Article

Not peer-reviewed version

# Bacterial Contamination in Dental Unit Water Lines at Primary Health Care Centers (2022–2023): A Nationwide Study

Abrar Jamal * and Eiman Alawadhi

Posted Date: 30 July 2025

doi: 10.20944/preprints202507.2532.v1

Keywords: dental unit waterlines; bacterial contamination; disinfection; water quality

 doi:10.20944/preprints202507.2532.v1



*Article*

# Bacterial Contamination in Dental Unit Water Lines at Primary Health Care Centers (2022–2023): A Nationwide Study

**Abrar Jamal [1,*] and Eiman Alawadhi [2]**

[1] Department of Preventive Medicine, Public Health Administration, Ministry of Health, Kuwait

[2] Department of Epidemiology and Biostatistics, Faculty of Public Health, Kuwait University, Kuwait City 13110, Kuwait

* Correspondence: abrar_jamal@yahoo.com

**Abstract**

**Background:** Dental unit water lines (DUWLs) can harbor microbial contamination, posing risks for cross-infection to patients and dental staff. This study assessed the prevalence of bacterial contamination in DUWLs at primary healthcare centers in Kuwait during 2022–2023 and examined variation by year, governorate, and sampling outlet. **Methods:** A retrospective cross-sectional analysis was conducted using 3,290 water test results from six governorates. Data were obtained from the Environmental Health Department, Ministry of Health, and analyzed using STATA 17. Contamination was defined as a total plate count (TPC) >100 CFU/mL or presence of coliforms, Escherichia coli, Pseudomonas aeruginosa, or fecal streptococci. Descriptive and logistic regression analyses were performed. **Results:** Overall contamination prevalence was 16.8%. Rates were higher in 2023 (19.8%) than in 2022 (13.7%), and higher in Mubarak Al-Kabeer governorate (23%) and cup filler outlets (18.9%). Logistic regression showed significantly increased odds of contamination in 2023 (OR = 1.6; 95% CI: 1.3–2.0), Mubarak Al-Kabeer (OR = 1.4; 95% CI: 1.1–1.9), and cup fillers (OR = 1.3; 95% CI: 1.1–1.6). P. aeruginosa was detected in 1.3% of samples. **Conclusion:** One in six DUWL samples exceeded Kuwait's strict microbial safety threshold. Findings highlight spatial and procedural variations in contamination and underscore the need for enhanced disinfection protocols, preventive maintenance, and targeted staff training to ensure waterline safety.

**Keywords:** dental unit waterlines; bacterial contamination; disinfection; water quality

## 1. Introduction

Dental Unit Water Lines (DUWLs) are essential for daily dental procedures but are known reservoirs for microbial contamination due to long tubing, low flow rates, and prolonged water stagnation [1,2]. These conditions facilitate biofilm formation—surface-attached bacterial colonies encased in a protective matrix, which can harbor opportunistic pathogens such as Pseudomonas aeruginosa, Escherichia coli, fecal streptococci, Legionella spp., and nontuberculous mycobacteria, posing risks to patients and dental personnel [3,4]. Water sources for dental units vary between municipal supplies and independent reservoirs using distilled water, yet both systems are susceptible to contamination [5]. Even in units supplied with distilled water, biofilm formation has been documented [6,7]. Microbes can detach from these biofilms and enter a patient's mouth via water irrigation or aerosol dispersion from handpieces [8]. Biofilm resilience complicates eradication, as standard disinfection methods often prove inadequate [9].

International guidelines differ in their microbial water safety thresholds. The U.S. CDC sets a limit of <500 CFU/mL for nonsurgical dental procedures [10], while the American Dental Association recommends a more stringent <200 CFU/mL [11]. Kuwait's Environmental Public Authority (EPA) adopts the strictest threshold of ≤100 CFU/mL and mandates the complete absence of E. coli, P.

 doi:10.20944/preprints202507.2532.v1



aeruginosa, and fecal streptococci in DUWLs [12,13]. These standards underscore the importance of regular testing and adherence to disinfection protocols. The prevalence of DUWL contamination has been reported as high as 96.7% globally, with levels fluctuating throughout the day depending on flushing practices and equipment design [14,15]. A 2022 local study at Kuwait University Dental Center found that basic adherence to manufacturer instructions was insufficient to reduce contamination unless supplemented with additional practices such as flushing, draining, and disinfectant use [16].

Despite the global and local recognition of these risks, there remains a lack of systematic data on DUWL contamination across Kuwait's six governorates. This study seeks to fill that gap by evaluating the prevalence of bacterial contamination in DUWLs across primary health care centers in Kuwait for the years 2022 and 2023. Contaminated water is defined as water samples with total plate count (TPC) exceeding 100 CFU/mL or positive findings for indicator pathogens including coliforms, E. coli, P. aeruginosa, and fecal streptococci [17]. Specifically, the study investigates whether contamination prevalence varies by year, governorate, or sampling outlet (cup filler, handpiece, or distillation unit), and examines the statistical relationship between these factors. We hypothesize that microbial contamination in Kuwait's DUWLs exceeds both local and international thresholds and exhibits significant variation across regions and outlet types. By identifying contamination patterns and predictors, this study contributes evidence needed to guide infection control protocols, improve disinfection strategies, and strengthen water quality monitoring in Kuwait's dental settings.

## 2. Materials and Methods

The study employed a retrospective cross-sectional design to assess bacteriological contamination in dental unit water lines (DUWLs) using secondary data collected from primary healthcare centers across the six governorates of Kuwait. The dataset, provided by the Environmental Health Department within the Public Health Administration of the Ministry of Health, comprised all routine "water sample inspection request forms" submitted between January 2022 and December 2023. Each record included the center name, governorate, sample date, outlet type (cup filler, handpiece, or distillation unit), and bacteriological test result. These water samples were categorized as bacteriologically non-contaminated (permissible) if they had a total plate count (TPC) of 100 colony-forming units per milliliter (CFU/mL) or less and no pathogenic findings. Contaminated (non-permissible) samples were defined as those with a TPC above 100 CFU/mL or a positive result for any of the following bacteria: Coliforms, Escherichia coli, Pseudomonas aeruginosa, Fecal streptococci, or other potential pathogens.

All data were manually entered into Microsoft Excel for cleaning and validation and subsequently analyzed using STATA 17. The full population of 3,290 water sample results was included, eliminating the need for sampling or power estimation. The main outcome variable was binary microbial contamination status (contaminated vs. non-contaminated). Independent variables included the year of testing (2022 or 2023), governorate (Al-Asima, Al-Ahmadi, Al-Farwaniya, Hawalli, Al-Jahra, and Mubarak Al-Kabeer), and the type of water sampling outlet. Descriptive statistics were generated as frequencies and percentages. Chi-square tests were conducted to examine associations between contamination status and the independent variables. Variables with a p-value less than 0.10 in univariate analysis were entered into a multivariable logistic regression model. This model was used to assess adjusted associations between microbial contamination and the independent variables, with a significance threshold of $p < 0.05$. Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). All analyses were conducted using STATA version 17.

## 3. Results

### *3.1. Prevalence of Bacteriological Contamination (DUWLs)*

A total of 3,290 results were analysed in our study (Table 1). The majority of samples were collected in 2023 (57.72%), primarily from the Capital Al-Asima governorate (27.9%) and the cup filler

 doi:10.20944/preprints202507.2532.v1



outlets (43.45%). Of these, 555 results (16.9%) showed microbial contamination. The highest proportion of contamination was observed in 2023 (19.9%), with Mubarak AlKabeer governorate showing 22.5% and the cup filler outlet 18.9%. Samples with bacterial findings of TPC over 100 CFU/mL constituted 16.6%, with a range from 110 to 82,000 CFU/mL, while Pseudomonas aeruginosa was detected in 1.3% of samples. A significant association was found between contamination and factors including the year of water testing, governorate, water sampling outlet, and the presence of Pseudomonas aeruginosa (p<.05).

**Table 1.** Descriptive characteristics of dental water results based on bacterial contamination status.

| Characteristic | Total | Contamination Status | | p-value |
|---|---|---|---|---|
| | | Uncontaminated | Contaminated | |
| | n (%) | n (%) | n (%) | |
| **Total** | 3,290 (100%) | 2,735 (83.1%) | 555 (16.9%) | |
| **Year** | | | | **<0.001*** ** |
| 2022 | 1,391 (42.3%) | 1,213 (87.2%) | 178 (12.7%) | |
| 2023 | 1,899 (57.7%) | 1,522 (80.2%) | 377 (19.8%) | |
| **Governorate** | | | | **0.001** ** |
| Asima | 919 (27.9%) | 774 (84.2%) | 145 (15.8%) | |
| Ahmadi | 734 (22.3%) | 635 (86.5%) | 99 (13.5%) | |
| Farwaniya | 394 (12.0%) | 317 (80.5%) | 77 (19.5%) | |
| Hawally | 603 (18.3%) | 507 (84.1%) | 96 (15.9%) | |
| Jahra | 156 (4.7%) | 127 (81.4%) | 29 (18.6%) | |
| Mubarak Alkabeer | 484 (14.7%) | 375 (77.5%) | 109 (22.5%) | |
| **Water Sampling Outlet** | | | | **0.023*** |
| Hand piece | 1,050 (31.9%) | 891 (84.9%) | 159 (15.1%) | |
| Cup filler | 1,428 (43.4%) | 1,158 (81.1%) | 270 (18.9%) | |
| Distillation unit | 812 (24.7%) | 686 (84.5%) | 126 (15.5%) | |
| **Bacterial Finding** | | | | **<0.01** ** |
| TPC[1] | 3,290 (100%) | 2,742 (83.4%) | 548 (16.6%) | |
| Coliform | 3,290 (100%) | 0 (0.0%) | 0 (0.0%) | |
| E.coli | 3,290 (100%) | 0 (0.0%) | 0 (0.0%) | |
| P.aerug | 3,290 (100%) | 3,246 (98.7%) | 44 (1.3%) | |
| F.Strep | 3,290 (100%) | 0 (0.0%) | 0 (0.0%) | |
| Others | 3,290 (100%) | 0 (0.0%) | 0 (0.0%) | |

*p-value <0.05, **p-value <0.01, ***p-value <0.001; p-value obtained by chi-square test. [1] TBC range = = 110-82,000 CFU/mL.

*3.2. Multivariable Logistic Regression Model*

The final adjusted multivariable logistic regression model (Table 2) indicated that, after accounting for the effects of other variables in the model, the odds of water bacteriological contamination in 2023 were 1.63 times that in 2022 (OR=1.63, 95% CI: 1.34–1.98, p<0.001). Among the six governorates, the odds of water bacteriological contamination in Mubarak Al-Kabeer were 1.42 times those in Al-Asima (OR = 1.42, 95% CI: 1.07–1.88). Additionally, water samples from the cup filler had 1.3 times the odds of bacterial contamination compared to samples from the handpiece (OR = 1.3, 95% CI: 1.3–1.62, p = 0.017).

**Table 2.** Adjusted logistic regression of contaminated dental water results.

| Characteristic | OR | (95% CI) | p-value |
|---|---|---|---|
| **Year** | | | |
| 2022 | Reference | | |
| 2023 | 1.6 | (1.3, 2.0) | **<0.001** |
| **Governorate** | | | |
| Asima | Reference | | |
| Ahmadi | 0.9 | (0.7, 1.2) | 0.332 |

 doi:10.20944/preprints202507.2532.v1



| | | | |
|---|---|---|---|
| Farwaniya | 1.3 | (1.0, 1.8) | 0.104 |
| Hawally | 1.0 | (0.8, 1.4) | 0.877 |
| Jahra | 1.2 | (0.8, 1.9) | 0.369 |
| Mubarak Alkabeer | 1.4 | (1.1, 1.9) | **0.014*** |
| **Water Sampling Outlet** | | | |
| Hand piece | Reference | | |
| Cup filler | 1.3 | (1.3, 1.6) | 0.017 |
| Distillation unit | 1.1 | (0.8, 1.4) | 0.593 |

*p-value <0.05, **p-value <0.01, ***p-value <0.001; p-value obtained by logistic regression test.

## 4. Discussion

This study aimed to assess the prevalence of bacteriological contamination in dental unit waterlines (DUWLs) in primary healthcare centers across Kuwait during 2022 and 2023. It also sought to identify the most frequently contaminated water sampling outlets and explore variations across time, location, and outlet type. To our knowledge, this is the first nationwide effort in Kuwait to evaluate bacteriological DUWL safety using routine environmental inspection data from nearly all public dental departments in primary healthcare settings. The findings provide critical insights into potential microbial hazards in general dental care and emphasize the need for continuous surveillance and tailored infection control interventions to protect both patients and dental professionals. Our study found a bacterial contamination prevalence of 16.9%, based on Kuwait's national and European C-100 standards, which require total plate counts (TPC) to remain below 100 CFU/mL. This level is notably lower than contamination rates reported in other regional and international settings [18]. For instance, a study in Saudi Arabia documented a prevalence of 42.2% [19], while systematic reviews covering Iran, Turkey, Iraq, and Jordan reported even higher rates [20]. In Iran, one meta-analysis found contamination rates as high as 69% using the same <100 CFU/mL threshold [21]. The relatively lower contamination rates observed in our study may reflect improved compliance with decontamination protocols or differing structural factors in Kuwait's public dental infrastructure.

The predominant contributor to contamination in our findings was elevated TPC. Across both years, 16.9% of DUWL samples exceeded the recommended microbial threshold, with HPC values ranging from 110 to 82,000 CFU/mL. These findings are lower than those reported in the Netherlands, where 61% of samples failed the EU benchmark [22], or in China, where 57.4% surpassed the threshold, with counts reaching 380,000 CFU/mL [23]. Differences across studies may reflect variations in study design, timing of sample collection, water line disinfection practices, or the specific sampling outlets tested. As for Pseudomonas aeruginosa, a known opportunistic pathogen in dental water systems, our study reported a low overall prevalence of 1.3%, which was not statistically significant. This compares favorably to other studies across the region and globally, where P. aeruginosa has been detected in 7.5% to 40% of DUWL samples [20,24–26]. These comparatively lower findings may point to effective chlorination or filtration in Kuwait's systems or improved maintenance procedures in the public sector. Nonetheless, the variation in P. aeruginosa prevalence across countries underscores the need for uniform diagnostic methods and cross-comparable standards for DUWL safety assessment.

Contamination prevalence rose from 12.8% in 2022 to 19.8% in 2023—a 63% increase in odds of contamination. Similar upward trends were observed in Germany, where contamination rose from 50% to 55% between the same years [24]. These highlight the dynamic nature of microbial contamination and the importance of contextual factors, including maintenance quality, seasonal influences, and the operational burden following COVID-19 recovery. Internationally, a Canadian 11-year retrospective study found that 21% of dental handpiece samples failed the 500 CFU/mL standard, and 19% failed the stricter 100 CFU/mL limit, comparable to our findings. That study emphasized the importance of retesting following failures, as nearly half of initial failures remained

 doi:10.20944/preprints202507.2532.v1



above the acceptable threshold even after follow-up [27]. These results support the implementation of structured retesting protocols and stress the need for continuous quality monitoring.

Significant differences in contamination rates were also observed across Kuwait's governorates. Mubarak Al-Kabeer governorate had a 42% higher contamination rate compared to Al-Asima. Although geographic variation in DUWL contamination has not been extensively studied in the literature, similar differences have been noted across dental specialties, with periodontics showing distinct microbial profiles [23]. Another study from Italy found that dental units relying on tank-based water systems had higher contamination (69%) than those connected to municipal water (56%) [28]. Such disparities may be related to unequal human resources, inconsistent training, or water supply differences between regions. These findings highlight the need to standardize DUWL maintenance procedures across all governorates and investigate regional resource gaps in dental infrastructure.

Our analysis revealed that bacterial contamination was 30% more likely in samples taken from cup-fillers compared to handpieces. This aligns with prior studies emphasizing the role of stagnant water in microbial proliferation [29]. Cup-fillers, being less frequently used, may foster water stagnation and biofilm formation, which increases bacterial loads. Even with the use of distilled water, contamination can persist if disinfection protocols are not rigorously maintained [30]. These findings reinforce the need for clearer guidance on routine outlet testing, line-specific disinfection schedules, and targeted protocols for less-used outlets. Further research is warranted to test the efficacy of anti-stagnation and automated flushing systems in reducing outlet-specific contamination risks.

*Limitations*

Several limitations should be considered. First, variations in microbial standards complicate comparison with other studies. While Kuwait enforces a strict 100 CFU/mL limit, many studies rely on the CDC's 500 CFU/mL or the ADA's 200 CFU/mL thresholds. This heterogeneity challenges regional benchmarking and interpretation. Additionally, most published studies focus on private clinics or specialized dental hospitals, while our study was limited to public primary care units, which operate under different conditions and protocols. Second, our data lacked key contextual variables such as the water source (e.g., municipal vs. tank), clinic patient volume, and DUWL maintenance schedules. This limits the ability to fully explain contamination differences across sites. Third, the study covered only a two-year period, restricting our ability to examine long-term trends or seasonal variation. Moreover, comparisons with studies from specialized units are limited, as our data represent general dental services without subspecialties. Despite these limitations, the study offers a valuable nationwide snapshot of DUWL contamination in Kuwait's primary care sector and highlights key areas for policy attention, including outlet-level surveillance, regional standardization, and data-driven disinfection protocols. Future studies should aim to include private sector data, incorporate longer timeframes, and assess additional predictors such as staff compliance, line flushing frequency, and types of water reservoirs used in clinics.

## 5. Conclusions

This study demonstrates that the prevalence of bacteriological contamination in dental unit waterlines (DUWLs) across Kuwait's primary healthcare centers is influenced by temporal, geographical, and outlet-specific factors. Notably, an overall increase in contamination was observed in 2023 compared to 2022, indicating a possible decline in maintenance efficiency or adherence to disinfection protocols post-pandemic. Geographic disparities were particularly evident in Mubarak Al-Kabeer governorate, where contamination rates were substantially higher than in other regions. These variations highlight the uneven implementation of infection control measures and underscore the necessity of standardizing protocols across governorates. A significant finding of this study was the elevated contamination rate in water samples collected from cup filler outlets compared to handpieces. This discrepancy may stem from the infrequent use of cup fillers, allowing for water

 doi:10.20944/preprints202507.2532.v1



stagnation and biofilm formation, and possibly from their omission in routine flushing or disinfection procedures. These insights call for enhanced disinfection efforts targeting less frequently used components and the incorporation of outlet-specific protocols in routine infection control guidelines. Despite the overall lower prevalence of Pseudomonas aeruginosa compared to international benchmarks, its presence remains clinically relevant due to its opportunistic pathogenicity and resistance to disinfectants. Regular microbial monitoring, rigorous flushing procedures, and adherence to EPA and EU bacteriological standards (<100 CFU/mL) should be enforced to sustain water safety in dental care settings. In light of these findings, further research is warranted to investigate the underlying causes of regional discrepancies, particularly with respect to water supply sources, clinic workloads, and maintenance infrastructure. A broader surveillance framework and continuous quality improvement initiatives are essential to ensure consistent water quality and reinforce patient safety across all dental units in Kuwait's public health system.

**Author Contributions:** Both authors have contributed to all stages of developing the paper. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Ministry of Health, Kuwait (protocol code 2521/2025, 21 November 2024).

**Acknowledgments:** The authors would like to thank Dr. Muna Alkhabbaz for granting access to the dataset used in this study and for her support in facilitating the data collection process.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| DUWLs | Dental Unit Water Lines used in dental units to supply water to handpieces and cup fillers. |
| CFU | Colony Forming Unit a standard used to measure bacterial concentration in water sample lab results. |
| IWR | Independent Water Reservoir an attachable bottle to the dental chair. |
| WHO | World Health Organization |
| CDC | Centers for Disease Control and Prevention |
| HPC | Hetero plate count |
| TPC | Total Plate Count |
| MOH | Ministry of Health |
| PHA | Public Health Administration |
| EHD | Environmental Health Department |
| ADA | American Dental Association |
| EPS | Extracellular polymeric substances |
| GCC | Gulf Cooperation Countries |
| Stata 17 | A statistical software used to analyze data for descriptive and regression analysis. |

## References

1. Odonnell, M.J.; Boyle, M.A.; Russell, R.J.; Coleman, D.C. Management of Dental Unit Waterline Biofilms in the 21st Century. *Future Microbiol* **2011**, *6*, 1209–1226, doi:10.2217/FMB.11.104.
2. Chen, P.; Zeng, J.; Hong, F.; Li, C.; Wang, H.; Yu, X. The Importance of Biofilm Contamination Control for Dental Unit Waterlines: A Multicenter Assessment of the Microbiota Diversity of Biofilm in Dental Unit Waterlines. *Journal of Oral Microbiology* **2023**, doi:10.1080/20002297.2023.2299496.
3. RM Donlan Biofilms: Microbial Life on Surfaces. *Emerg Infect Dis* **2002**.

 doi:10.20944/preprints202507.2532.v1




4. Wirthlin, M.R.; Marshall, G.W.; Rowland, R.W. Formation and Decontamination of Biofilms in Dental Unit Waterlines. *J Periodontol* **2003**, *74*, 1595–1609, doi:10.1902/JOP.2003.74.11.1595.
5. Coleman, D.C.; O'Donnell, M.J.; Shore, A.C.; Russell, R.J. Biofilm Problems in Dental Unit Water Systems and Its Practical Control. *J Appl Microbiol* **2009**, *106*, 1424–1437, doi:10.1111/J.1365-2672.2008.04100.X.
6. Palenik, C.J.; Miller, C.H. The Effect of Distillation and Line Cleaning on the Quality of Water Emitted from Dental Units. *Am J Dent* **2003**, *16*, 385–389.
7. Shuai, T.; Shao, T.; Yi, L.; Han, S.; Jiménez-Herrera, M.F.; Wang, Z.; Li, X. The Effect of Different Types of Water Sources on Dental Unit Waterline Contamination: A Systematic Review and Meta Analysis. *Heliyon* **2024**, *10*, e35745, doi:10.1016/j.heliyon.2024.e35745.
8. Sauer, K.; Camper, A.K.; Ehrlich, G.D.; William Costerton, J.; Davies, D.G. Pseudomonas Aeruginosa Displays Multiple Phenotypes during Development as a Biofilm. *J Bacteriol* **2002**, *184*, 1140–1154, doi:10.1128/JB.184.4.1140-1154.2002.
9. Spagnolo, A.M.; Sartini, M.; Cristina, M.L. Microbial Contamination of Dental Unit Waterlines and Potential Risk of Infection: A Narrative Review. *Pathogens 2020, Vol. 9, Page 651* **2020**, *9*, 651, doi:10.3390/PATHOGENS9080651.
10. Kohn, W.G.; Collins, A.S.; Cleveland, J.L.; Harte, J.A.; Eklund, K.J.; Malvitz, D.M.; Centers for Disease Control and Prevention (CDC) Guidelines for Infection Control in Dental Health-Care Settings--2003 . *Recommendations and Reports: Morbidity and Mortality Weekly Report* **2003**, *52*, 1–61.
11. Marino, F.; Mazzotta, M.; Pascale, M.R.; Derelitto, C.; Girolamini, L.; Cristino, S. First Water Safety Plan Approach Applied to a Dental Clinic Complex: Identification of New Risk Factors Associated with Legionella and P. Aeruginosa Contamination, Using a Novel Sampling, Maintenance and Management Program. *J Oral Microbiol* **2023**, doi:10.1080/20002297.2023.2223477.
12. European Council Directive 93/42/EEC Medical Devices Available online: https://eur-lex.europa.eu/legal-content/DE/ALL/?uri=celex%3A31993L0042 (accessed on 22 July 2025).
13. Enivronment Public Authority - Kuwait Executive Lists Available online: https://epa.gov.kw/ExecutiveLists (accessed on 22 July 2025).
14. Ma, S.Y.; Al-Hiyasat, A.S.; Yacoub Hindiyeh, M.; Saleh Khader, Y. Legionella Pneumophila Contamination of a Dental Unit Water Line System in Dental Teaching Centre. *Article in International Journal of Dental Hygiene* **2008**, *6*, 48–55, doi:10.1111/j.1601-5037.2007.00280.x.
15. Lal, B.; Ravindra, K.; Biswal, M. Appraisal of Microbial Contamination of Dental Unit Water Systems and Practices of General Dental Practitioners for Risk Reduction. *Environmental Science and Pollution Research* **2018**, *25*, 33566–33572, doi:10.1007/S11356-018-3298-Y/METRICS.
16. Hussain Akbar, J.; Behbehani, J.; Karched, M. Biofilm Growth and Microbial Contamination of Dental Unit Waterlines at Kuwait University Dental Center. *Frontiers in Oral Health* **2023**, *3*, doi:10.3389/FROH.2022.1071018/FULL.
17. Rodrigues, S.; Suvarna, S.; Suvarna, J.; Saralaya, V.; Saldanha, S.; Shenoy, V.K. Microbial Assessment of Dental Unit Waterlines in an Institutional Setup in Karnataka, South India. *Indian Journal of Dental Research* **2017**, *28*, 555–559, doi:10.4103/IJDR.IJDR_775_16.
18. Okubo, K.; Ito, T.; Shiota, Y.; Kawata, Y.; Yamamoto, T.; Takashiba, S. Effectiveness and Safety of Low-Concentrated Ozonized Water for the Reduction of Contamination in Dental Unit Water Lines. *Heliyon* **2019**, *5*, e02306, doi:10.1016/J.HELIYON.2019.E02306.
19. Khajezadeh, M.; Mohseni, F.; Khaledi, A.; Firoozeh, A. Contamination of Dental Unit Water Lines (DUWL) with Legionella Pneumophila and Pseudomonas Aeruginosa; A Middle East Systematic Review and Meta-Analysis. *Eur J Microbiol Immunol (Bp)* **2023**, *12*, 93–99, doi:10.1556/1886.2022.00023.
20. Vinh, R.; Azzolin, K.A.; Stream, S.E.; Carsten, D.; Eldridge, L.A.; Estrich, C.G.; Lipman, R.D. Dental Unit Waterline Infection Control Practice and Knowledge Gaps. *Journal of the American Dental Association* **2024**, *155*, 515-525.e1, doi:10.1016/j.adaj.2024.02.011.
21. Hoogenkamp, M.A.; Brandt, B.W.; Laheij, A.M.G.A.; de Soet, J.J.; Crielaard, W. The Microbiological Load and Microbiome of the Dutch Dental Unit; 'Please, Hold Your Breath.' *Water Res* **2021**, *200*, 117205, doi:10.1016/J.WATRES.2021.117205.

 doi:10.20944/preprints202507.2532.v1




22. Gawish, S.; Abbass, A.; Abaza, A. Occurrence and Biofilm Forming Ability of Pseudomonas Aeruginosa in the Water Output of Dental Unit Waterlines in a Dental Center in Alexandria, Egypt. *Germs* **2019**, *9*, 71, doi:10.18683/GERMS.2019.1160.
23. Dang, Y.; Zhang, Q.; Wang, J.; Wang, Q.; Han, M.; Niu, Y.; Li, H.; Li, X. Assessment of Microbiota Diversity in Dental Unit Waterline Contamination. *PeerJ* **2010**, doi:10.7717/peerj.12723.
24. Arvand, M.; Hack, A. Microbial Contamination of Dental Unit Waterlines in Dental Practices in Hesse, Germany: A Cross-Sectional Study. *Eur J Microbiol Immunol (Bp)* **2013**, *3*, 49, doi:10.1556/EUJMI.3.2013.1.7.
25. Barbot, V.; lie Robert, A.; lè ne Rodier, M.-H.; Imbert, C. Update on Infectious Risks Associated with Dental Unit Waterlines. *FEMS Immunol Med Microbiol* **2012**, *65*, 196–204, doi:10.1111/j.1574-695X.2012.00971.x.
26. Costa, D.; Mercier, A.; Gravouil, K.; Lesobre, J.; Delafont, V.; Bousseau, A.; Verdon, J.; Imbert, C. Pyrosequencing Analysis of Bacterial Diversity in Dental Unit Waterlines. *Water Res* **2015**, *81*, 223–231, doi:10.1016/J.WATRES.2015.05.065.
27. Siqueira, M.F.; Buitrago, J.M.; Kolbe, R.J. Dental Unit Waterline Testing Practices: An 11-Year Retrospective Study. *BMC Oral Health* **2023**, *23*, 867, doi:10.1186/s12903-023-03590-y.
28. Lizzadro, J.; Mazzotta, M.; Girolamini, L.; Dormi, A.; Pellati, T.; Cristino, S. Comparison between Two Types of Dental Unit Waterlines: How Evaluation of Microbiological Contamination Can Support Risk Containment. *International Journal of Environmental Research and Public Health 2019, Vol. 16, Page 328* **2019**, *16*, 328, doi:10.3390/IJERPH16030328.
29. Hikal, W.M.; Kačániova, M.; Hussein, D.E.E.; Ghit, A.; Smaoui, S.; Aleem, M.T.; Čmiková, N.; Said-Al Ahl, H.A.H. Dental Unit Waterlines and Health Risks of Pathogenic Microbial Contamination: An Update Review. *Journal of Biological Studies* **2023**, *6*, 282–298, doi:10.62400/JBS.V6I4.8729.
30. Kettering, J.D.; Muñoz-Viveros, C.A.; Stephens, J.A.; Patrick Naylor, W.; Zhang, W. Reducing Bacterial Counts in Dental Unit Waterlines: Distilled Water vs. Antimicrobial Agents. *J Calif Dent Assoc* **2002**, *30*, 735–740, doi:10.1080/19424396.2002.12223313.