36.67)
Total	318	0–1,700,000	190 (27.75–1,825)	186 (58.49)	165 (51.89)	293	0–1,800,000	410 (66.5–4,100)	157 (53.58)	126 (43.00)	611	0–1,800,000	280 (35–2,450)	343 (56.14)	291 (47.63)

weekly disinfection (OR = 0.518; 95% CI: 0.270–0.994) were statistically significant compared with non-disinfection regarding whether the DUWL output water was ≤500 CFUs/mL. Daily disinfection was better than weekly disinfection. There was no statistical difference between monthly disinfection and non-disinfection.

Similarly, in the three categories of DCU supply water, hospital self-made purified water (OR = 0.417; 95% CI: 0.242–0.716) and purchased purified bottled water (OR = 0.368; 95% CI: 0.162–0.837) showed a statistically significantly ($P < 0.05$) lower concentration of bacteria in the DUWL output water compared with municipal water used as the reference.

The difference between the above two factors was that DCU anti-retraction is a binary classification variable. It can be seen from the model results that the valid anti-retraction of the DCU (OR = 0.084; 95% CI: 0.038–0.186) compared with invalid anti-retraction was statistically significant for DUWL output water.

Pathogen detection

A total of 112 strains of bacteria were isolated from the output water samples of DUWLs, including *Bacillus cereus* (*n* = 34), *Burkholderia cepacia* (*n* = 10), *Pseudomonas aeruginosa* (*n* = 7), *Alcaligenes faecalis* (*n* = 6), *Staphylococcus epidermidis* (*n* = 5) and *Pseudomonas fluorescens* (*n* = 5). The other strains isolated were from 14 genera, mainly *Micrococcus*, *Comamonas* and *Staphylococcus*.

DISCUSSION

To our knowledge, this is the longest sampling study of DUWL output water contamination in China. The output water from dental handpieces and air/water syringes used in patient treatment is more likely to affect patients than dental personnel. However, it readily forms microaerosols, putting both patients and dental personnel at risk for inhalation of potentially pathogenic bacteria[3,9].

Contamination of DUWLs with bacteria has been documented by scientific evidence[7,25–30]. Microbial levels of 10^4–10^6 CFUs/mL are frequently reported in water samples from DUWLs[25–28]. The maximum concentration of bacteria in DUWL output water was 1.8×10^6 CFUs/mL in our study, which is higher than the concentrations most frequently obtained in other studies[25–28]. In our samples, 56.14% (343/611) complied with the threshold recommended by ADA or CDC guidelines, and 47.63% (291/611) met the China drinking water standards, that is, the number of bacteria should be ≤100 CFUs/mL. Regarding numerical values the results of this study showed that the percent of DUWL samples below the threshold

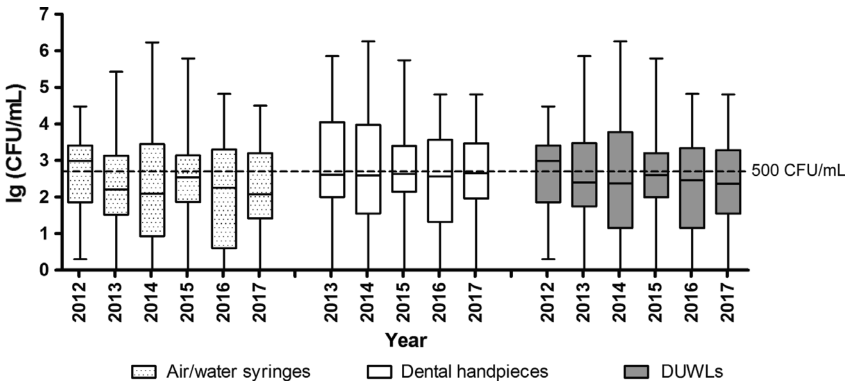

Figure 2. The values of microbial loads were converted into log10 × to compare the results over the years using box-and-whisker plots. A box-and-whisker plot shows a 'box' with a low edge at lower quartile, the high edge at upper quartile, the 'middle' of the box at the median and the maximum and minimum as 'whiskers'. The dotted line in the figure corresponds to lg500 colony-forming units (CFUs)/mL. DUWLs, dental unit waterlines.

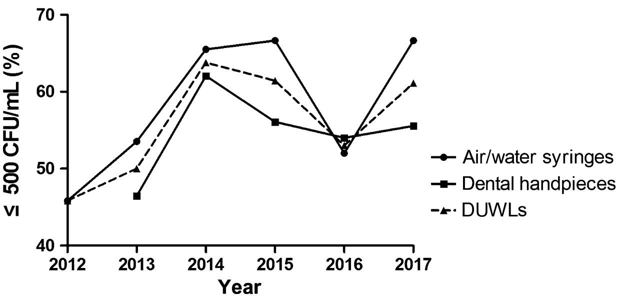

Figure 3. The lines demonstrate the trends of the output water samples that were below the threshold for dental unit waterlines, handpieces and air/water syringes from 2012 to 2017. CFU, colony-forming unit; DUWLs, dental unit waterlines.

values were lower than reported by Mardjan Arvand *et al.*[31]: 58 (64.44%) of 90 samples complied with the German drinking water standards of a total colony count of ≤100 CFU/mL for all dental units.

The high CFUs/mL and low percent of samples below the threshold were unexpected. There are several possible reasons. The most important, at present, China has not enacted any national standards or industry standards in this field. In China, many studies have evaluated DUWL output water either by the Standards for Drinking Water Quality of China (≤100 CFUs/mL)[32] or by the CDC guidelines (≤500 CFUs/mL)[24]. In addition to the lack of indicator thresholds, there is also a lack of management regulations and control measures for DUWLs, leading to a lack of supervision in hospitals and a lack of knowledge and attention regarding DUWL contamination of dental personnel[21].

Besides the lack of national standards, the causes of microbial contamination of DUWL output water are multifactorial[7]. These include DCU type, sampling season, type of hospital, DCU supply water, DUWL disinfection, DUWL disinfection frequency, bottle disinfection frequency and DCU anti-retraction valve validity[1,8,24]. These factors play essential roles in the formation of biofilms, which develop in DUWLs and function as a reservoir for continuous contamination of DUWL output water[7]. In our multifactor logistic regression study, the OR of non-compliance with the standards for the DCU with valid anti-retraction valve *versus* invalid anti-retraction valve was 0.084. This suggests that the odds of having a DCU with a valid anti-retraction valve were 11.9 (1/OR, 1/0.084) times larger than the odds of a DCU failing anti-retraction testing. Berlutti showed that the overwhelming majority (74%) of anti-retraction devices did not prevent retraction when the turbine stopped running, leading to contamination of the water lines and to consequent possible cross-contamination of patients[10]. Also, analysis of our preliminary study showed a significant, positive correlation ($P < 0.05$) between increased concentration of bacteria in the water sample and retracted volume[21]. In conclusion, DCUs equipped with anti-retraction valves should be periodically monitored and should undergo preventive maintenance to minimise instances of anti-retraction valve failure[7].

The exponent of the coefficient of DCU supply water has an OR of 0.368, meaning that the odds of a DCU supplied by purchased bottled water were 2.72 (1/0.368) times larger than the odds of DCU supplied by non-purified municipal water (the reference category). Similarly, the odds of a DCU being supplied by the hospital self-made purified water were 2.39 (1/0.417) times larger than the odds of a DCU being supplied by non-purified municipal water. The result obtained is in agreement with CDC guidelines for infection control in dental health-care settings[24].

In our multifactorial logistic regression study, the odds of DUWL disinfection once daily were 4.07 (1/0.246) times larger than the odds of DUWLs without disinfection (the reference category), and the odds of DUWLs disinfection once weekly were 1.93 (1/0.518) times larger than the odds of DUWL without disinfection. The OR of complying with standards for DUWL disinfection monthly *versus* non-disinfection was 6.494, with no statistical significance. Some studies

Table 2 Single-factor analysis for bacterial contamination of dental unit waterlines with the threshold value [500 colony-forming units (CFUs)/mL]

Factor	Category	≤500 *n* (%)	>500 *n* (%)	χ^2	*P*
Distinct category	Downtown area	140 (58.33)	100 (41.67)	0.774	0.379
	Agricultural area	203 (54.72)	168 (45.28)		
Hospital category	Stomatological hospital	43 (49.43)	44 (50.57)	1.856	0.173
	General hospital	300 (57.25)	224 (42.75)		
Anti-retraction handpiece	Y	282 (55.4)	227 (44.6)	0.668	0.414
	N	61 (59.8)	41 (40.2)		
Hot water	Y	134 (52.14)	123 (47.86)	2.879	0.090
	N	209 (59.04)	145 (40.96)		
Imported DCU	Y	135 (64.29)	75 (35.71)	8.628	0.003
	N	208 (51.87)	193 (48.13)		
Sampling season	Spring	79 (57.66)	58 (42.34)	8.886	0.012
	Summer	170 (61.37)	107 (38.63)		
	Autumn	94 (47.72)	103 (52.28)		
Hospital level	Level 1 hospital	88 (47.57)	97 (52.43)	25.279	0.000
	Level 2 hospital	89 (48.37)	95 (51.63)		
	Level 3 hospital	166 (68.6)	76 (31.4)		
DCU supply water	Municipal water	36 (35.29)	66 (64.71)	22.958	0.000
	Hospital self-made purified water	244 (59.08)	169 (40.92)		
	Purchased bottled water	63 (65.63)	33 (34.38)		
DUWL disinfection	Not disinfect	256 (53.33)	224 (46.67)	10.042	0.007
	Chlorine disinfectant	76 (69.72)	33 (30.28)		
	Other disinfectant	11 (50)	11 (50)		
Frequency of DUWLs disinfection	Not disinfect	256 (53.33)	224 (46.67)	16.945	0.001
	Once a day	16 (69.57)	7 (30.43)		
	Once a week	63 (73.26)	23 (26.74)		
	Once a month	8 (36.36)	14 (63.64)		
Bottle disinfection	Not disinfect	22 (57.89)	16 (42.11)	3.435	0.180
	Chlorine disinfectant	98 (63.64)	56 (36.36)		
	Other disinfectant	8 (42.11)	11 (57.89)		
Frequency of bottle disinfection	Once a day	30 (88.24)	4 (11.76)	16.478	0.000
	Once a week	82 (58.99)	57 (41.01)		
	Not disinfect	16 (42.11)	22 (57.89)		
Water monitoring	Y	232 (53.83)	199 (46.17)	3.168	0.075
	N	111 (61.67)	69 (38.33)		
DCU anti-retraction valve	Valid	224 (67.07)	110 (32.93)	180.706	0.000
	Invalid	29 (11.51)	223 (88.49)		

DCU, dental chair unit; DUWL, dental unit waterline; N, no; Y, yes.

Table 3 Multifactorial logistic analysis of bacterial contamination of dental unit waterlines (DUWLs)

Factor	β	SE	Wals	*P*	OR (95% CI)
Frequency of DUWLs disinfection					
Not disinfect					1.000
Once a day	−1.401	0.677	4.279	0.039	0.246 (0.065–0.929)
Once a week	−0.657	0.332	3.919	0.048	0.518 (0.270–0.994)
Once a month	1.871	1.165	2.577	0.108	6.494 (0.661–63.760)
χ^2 (df = 3)			10.502	0.015	
DCU supply water					
Municipal water					1.000
Hospital self-made purified water	−0.875	0.276	10.027	0.002	0.417 (0.242–0.716)
Purchased purified bottled water	−1.000	0.420	5.683	0.017	0.368 (0.162–0.837)
χ^2 (df = 2)			10.965	0.004	
DCU anti-retraction (invalid = 0, valid = 1)	−2.473	0.404	37.523	0.000	0.084 (0.038–0.186)

DCU, dental chair unit; df, degrees of freedom; OR, odds ratio.

reported that biofilms could form within the DUWLs of new DCUs within several hours of connection to a water supply[33,34] and reform rapidly following an intermittent treatment. Although continuously applied agents performed better than those used periodically, patients are exposed to residual products[7]. In the present study, we showed that DUWL disinfection weekly was better than the other frequencies of disinfection. Therefore, when formulating standards in the future, in addition to considering the effect after the implementation of control measures, it is necessary to consider compliance of the clinic staff. Logistic regression and other related categorical-data regression methods have often been used to assess risk factors for various diseases[35]. However, the authors did not find any reports in which logistic regression models were used to analyse the factors influencing contamination of DUWL output water at levels exceeding the CDC standards. The model developed here for predicting the quality of DUWL output water suggests that DUWLs should be disinfected at least once a week. The water supply for DCUs should be purified water (purchased purified bottled water is better) and the effectiveness of the DCU anti-retraction valve should be maintained.

The bacterial species found in the present study were mostly environmental aerobes, which were also present at low levels in municipal water[36]. Most of the genera isolated (*Micrococcus* spp.[37], *Comamonas* spp.[38] and *Staphylococcus* spp.[39]) are known opportunistic pathogens, as also reported in many other studies[40–42]. *Bacillus cereus*, occasionally isolated from human dental plaque[43], was the strain most frequently detected. It is also indirect proof of the existence of retraction. It is important to note that several strains of *B. cereus* can enhance biofilm formation. Within established biofilms, *B. cereus* can form spores, which may lead to contamination of DUWL output water, but this microbe is not common in oral infections[43,44]. *Burkholderia cepacia*, isolated in various DUWL output water investigations[25,45,46], is a known opportunistic human pathogen. There is evidence for transmission of *B. cepacia* to cystic fibrosis patients via pulmonary test equipment, nebulisers and other types of respiratory equipment used both in cystic fibrosis centres and for home-care, but there is little evidence of spread through aerosols, dental equipment, hands, contaminated disinfectants or water supplies[41]. Further research is needed. Al-Hiyasat *et al.* reported that *P. aeruginosa* was detected in 86.7% (26/30) of the DCUs at the beginning of the work day. The high percentage of contamination of the DUWLs tested in that study can be related mainly to the low level of efficiency of the anti-retraction valves and also to the heating system in the DCUs, in addition to the presence of softener that may act as a source of contamination when the water passes through[47].

As we did not choose a selective culture medium for the culture of specific bacteria, we did not identify *Legionella pneumophila*, which has been reported to be associated with DUWL infection[14,15,17]. Serological studies have shown higher titres of antibodies specific to *L. pneumophila* in healthy dental personnel than general population[9]. According to the results of the present study, the presence of bacteria in DUWL is not a matter of grave concern, but their quantity and the presence of potential pathogens and microbial flora in the oral cavity of the patients as a result of retraction are of concern[48].

In the future, research should be focused on the health economics of the DUWL problem (cost of testing, disinfection), risks to patients and staff, surveillance of adverse events related to dental treatment and importance of following the advice of dental unit manufacturers.

CONCLUSION

The high rate of contamination in DUWLs highlights the need to develop national standards in China. There is a need to disinfect DUWLs regularly and use a cleaner source of water; more attention should be paid to the efficacy of DCU anti-retraction valves.

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Conflict of interest

None declared.

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