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Original Article

Infection control-related factors associated with microbial contamination in dental unit waterlines

Ju-Yeon Cho[1*], Geun-Yeong Kim[2*], Joon Sakong[3], Eun-Kyong Kim[4†], Eun-Young Park[5†]

[1]Department of Dentistry, Dongsan Hospital, Keimyung University

[2]Department of Public Health, Graduate School of Environment and Public Health Studies, Yeungnam University

[3]Department of Preventive Medicine and Public Health, College of Medicine, Yeungnam University

[4]Department of Preventive Dentistry, School of Dentistry, Kyungpook National University

[5]Department of Dentistry, College of Medicine, Yeungnam University

Corresponding Author 1: Eun-Kyong Kim, Department of Preventive Dentistry, School of Dentistry, Kyungpook National University, 2177 Dalgubeol-daero, Jung-gu, Daegu-si, 41944, Korea. Tel: +82-53-660-6870, Fax: +82-53-423-2947, E-mail: ekkim99@knu.ac.kr

Corresponding Author 2: Eun-Young Park, Department of Dentistry, College of Medicine, Yeungnam University, 170 Hyeonchung-ro, Nam-gu, Daegu-si, 42415, Korea. Tel: +82-53-620-3282, Fax: +82-53-629-1772, Email: acidic@yu.ac.kr

*The author is a co-first author.

ABSTRACT

Objectives: Although dental unit waterlines (DUWLs) could harbor biofilms that pose an infection risk to patients and staff, data on infection control factors related to the microbial contamination of DUWLs in Korea remains limited. Therefore, in this study, we aimed to analyze the microbial load in DUWL according to infection control factors using a survey. **Methods:** In this cross-sectional study, we surveyed 58 dental institutions for characteristics (chair number, daily patient load, and accreditation status) and infection-control status (written guidelines, designated managers, monitoring, and staff training). Simultaneously, we examined the microbial contamination levels of the high-speed handpieces and three-way air-water syringes via water sampling, expressing contamination as arithmetic and geometric means (GM)±geometric standard deviation (GSD). We used the Mann–Whitney U test to compare bacterial contamination according to institutional characteristics and infection control factors ($p<0.05$). **Results:** Overall GM contamination was 1,141 and 411 CFU/mL for high-speed handpieces and syringes, respectively, exceeding the CDC guideline of ≤500 CFU/mL. We observed significant differences in microbial loads according to the institution type, unit chair count, and average patient count. Moreover, the existence of infection control guidelines revealed significant effects. **Conclusions:** DUWL microbial quality varied according to the clinic size and infection control program quality. Institutions with detailed guidelines, routine surveillance, and skill-based training have achieved better microbial control. Standardized guidelines and incentivized training could help reduce infection control gaps, especially in small private clinics.

Key Words: Biofilm, Dental clinic water quality, Dental unit waterlines, Infection control

Introduction

The waterline of dental clinics are critical components that require careful management to ensure patient safety [1]. Dental unit waterlines (DUWLs) can harbor dangerous microbial biofilms, which might proliferate rapidly in the stagnant environment of these systems [2,3]. These biofilms might present substantial risks to both patients and the medical team, underscoring the need for effective waterline management to uphold rigorous infection control standards in dental practices [4-7].

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Although the importance of maintaining proper water quality is widely recognized, significant variation exists in how dental clinics implement guidelines for cleaning, flushing, and monitoring their waterlines [8,9]. Some previous studies conducted across multiple countries have revealed significant discrepancies between recommended protocols and actual practice [10]. Resource limitations and inconsistent protocols in dental offices often result in inadequate management of dental unit waterlines (DUWLs) [11]. The study by the ADA Science & Research Institute highlighted that while many dental professionals acknowledge the importance of infection control, they encounter barriers such as knowledge gaps, time constraints, financial limitations, and staffing shortages, which impede proper DUWL management [12]. Consequently, these challenges may lead to suboptimal management practices for DUWL, increasing the risk of infection for both patients and dental healthcare workers.

Addressing these barriers requires a multi-faceted approach that includes improved training programs, better resource allocation and the development of standardized protocols to ensure consistent practices across all dental settings. [12]. To this end, it can be said that the first task is to identify vulnerable conditions for infection control related to microbial loads of DUWLs. Gathering data through surveys as well as microbial contamination examinations might be helpful to understand the challenges faced by dental professionals in maintaining optimal waterline management.

For example, it may be useful to consider various factors such as the size and operation of the facility, the presence of dedicated infection managers, and the availability of relevant manuals and training programs. Especially, trained infection control managers have been demonstrated to have a substantial impact on enhancing compliance with guidelines and protocols [13]. In addition, access to up-to-date manuals and effective training programs is crucial for ensuring that dental professionals are well-informed about infection control measures [12].

Therefore, by analyzing microbial contamination level in DUWLs in conjunction with survey data, oral healthcare providers or dental infection control specialists can better understand the impact of their efforts and identify gaps that need to be addressed. To achieve this objective, we conducted a study aimed at evaluating the relationship between various factors associated with infection control and the bacterial load of water used for dental treatment in clinics.

Methods

1. Study design

This cross-sectional study was designed to assess waterline management related factors including practices, guidelines, and existence of assigned managers in dental clinics through a comprehensive questionnaire survey, which had been conducted over 2-month period from 2020 Feb. This study was approved by the Institutional Review Board (IRB) of Yeungnam University (IRB No. YU2019-06-008-002). Informed consents were obtained from a representative director of dental clinics prior to their involvement in the study. Finally, 58 dental clinics in Daegu metropolitan city were included in this study. The required sample size was calculated using G*power 3.1 software. With a significance level (α) of 0.05, an effect size of 0.8, and a statistical power of 0.8, the minimum required sample size for t-test was determined to be 52 dental clinics. To account for potential non-responses and incomplete surveys, the target sample size was decided as 58 clinics.

2. Questionnaire survey

The survey was conducted using a self-administered method through in-person visits, following sufficient prior explanation and understanding about study. Participants completed and sealed the questionnaire by themselves. The questionnaire comprised a total of 13 items and consists of three main sections addressing key aspects of waterline management The first section focuses on general characteristics of dental institutions, including type of institution, dental chair count, operation period, average patient count per day, and participation in medical institution accreditation assessment (7 items). The second section examines specific waterline management practices, including monitoring for infectious control status, existence of assigned infection control manager, and education for infectious control (3 items). The third section assesses knowledge and attitudes toward waterline management, including existence of infectious control guidelines, detailed guidelines according to infectious control target, and type of education for infectious control such as lecture only, lecture with practice or e-lecture only (3 items). Most questions were multiple-choice or yes/no type, while a few required short written answers (e.g., year of establishment, replacement period of dental units).

At each participating institution, one infection control manager was asked to complete the questionnaire; if no such staff was assigned, a worker responsible for sterilization or environmental hygiene was designated as the respondent. The completed forms were sealed in envelopes and collected on-site by the research team.

3. Water sampling

In addition to questionnaire data collection, water samples were collected to assess microbial contamination levels. Samples were obtained from two components of each dental unit: high-speed handpieces and three-way air-water syringes. Prior to sample collection, a 30-second flush was performed to eliminate stagnant water from each component. Water samples were collected in sterile 100 mL bottles, with two samples taken from each dental clinic (one from each component). All samples were stored at 4℃ in sterile cooling bags and transported to the laboratory within four hours to examine bacterial loads.

4. Microbiological analysis

The collected water samples underwent standardized microbiological analysis to assess bacterial contamination levels. First, the samples were serially diluted corresponding to plates yielding 30 to 300 colonies. Then, R2A agar (Difco™, Becton, Dickinson and Company, Sparks, MD, USA), which was pre-sterilized and maintained at 44-46 ℃, was dispensed into petri dishes and mixed with the diluted samples. R2A agar is a low-nutrient medium specifically developed for the recovery of heterotrophic bacteria from potable water systems such as tap water. After solidification of the medium at 21.0±1.0℃ for 72±3 hours. Although R2A agar is commonly incubated for 5-7 days, previous studies have demonstrated that stable colony counts can be obtained within 72 h under these conditions; therefore, a 3-day incubation was adopted in this study. [13,14] The number of bacterial colonies formed on each plate was counted. Colony counts within the valid dilution range were averaged, and the result was multiplied by the corresponding dilution factor to calculate the number of colony-forming units (CFU) per mL to yield bacterial load.

5. Statistical analysis

All collected data was analyzed using IBM SPSS program (ver. 19.0; IBM Corp., Armonk, NY, USA). Mann-Whiteny U or Kruskal-Wallis test was employed to compare bacterial contamination levels according to general characteristics or infection control characteristics of dental institutions, respectively. After Kruskal-Wallis test, if result was significant, Mann-whitney + Bonferroni was analyzed for nonparametric post-hoc tests. As bacterial loads, Arithmetic mean as well as Geometric mean±Geometric standard deviation was used. Because when the data distribution is skewed or exhibits high variability, the geometric mean and standard deviation (STD) could be used as statistical indicators to more accurately represent the central tendency and dispersion than the simple arithmetic mean [15]. Statistical significance will be set at $p<0.05$ for all analyses.

Results

At <Table 1>, microbial contamination levels were compared according to general characteristics of dental institutions. Dental clinics exhibited significantly higher microbial loads in handpieces (AM: 13,073 CFU; GM±GSD: 1,885±7.40) compared to dental hospitals (AM: 2,263 CFU; GM±GSD: 373±10.96) (p<0.05). Similarly, air-water syringes in dental clinics showed higher contamination levels (AM: 7,995 CFU; GM±GSD: 635±11.28) compared to larger hospitals (AM: 1,271 CFU; GM±GSD: 156±13.24), though this difference did not reach statistical significance. Also, according to both unit chair count and patient count per day, there were significant differences in microbial contamination levels. Dental institutions with 11-20 unit-chairs or having fewer patients than 50 patients per day showed the most contaminated handpiece and air-water syringe in geometric mean (p<0.05). However, operation period or healthcare accreditation did not relate to microbial contamination levels at both handpiece and air-water syringe.

The microbial contamination levels demonstrated significant variation depending on infection control characteristics implemented in dental institutions, as summarized in <Table 2>. Institutions with designated infection control personnel exhibited lower microbial loads in handpieces and air-water syringes compared to clinics without designated personnel. However, these differences were not statistically significant. In contrast, the presence of infection control guidelines yielded statistically significant reductions in microbial contamination. Institutions with infection control guidelines reported substantially lower microbial loads in handpieces (GM±GSD: 562±11.56) compared to those without guidelines (GM±GSD: 2,754±4.99, p=0.018). Similarly, air-water syringes in Institutions following guidelines had lower contamination levels (GM±GSD: 215±1,260) than those without guidelines (GM±GSD: 912±10.47, p=0.032). In addition, detailed guidelines according to infectious control target, monitoring for Infectious control status, and education for infectious control showed similar significant results, in which institution having detailed guidelines, doing monitoring for infectious control status, or education for infectious control showed lower microbial loads in both handpiece and air-way-syringe than those did not.

Table 1. Microbial load according to general characteristics of dental institution

Characteristics	N(%)	Handpiece			Air-water syringe		
		AM	GM±GSD	p*	AM	GM±GSD	p*
Type of dental institution							
Clinic	40(69.0)	13,073	1,885±7.40	0.014	7,995	635±11.28	0.067
Hospital	18(31.0)	2,263	373±10.96		1,271	156±13.24	
Operation period (yr)							
≤5	10(17.2)	7,987	1,184±24.74		3,525	438±31.04	
6-10	11(19.0)	1,902	478±12.03	0.505	1,291	385±14.11	0.445
11-15	16(27.6)	24,230	1,800±5.67		15,548	307±17.78	
≥16	21(36.2)	3,580	1,248±1.25		2,118	514±5.97	
Dental chair count							
≤10	39(67.2)	12,934	1,637±8.17[b]		7,915	601±10.65[b]	
11-20	6(10.3)	7,470	2,383±6.21[ab]	0.047	4,663	2,867±2.87[b]	0.000
≥21	13(22.4)	1,108	275±10.64[a]		464	53±10.37[a]	
Average count of patients per day							
≤50	37(63.8)	14,607	2,045±8.96[b]		8,861	761±11.74[b]	
51-100	8(13.8)	1,158	871±2.30[b]	0.01	1,006	416±6.11[ab]	0.012
≥101	13(22.4)	1,071	256±10.43[a]		522	70±11.38[a]	
Participation in medical institution accreditation assessment							
Yes	4(6.9)	963	288±6.76		93	89±1.41	
No	54(93.1)	10,367	1,263±9.44	0.141	6,339	460±13.38	0.061
Total	58(100.0)	9,718	1,141±9.40		5,909	411±12.64	

N: Number of responding dental institutions
AM: Arithmetic means of microbial load
GM, GSD: Geometric mean and Geometric standard deviation of microbial load
*by mann-whiteny U or Kruskal-wallis test according to the number of groups compared (2 or 3, 4)
Bonferroni method post hoc test (a<b)

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Table 2. Microbial load according to infection control characteristics of dental institution

Characteristics	N(%)	Handpiece			Air-water syringe		
		AM	GM±GSD	*p**	AM	GM±GSD	*p**
Assigned infection control manager							
Yes	33(56.9)	13,925	817±14.54	0.258	8,974	319±18.75	0.233
No	25(43.1)	4,166	1,771±4.13		1,862	571±6.73	
Infectious control guidelines							
Yes	32(55.2)	2,972	562±11.56	0.018	1,616	215±12.60	0.032
No	26(44.8)	18,022	2,754±4.99		11,191	912±10.47	
Detailed guidelines according to infectious control target							
Yes	27(46.6)	2,967	505±12.33	0.021	1,766	183±14.70	0.024
No	31(53.4)	15,598	2,317±5.57		9,516	830±9.09	
Monitoring for infectious control status							
Yes	28(48.3)	1,740	409±9.61	0.001	1,195	132±14.20	0.002
No	30(51.7)	17,164	2,968±5.88		10,308	1,180±6.86	
Education for infectious control							
Yes	36(62.1)	12,255	610±10.65	0.001	5,771	239±16.05	0.052
No	22(37.9)	7,203	3,172±4.92		6,133	998±6.14	
Type of education for infectious control							
No	22(37.9)	7,203	3,172±4.91	0.009	6,133	998±6.14[b]	0.043
Lecture only	14(24.1)	2,502	728±5.28		1,915	442±6.27[b]	
Lecture/practice	9(15.5)	1141	233±14.35		422	48±14.45[a]	
E-lecture only	13(22.4)	27,684	981±15.78		13,627	371±29.62[b]	
Total	58(100.0)	9,718	1,141±9.40		5,909	411±12.64	

N: Number of responding dental institutions
AM: Arithmetic means of microbial load
GM, GSD: Geometric mean and Geometric standard deviation of microbial load
*by mann-whiteny U or Kruskal-wallis test according to the number of groups compared (2 or 3, 4)
Bonferroni method post hoc test (a<b)

Discussion

This study evaluated microbial contamination in dental unit waterlines (DUWLs) across 58 dental institutions and analyzed how their general characteristics and infection-control–related factors influenced contamination levels of handpiece and air-water syringe. The overall geometric mean (GM) of microbial lode was 1,141 CFU/mL for handpieces and 411 CFU/mL for air–water syringes, in which microbial lode of handpieces exceeded the U.S. CDC guideline of ≤500 CFU/mL [16]. In case of GM in handpiece, contamination was relatively high in the clinics (11-20 chairs) or treated ≤50 patients per day, which is exceeding U.S. CDC guideline [16]. In contrast, large facilities with ≥21 chairs met the safety criteria.

According to previous study, bacterial loads in DUWLs can exceed 10^4 CFU/mL [17]. Several institutions in the present study also surpassed this threshold. Pankhurst et al. [4] highlighted the risk of opportunistic pathogens such as Legionella spp. during routine dental procedures, and a recent multinational survey by Vinh et al. [10] reported that 46% of facilities continued treatment even when counts exceeded 500 CFU/mL. The high exceedance rate in Korean small-scale clinics mirrors these global shortcomings. The CDC and American Dental Association recommend maintaining <500 CFU/mL even for non-surgical care [16]. Small facilities treating ≤50 patients per day showed microbial load of GM= 2,045 CFU/mL, far above the standard. These clinics often have limited staff and budgets, which might make it difficult to maintain consistent flushing or chemical disinfection protocols [18,19]. Deploying automated DUWL maintenance systems and offering financial or regulatory incentives could help sustain infection-control activities.

Also, dental institutions having infectious control guidelines exhibited 4.9-fold and 4.2-fold lower contamination in handpieces and air–water syringes, respectively ($p<0.05$). Regular monitoring, detailed protocols, and staff education were likewise associated with significant reductions, underscoring the importance of infection control programs. The mere presence of an assigned infection control manager did not reach statistical significance, suggesting that human resources alone are insufficient without accompanying detailed infection control programs. These findings therefore corroborate the core CDC guidance that written protocols alone are insufficient unless coupled with ongoing surveillance and feedback loops [20,21]. Therefore, the data might support a multicomponent strategy—written, detailed protocols, ongoing surveillance, and hands-on education acting synergistically to minimize reservoirs of pathogens in DUWLs.

Strengths of the present study include the integration of field-collected water samples with organizational survey data, enabling simultaneous assessment of structural determinants and microbial contamination outcomes. Especially, all DUWL samples were stored at 4℃ and processed within 4 h, limiting bacterial regrowth during the sampling-transport-analysis chain and improving data reliability [22-24]. Finally, this is the largest Korean DUWL microbial survey to date to examine 58 dental institutions, to our knowledge.

However, limitations are: (1) the cross-sectional design precluded evaluation of seasonal or longitudinal trends; (2) the regional sample (one city) may limit generalizability; (3) CFU enumeration did not capture viral or fungal constituents of the biofilm; and (4) Logistic regression analysis was not performed due to the limited sample size; therefore, further studies with a larger number of institutions are necessary. Future multicenter cohort studies incorporating molecular techniques (e.g., 16S rRNA sequencing) are warranted.

In conclusion, several suggestions could be made based on the result of this study. First, given that infectious control guidelines were the most affecting factor, national dental associations should distribute and mandate standardized manuals. In addition, specialized hands-on education program blended learning (lecture + practice) could attribute to lower contamination.

Conclusions

This study evaluated microbial contamination levels in dental unit waterlines (DUWLs) across multiple dental institutions to identify key infection control factors associated with water quality. The findings provide important insights into how institutional size and infection control comprehensiveness affect microbial contamination in clinical environments.

1. Microbial contamination of DUWLs varies significantly by institution size and the comprehensiveness of infection-control systems.
2. Only the combination of written protocols, regular monitoring, and skills-based training achieved water quality ≤500 CFU/mL.

In conclusion, the results highlight the need for integrated infection-control frameworks that combine structured protocols, continuous surveillance, and practical staff education. Establishing standardized guidelines and expanding training programs nationwide will be crucial to sustaining safe and reliable dental waterline management.

Notes

Author Contributions

Conceptualization: J Sakong; Data collection: KY Kim; Formal analysis: EK Kim; Writing-original draft: KY Kim; Writing-review & editing: JY Cho, EY Park, EY Kim

Conflicts of Interest

The authors declared no conflicts of interest.

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Ethical Statement

This study was approved by the Institutional Review Board (IRB) of Yeungnam University (IRB No: YU2019-06-008-002).

Data Availability

Data can be obtained from the coauthor.

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치과 유니트 수관에서 미생물 오염과 관련된 감염 관리 요인

초록

연구목적: 본 연구는 설문조사를 통해 감염관리 요인에 따른 치과 유니트 수관의 미생물 오염 정도를 분석하고자 하였다. **연구방법:** 국내 58개 치과 의료기관을 대상으로 유니트 의자 수, 일일 환자 수, 인증 여부 등의 기관 특성과 감염관리 상태(문서화된 지침 보유 여부, 전담 관리자 지정 여부, 모니터링 여부, 직원 교육 여부 등)를 설문조사하였다. 동시에 고속 핸드피스와 3-way 시린지에서의 채수한 시료의 미생물 오염도를 분석하였다. 오염 수준은 산술평균 및 기하평균(Geometric mean, GM)±기하표준편차(GSD)로 표현하였으며, 기관 특성과 감염 관리 요소에 따른 세균 오염 차이는 Mann-Whitney U 검정을 통해 분석하였다($p<0.05$). **연구결과:** 고속 핸드피스에서의 기하평균 오염도는 1,141 CFU/mL, 시린지에서는 411 CFU/mL로, CDC 권장 기준치(≤500 CFU/mL)를 초과하였다. 기관 유형, 유니트 의자 수, 일일 환자 수 등에 따라 유의한 차이가 있었으며, 감염관리 지침 보유 여부 또한 미생물 오염도에 유의한 영향을 미쳤다. **결론:** DUWL의 미생물 수질은 의료기관의 규모와 감염관리 프로그램의 질에 따라 달라졌다. 세부 지침이 마련되어 있고, 정기적 감시와 실무 중심 교육이 시행되는 기관에서 오염 관리 수준이 더 우수하였다. 특히 소규모 개인치과에서 나타나는 감염관리 격차를 줄이기 위해 표준화된 지침 마련과 교육 참여를 유도할 수 있는 보상 제도가 필요하다.

색인: 생물막, 치과 진료실 수질, 치과 유니트 수관, 감염 관리