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O R I G I N A L A R T I C L E

Microbial contamination patterns in dental unit waterlines and evaluation of a centralized automated cleaning and disinfection system

Tingting Yin[1*], Miao He[2*], Boyi Ma[1], Mingmei Zhou[1], Lumin Luo[1], Rong Tang[1], Jingdi Huang[1], Pingjing Sun[1], Li Wang[1], Qiaowen Li[1]

1 State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University,Wuhan 430079, Hubei Province, China.
2 Department of Stomatology, People's Hospital of Wuhan University, Wuhan 430060, Hubei Province, China.

*These authors contributed equally to this study.

Correspondence to: Qiaowen Li, BS
State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University, No. 237 loyu road, Hongshan District, Wuhan 430079, Hubei Province, China.
TEL.: +86 15907152223; E-MAIL: liqiaowenqwl@126.com

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Abstract

OBJECTIVE: This study aimed to quantitatively characterize microbial contamination patterns in dental unit waterlines (DUWLs) and to evaluate a centralized, data-driven cleaning and disinfection system in a university dental hospital.
METHODS: We conducted a cross-sectional survey combined with longitudinal monitoring of DUWL samples from 56 dental chairs in three clinical departments of a university hospital. One-way analysis of variance, Kruskal–Wallis tests, paired t tests, linear regression, correlation analysis, and multiple linear regression were used to evaluate interdepartmental differences, sampling site–specific contamination risk, temporal trends, and independent predictors. Based on the statistical findings, a centralized cleaning and disinfection system was designed and subsequently validated.
RESULTS: A total of 726 water samples were analyzed. Statistically significant differences in microbial colony counts were observed between departments ($p < 0.001$), with the Optics Valley Teeth Cleaning Center exhibiting the highest contamination levels. Significant differences were also detected among sampling points ($p < 0.001$), with the handpiece water outlet identified as the highest-risk location. Active dental procedures were associated with a significant increase in microbial colony counts ($p < 0.001$). Under conventional disinfection, contamination levels increased progressively from April to July ($p < 0.01$). Significant positive correlations were identified among contamination levels at different sampling points. Multiple linear regression identified month, sampling site, treatment

status, and department as independent predictors of microbial contamination. Implementation of the centralized cleaning and disinfection system significantly extended the duration of compliance with established water quality standards from 1.2 ± 0.4 days to 4.1 ± 0.7 days ($p < 0.001$).

CONCLUSION: We observed significant interdepartmental variation, site-specific contamination **risk profiles**, and clear temporal patterns in DUWL microbial contamination. The centralized cleaning and disinfection system, developed based on these statistical findings, significantly improved microbial control and prolonged compliance with water quality standards.

INTRODUCTION

Microbial contamination of dental unit waterlines (DUWLs) poses a significant risk of cross-infection during dental procedures (Spagnolo *et al.* 2021). Although the clinical importance of this issue has been widely recognized, many investigations have focused on descriptive contamination surveys and have provided limited quantitative assessment using systematic statistical evaluation or targeted control strategies (O'Donnell *et al.* 2011; Zhang *et al.* 2017; Pappas *et al.* 2005). This limitation has restricted the precision and effectiveness of current infection-prevention and control measures in dental clinical settings.

Our previous investigations have confirmed the widespread presence of microbial contamination in DUWLs. However, the multidimensional characteristics of this contamination have not been comprehensively elucidated. Specifically, several key questions remain unresolved: (1) whether statistically significant differences in contamination levels exist among clinical departments and how contamination is distributed; (2) the relative contamination risk of individual waterline sampling points and the extent to which these can be quantitatively ranked; and (3) the influence of dental treatment activities and temporal factors on microbial contamination dynamics. Addressing these issues is essential for establishing an accurate, efficient, and evidence-based infection control system for DUWLs.

Accordingly, we applied a multidimensional statistical framework integrating cross-sectional surveys and longitudinal monitoring. Our objectives were to: (1) assess interdepartmental variability and sampling point–specific contamination risk using one-way analysis of variance and Kruskal–Wallis tests; (2) evaluate the effects of dental treatment activities and temporal variation on contamination dynamics using paired t tests and linear regression; (3) identify independent predictors of microbial contamination using correlation analysis and multiple linear regression; and (4) develop and validate a data-driven centralized cleaning and disinfection system based on these statistical findings.

MATERIALS AND METHODS

Study design and participants

We used a mixed-methods approach integrating cross-sectional surveys and longitudinal monitoring. The study included 56 dental units in active use in the Department of Endodontics I, the Surgery Department, and the Optics Valley Teeth Cleaning Center at the Hospital of Stomatology, Wuhan University. Sample size estimation, based on preliminary data with an effect size of $f = 0.35$, $\alpha = 0.05$, and $1-\beta = 0.90$, was conducted using G*Power version 3.1. A minimum of 48 dental units was calculated to achieve sufficient power. Including 56 units ensured adequate statistical power for subsequent analyses.

Sampling points comprised the handpiece water outlet, three-way syringe, cup filler, ultrasonic scaler connection, and floor box water supply. These locations were selected because they represent key sites associated with microbial contamination risk within DUWLs.

Water sampling and testing

From April to August 2025, DUWL water samples were collected at predefined time points before and after dental procedures using a standardized sampling protocol. Sampling was conducted twice daily to capture diurnal variation and ensure temporal balance. All procedures were performed in strict accordance with aseptic requirements and the *Standard for Prevention and Control of Healthcare-Associated Infection in Dental Outpatient Department and Clinic* (WS/T 842-2024) (National Health Commission, 2024). Total bacterial counts were determined using the plate count method. All microbiological analyses were completed within two hours of sample collection to maintain analytical validity.

Statistical analysis

Statistical analyses were performed using SPSS software, version 23.0. Quantitative data are presented as mean ± standard deviation. Statistical methods were selected according to data distribution and analytical objectives. Interdepartmental differences were assessed using one-way analysis of variance followed by Tukey HSD post hoc test. Differences among sampling points were evaluated using the Kruskal–Wallis nonparametric test with subsequent Dunn Bonferroni correction. Comparisons between pre-treatment and post-treatment samples were conducted using paired *t* tests after logarithmic transformation. Temporal trends were assessed using simple linear regression analysis. Associations among contamination levels at different sampling points were examined using Pearson's correlation coefficients. Multiple linear regression analysis was performed to identify independent predictors of microbial contamination. In this model, month was treated as a continuous variable, sampling point and department as categorical variables, and treatment status as a binary variable.

Tab. 1. Comparison of microbial colony counts in dental unit waterlines across clinical departments in a university hospital (CFU/mL)

Department	Number of samples	Mean ± SD	Median	Range (Min-Max)	F value	*p* value	η^2
Department of Endodontics I	380	2845±4216	992	0-24192	28.45	<0.001	0.143
Surgery Department	64	3892±5128	1456	0-15168			
Optics Valley Teeth Cleaning Center	108	6284±7342	3456	0-32768			

The table shows the number of samples, mean ± standard deviation, median, range (minimum–maximum), F value, *p* value, and η^2 for the Department of Endodontics I, the Surgery Department, and the Optics Valley Teeth Cleaning Center.

Tab. 2. Distribution of microbial colony counts across dental unit waterline sampling points (CFU/mL) in 56 dental units

Sampling point	Number of samples	Mean ± SD	Median	H value	*p* value	ε^2
Handpiece water outlet	156	4892±6128	2336	45.28	<0.001	0.188
Three-way syringe	156	3456±4892	1568			
Ultrasonic scaler connection	124	3128±4216	1024			
Cup filler	156	2845±3892	896			
Floor box water supply	124	1256±2184	288			

The table presents the number of samples, mean ± standard deviation, median, H value, p value, and ε^2 for the handpiece water outlet, three-way syringe, ultrasonic scaler connection, cup filler, and floor box water supply.

Development and validation of the centralized cleaning and disinfection system

A centralized automated cleaning and disinfection system was designed based on contamination characteristics identified through statistical analysis. The system was installed in series at the upstream segment of the DUWL water supply. The system adopted a modular architecture with disinfectant dosing, circulation, soaking, rinsing, and real-time water quality monitoring. System efficacy was evaluated using a randomized controlled design by comparing indicators, including the duration of compliance with water quality standards, between the centralized system and conventional single-unit disinfection.

RESULTS

Interdepartmental differences in DUWL microbial contamination

A total of 726 DUWL water samples were analyzed. One-way analysis of variance demonstrated significant differences in microbial colony counts among departments ($F = 28.45$, $p < 0.001$; Table 1). Post hoc Tukey HSD analysis indicated that the Optics Valley Teeth Cleaning Center exhibited significantly higher mean colony counts than those observed in the Department of Endodontics I ($p < 0.001$) and the Surgery Department ($p = 0.023$). No significant difference was observed between the Department of Endodontics I and the Surgery Department ($p = 0.215$).

Contamination risk at different sampling points

The Kruskal–Wallis test identified a statistically significant difference in microbial colony counts among sampling points ($H = 45.28$, $p < 0.001$), with detailed results presented in Table 2. Post hoc pairwise comparisons demonstrated that contamination levels at the handpiece water outlet were significantly higher than those at all other sampling points ($p < 0.01$). Based on relative contamination levels, risk ranked as follows: handpiece water outlet > three-way syringe > ultrasonic scaler connection > cup filler > floor box water supply.

Immediate impact of dental treatment on DUWL contamination

Paired *t* tests indicated that dental treatment activities significantly affected microbial colony counts in DUWLs (Table 3). In the Department of Endodontics I and the Surgery Department, colony counts increased significantly following treatment (both $p < 0.001$). In contrast, an opposite trend was observed in the Optics Valley Teeth Cleaning Center, where post-treatment colony counts were significantly reduced, with a mean difference of −1568 CFU/mL ($p = 0.008$).

Temporal trends and effects of disinfection intervention

Linear regression demonstrated a significant upward trend in microbial contamination from April to July, with an estimated monthly increase of 1256.8 CFU/mL ($p < 0.01$; Table 4). Month-to-month variations are

Tab. 3. Comparison of microbial colony counts in dental unit waterlines before and after dental treatment by department

Department	Number of paired samples	Pre-treatment mean (CFU/mL)	Post-treatment mean (CFU/mL)	Mean difference (CFU/mL)	*t* value	*p* value	Cohen's *d*
Department of Endodontics I	76	952±1245	2844±3216	+1892±1567	3.45	<0.001	0.62
Surgery Department	16	1047±1456	3892±4234	+2845±1989	4.12	<0.001	0.78
Optics Valley Teeth Cleaning Center	24	7852±6345	6284±5234	-1568±1678	-2.89	0.008	0.32

The table summarizes the number of paired samples, pre-treatment and post-treatment mean colony counts (CFU/mL), mean differences, *t* values, *p* values, and Cohen's *d* for the Department of Endodontics I, the Surgery Department, and the Optics Valley Teeth Cleaning Center.

Tab. 4. Monthly variation in microbial colony counts in dental unit waterlines in the Department of Endodontics I under conventional disinfection (April–August 2025)

Month	Number of samples	Mean (CFU/mL)	Standard deviation	Coefficient of regression	Standard error	*t* value	*p* value	R^2
April	48	1892	2456	1256.8	144.8	8.67	<0.01	0.89
May	240	2845	3892					
June	160	3456	4216					
July	352	4892	5684					
August	40	892	1284					

The table shows the number of samples, mean colony counts (CFU/mL), standard deviation, regression coefficient, standard error, *t* value, *p* value, and R^2 for each month.

presented in Table 4. Following the implementation of enhanced disinfection measures in August, colony counts decreased significantly compared with July levels ($p < 0.001$).

Correlation and systemic contamination patterns across sampling points

Correlation analysis identified significant positive associations among microbial colony counts at all sampling points (all $p < 0.05$), as detailed in the correlation matrix in Table 5. Strong positive correlations were observed between the handpiece water outlet and both the three-way syringe and the ultrasonic scaler connection, indicating coordinated contamination patterns across components of the DUWL system.

Multivariate regression analysis of determinants of contamination

Multiple linear regression analysis was performed to identify independent determinants of microbial colony counts, with results presented in Table 6. The overall model was statistically significant (F[4, 721] = 67.34, $p < 0.001$), and the adjusted R^2 was 0.724. Significant independent predictors of elevated microbial contamination included: Month ($\beta = 0.452$, $p < 0.001$); sampling point type, with the handpiece water outlet showing significantly higher contamination compared to the floor box water supply ($\beta = 0.892$, $p < 0.001$); post-treatment status ($\beta = 0.345$, $p = 0.002$); and department type with samples from the Optics Valley Teeth Cleaning Center exhibiting significantly higher counts compared to the Department of Endodontics I ($\beta = 0.567$, $p < 0.001$).

Validation of the centralized cleaning and disinfection system

Field validation demonstrated that the centralized cleaning and disinfection system, developed based on statistical findings, significantly enhanced DUWL contamination control. Compared with conventional single-unit disinfection, the centralized system extended the mean duration of compliance with water quality standards (≤ 100 CFU/mL) from (1.2 ± 0.4) days to (4.1 ± 0.7) days (t[54] = 15.23, $p < 0.001$). Additionally, the proportion of high-risk sampling points, specifically handpiece water outlets, maintaining microbial levels below the safety threshold increased from 45.6% to 92.3% ($\chi^2 = 36.78$, $p < 0.001$).

DISCUSSION

We employed a multidimensional statistical approach to quantitatively characterize microbial contamination patterns in DUWLs across departments, sampling points, and time. This approach enabled detailed evaluation of contamination patterns across departments, sampling points, and time, as well as identification of key determinants relevant to infection control.

One-way analysis of variance (Table 1) showed significant interdepartmental heterogeneity in

DUWL contamination. The higher microbial burden observed at the Optics Valley Teeth Cleaning Center may be related to its procedure mix and workflow, including frequent scaling procedures and extensive use of high-speed air turbine handpieces, which have been associated with increased aerosol generation and potential backflow into DUWLs (Mills *et al.* 2021; Leggat & Kedjarune, 2001; Centers for Disease Control and Prevention (CDC), 2003; Pankhurst & Coulter, 2007). These findings suggest that infection-prevention and control strategies should be tailored to department-specific clinical practices and operational characteristics rather than applying uniform protocols across departments..

The Kruskal–Wallis test (Table 2) demonstrated statistically significant differences in contamination among sampling points. The handpiece water outlet was the highest-risk site and should be prioritized in routine monitoring and disinfection. This observation is consistent with prior laboratory studies indicating that terminal water outlets often retain microbial contamination despite disinfection efforts (Walker *et al.* 2003).

Temporal analysis using linear regression (Table 4) showed a progressive increase in microbial contamination from April to July under conventional disinfection. This finding is consistent with existing evidence on microbial diversity and biofilm persistence in DUWLs, particularly in systems with intermittent stagnation (Singh *et al.* 2003; Lautenschlager *et al.* 2010). Paired *t* test results (Table 3) indicated that clinical procedures influence contamination dynamics, a finding consistent with the concept that DUWL biofilms can be disturbed during use, leading to transient microbial release into the water stream (Barbeau & Gagnon, 1997; Zhang *et al.* 2018). A reverse trend was observed at the Optics Valley Teeth Cleaning Center, where post-treatment contamination levels decreased significantly. This may reflect the impact of continuous high-volume water flushing during clinical procedures, which has been associated with reduced contamination under sustained flow conditions (Moffitt *et al.* 2020).

Positive correlations across sampling points (Table 5), together with the strong performance of the multivariable regression model (Table 6), indicate that

Tab. 5. Correlation analysis of microbial colony counts among dental unit waterline sampling points

Point	Handpiece water outlet	Three-way syringe	Ultrasonic scaler connection	Cup filler	Floor box water supply
Handpiece water outlet	1.00	0.78*	0.65*	0.52*	0.31*
Three-way syringe	-	1.00	0.71*	0.58*	0.28*
Ultrasonic scaler connection	-	-	1.00	0.49*	0.25*
Cup filler	-	-	-	1.00	0.42*
Floor box water supply	-	-	-	-	1.00

The table presents Pearson correlation coefficients for colony counts (CFU/mL) between the handpiece water outlet, three-way syringe, ultrasonic scaler connection, cup filler, and floor box water supply; an asterisk (*) indicates $p < 0.05$ for the corresponding correlation.

Tab. 6. Multiple linear regression analysis of factors associated with microbial colony counts in dental unit waterlines

Independent variable	Coefficient of regression (β)	Standard error	Standardized β	_t_ value	_p_ value	95% CI	VIF
Constant term	892.45	156.32	-	5.71	<0.001	585.62-1199.28	-
Month	1256.8	189.45	0.452	6.63	<0.001	885.12-1628.48	1.23
Handpiece water outlet (Ref: Floor box water supply)	3636.2	312.67	0.892	11.63	<0.001	3021.56-4250.84	2.45
Post-treatment status	1892.4	245.89	0.345	7.70	0.002	1409.85-2374.95	1.56
Optics Valley Teeth Cleaning Center (Ref: Department of Endodontics I)	3439.1	298.34	0.567	11.53	<0.001	2851.92-4026.28	2.12

The table reports regression coefficients (β), standard errors, standardized β, *t* values, *p* values, 95% confidence intervals, and variance inflation factors (VIF) for month, sampling point type (handpiece water outlet versus floor box water supply as reference), treatment status (post-treatment versus pre-treatment), and department (Optics Valley Teeth Cleaning Center versus Department of Endodontics I as reference).

DUWL contamination follows a systemic rather than isolated pattern (Szymańska *et al.* 2008). These findings support the rationale for centralized and system-level control strategies. The centralized cleaning and disinfection system developed on the basis of these statistical findings demonstrated sustained improvement in water quality control. These outcomes are consistent with empirical evaluations indicating that continuous, automated disinfection systems maintain effective antimicrobial concentrations throughout the complex pipeline networks (Coleman *et al.* 2009).

The principal contribution of this study lies in the comprehensive quantification of DUWL contamination using multidimensional statistical methods, defining contamination risk levels and identifying key predictors through regression modeling. From clinical and operational perspectives, these findings provide an evidence base to support differentiated infection control strategies, more efficient allocation of monitoring resources, and data-driven determination of optimal intervention timing within dental clinical settings.

This study has several limitations. First, it was conducted in a single university hospital, which may limit generalizability to other practice settings with different infrastructure and infection control policies. Second, we quantified total bacterial counts rather than specific pathogens, so we could not assess organism-specific risks or biofilm composition. Third, we did not collect clinical outcome data, and therefore cannot directly link DUWL contamination levels or the centralized disinfection system to patient infection rates. Finally, although we controlled for key factors such as department, sampling point, treatment status, and month in the regression models, residual confounding from unmeasured operational or environmental variables cannot be ruled out.

CONCLUSION

Our statistical analyses demonstrated pronounced interdepartmental heterogeneity, distinct sampling point–specific contamination risk profiles, and clear temporal patterns in DUWL microbial contamination. Treatment-related clinical activities were identified as a significant short-term factor associated with increased contamination levels. The consistently positive correlations observed across sampling points further support the presence of systemic, rather than localized, contamination within DUWLs. The centralized cleaning and disinfection system, developed on the basis of these quantitative findings, significantly extended the duration of compliance with established water quality standards and provides an effective, practical strategy for DUWL microbial control.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Authors' contributions

Conception and design of the research: Qiaowen Li, Li Wang
Acquisition of data: Tingting Yin, Li Wang
Analysis and interpretation of the data: Tingting Yin, Miao He
Statistical analysis: Mingmei Zhou, Boyi Ma, Lumin Luo, Rong Tang, Jingdi Huang, Pingjing Sun
Obtaining financing: Qiaowen Li
Writing of the manuscript: Miao He, Boyi Ma
Critical revision of the manuscript for intellectual content: Mingmei Zhou, Boyi Ma, Lumin Luo, Rong Tang, Jingdi Huang, Pingjing Sun
All authors read and approved the final draft.

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Clinical trial number

Not applicable.

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