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Longitudinal Restoration of Regulatory Compliance in Dental Unit Waterlines Following Continuous Hypochlorous Acid Disinfection: A Real-World Evaluation

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Abstract

Background:
Dental unit waterlines (DUWLs) are prone to biofilm formation and recurrent microbial amplification, frequently resulting in heterotrophic plate counts exceeding regulatory thresholds. Continuous hypochlorous acid (HOCl) disinfection has been proposed as a strategy to achieve sustained microbiological control; however, longitudinal real-world evidence under regulated European clinical conditions remains limited. This study evaluated microbiological evolution and regulatory compliance following implementation of continuous HOCl disinfection in a German pediatric dental clinic.

Methods:
A longitudinal environmental microbiological evaluation was conducted in two dental units (BE1, BE2) under routine clinical conditions. Water samples were collected at three predefined timepoints: baseline (T0), early follow-up at 3 weeks (T1), and late follow-up at ≥4 months (T2). Heterotrophic plate counts at 36°C and *Legionella spp.* detection were performed by an ISO 17025-accredited laboratory according to DIN EN ISO 11731. Percentage and $\log_{10}$reductions were calculated relative to baseline. Regulatory compliance was defined according to Robert Koch Institute (RKI) standards (<100 CFU/mL; no detectable *Legionella spp.*).

Results:
At baseline, heterotrophic counts were 234 CFU/mL (BE1) and 136 CFU/mL (BE2), exceeding regulatory thresholds. At T1, counts decreased to 65 CFU/mL and 48 CFU/mL, respectively, corresponding to 72.2% and 64.7% reductions and restoration of compliance. At T2, counts were below detection limits (<1 CFU/mL) in both units, corresponding to cumulative reductions of ≥99.57% (≥2.37 $\log_{10}$) in BE1 and ≥99.26% (≥2.13 $\log_{10}$) in BE2. *Legionella spp.* remained undetectable at all timepoints.

Conclusion:
Continuous HOCl disinfection was associated with progressive and sustained suppression of heterotrophic bacterial load, resulting in restoration and maintenance of regulatory compliance over a four-month observation period. These findings support continuous antimicrobial dosing as a potentially reliable long-term DUWL management strategy in regulated clinical environments.

INTRODUCTION

Dental unit waterlines (DUWLs) are recognized as complex engineered aquatic systems highly susceptible to microbial colonization and biofilm formation. Structural characteristics—including narrow internal lumen diameter (typically 2–3 mm), high surface-to-volume ratio, intermittent water flow, extended stagnation periods, and polymeric tubing materials—create an environment conducive to rapid bacterial adhesion and extracellular polymeric substance (EPS) production [1–3]. Once established, biofilms in DUWLs demonstrate remarkable resistance to mechanical flushing and episodic chemical disinfection, serving as persistent microbial reservoirs within dental treatment units [4, 5].

Even when incoming municipal water complies with potable standards, bacterial amplification within DUWL systems frequently leads to heterotrophic plate counts exceeding recommended thresholds for clinical use [6, 7]. Multiple investigations across Europe have documented persistent contamination in dental water systems, with reported detection of opportunistic microorganisms including *Pseudomonas aeruginosa*, *Legionella pneumophila*, and non-tuberculous mycobacteria (NTM) [8–11]. Although severe infections in dental settings remain uncommon, aerosolization during high-speed instrumentation and ultrasonic scaling may facilitate environmental dissemination and occupational exposure, particularly in enclosed clinical environments [12, 13].

In Germany, microbiological water quality in dental units is regulated according to recommendations issued by the Robert Koch Institute (RKI), which stipulate heterotrophic plate counts below 100 colony-forming units per milliliter (CFU/mL) and strict monitoring for *Legionella spp.* [14]. Non-compliance with these standards may result in regulatory intervention and operational consequences. Recent German surveillance data indicate that DUWL contamination remains a relevant challenge despite routine maintenance protocols [10].

Traditional DUWL management strategies predominantly rely on intermittent “shock” disinfection protocols employing oxidizing agents such as sodium hypochlorite, hydrogen peroxide, chlorine dioxide, or ozone [15–17]. Although such interventions may temporarily reduce planktonic bacterial counts, several studies have demonstrated rapid recolonization due to incomplete biofilm disruption [18, 19]. Biofilm matrices exhibit structural heterogeneity and diffusion-limited penetration, reducing antimicrobial efficacy during short-duration treatments [4, 20]. Consequently, intermittent shock approaches may fail to ensure sustained microbiological stability.

Continuous low-level antimicrobial dosing has therefore emerged as a preventive strategy aimed at ecological stabilization rather than episodic decontamination. Among available agents, hypochlorous acid (HOCl) has attracted increasing attention. HOCl represents the physiologically active antimicrobial species generated by neutrophils during innate immune responses and exhibits high oxidative potential against bacteria, viruses, and fungi [21]. At neutral pH, HOCl demonstrates greater membrane permeability and bactericidal efficacy compared with hypochlorite ion (OCl^-), due to its non-ionized molecular structure [22].

Recent laboratory and pilot clinical investigations (2022–2025) have reported significant heterotrophic count reduction and biofilm suppression following continuous HOCl application in DUWL systems [23–25]. These studies suggest that sustained oxidative exposure may inhibit recolonization cycles and promote microbiological stabilization over time. However, most published evidence derives from experimental settings, short-term evaluations, or laboratory simulations. Longitudinal real-world assessments conducted under highly regulated European clinical conditions remain limited. In particular, the temporal trajectory of microbial suppression and restoration of regulatory compliance—rather than isolated CFU reduction at a single follow-up point—has rarely been evaluated as a primary outcome.

Given the operational and regulatory implications of water quality management in dental healthcare, robust real-world longitudinal data are needed to determine whether continuous HOCl disinfection can achieve progressive microbiological suppression and sustained compliance under routine clinical conditions.

Therefore, the aim of the present longitudinal study was to evaluate heterotrophic bacterial load and regulatory compliance across predefined timepoints following implementation of continuous HOCl disinfection in a German pediatric dental clinic operating under RKI hygiene standards.

MATERIALS AND METHODS

Study design and setting

This investigation was designed as a longitudinal real-world environmental microbiological evaluation conducted under routine clinical conditions in a pediatric dental clinic located in Konstanz, Germany. The clinic operates under national hygiene regulations and follows recommendations established by the Robert Koch Institute (RKI) for microbiological quality of dental unit water.

The primary outcome was restoration and sustained maintenance of regulatory compliance, defined as heterotrophic plate counts below 100 CFU/mL at 36°C and absence of detectable *Legionella spp.*, in accordance with RKI recommendations [14].

Two dental treatment units (identified as BE1 and BE2) were included in the evaluation. Units were selected based on routine microbiological monitoring indicating prior non-compliance.

Study timeline

Microbiological sampling was performed at three predefined timepoints:

- T0 (Baseline): 04 July 2025 – prior to activation of continuous disinfection
- T1 (Early follow-up): 24 July 2025 – approximately three weeks after activation
- T2 (Late follow-up): 04 November 2025 – after ≥4 months of uninterrupted continuous operation

This structure enabled assessment of immediate microbiological response (T0→T1) and long-term stabilization (T0→T2).

Pre-intervention assessment

Baseline sampling was performed prior to activation of continuous hypochlorous acid (HOCl) disinfection. No chemical shock disinfection was performed immediately before baseline sampling to avoid transient suppression effects that could artificially influence initial microbiological measurements.

Water samples were collected during routine clinical workflow, after standard daily flushing procedures, to reflect real-world exposure conditions rather than artificially induced stagnation scenarios.

Maintenance procedures prior to intervention

Before implementation of continuous HOCl disinfection, standardized mechanical maintenance procedures were conducted. These included inspection of internal tubing integrity, servicing of filters, routine mechanical flushing, and verification of hydraulic functionality.

No structural replacement of entire tubing systems was performed. This methodological decision ensured that observed microbiological changes could be attributed to continuous HOCl exposure rather than complete infrastructural substitution.

Continuous HOCl disinfection system

Following maintenance, a continuous in situ electrochemical activation (ECA) system was installed. The system generates hypochlorous acid (HOCl) from saline solution (NaCl + H_2O) via controlled electrochemical processes.

Operational concentrations were maintained within manufacturer-specified and regulatory-approved limits (low mg/L range), ensuring antimicrobial efficacy while preserving material compatibility and patient safety.

Continuous dosing was maintained uninterrupted between T0 and T2. No additional shock disinfection procedures were performed during the observation period.

Sampling protocol

At each timepoint, 250 mL of procedural water was aseptically collected from each dental unit in sterile, single-use sampling bottles containing sodium thiosulfate to neutralize residual oxidizing agents.

Samples were transported under cooled conditions (mean transport temperature 6.4°C) and processed within the timeframe recommended by laboratory standards.

Microbiological analysis

All microbiological analyses were conducted by an independent laboratory accredited according to ISO 17025 standards (IfP Privates Institut für Produktqualität GmbH, Berlin, Germany).

The following analyses were performed:

- Heterotrophic plate count at 36°C (CFU/mL)
- *Legionella spp.* detection according to DIN EN ISO 11731:2019-03

The detection threshold was <1 CFU/mL. Results below this threshold were reported as “<1 CFU/mL.”

Regulatory compliance was defined as:

- <100 CFU/mL at 36°C
- No detectable *Legionella spp.*

Statistical analysis

Given the limited number of evaluated units (n = 2), inferential parametric testing was not appropriate. Instead, descriptive longitudinal analysis was performed.

Absolute values were reported for each timepoint (T0, T1, T2). Percentage reduction and $\log_{10}$ reduction were calculated relative to baseline (T0).

Percentage reduction was calculated as:

$$Reduction(\%) = \frac{T0 - Tx}{T0} \times 100$$

Log_{10} reduction was calculated as:

$$Log_{10}reduction = log_{10}(T0) - log_{10}(Tx)$$

For conservative estimation, values reported as <1 CFU/mL were calculated using 1 CFU/mL as the upper detection limit.

Compliance status was evaluated categorically (non-compliant vs compliant) at each timepoint.

RESULTS

Baseline microbiological status (T0)

Baseline microbiological assessment demonstrated heterotrophic plate counts exceeding the regulatory threshold established by the Robert Koch Institute (<100 CFU/mL) in both evaluated dental units.

At T0, BE1 presented 234 CFU/mL and BE2 136 CFU/mL at 36°C incubation. Both measurements surpassed the RKI compliance limit and were therefore categorized as non-compliant. No *Legionella spp.* were detected (<1 CFU/mL) at baseline.

These findings indicate persistent heterotrophic amplification within DUWL systems despite routine mechanical maintenance and daily flushing procedures, confirming the necessity for sustained antimicrobial intervention rather than episodic correction.

Early microbiological response (T1)

At approximately three weeks following initiation of continuous hypochlorous acid (HOCl) disinfection, a substantial reduction in heterotrophic bacterial load was observed in both units.

BE1 decreased from 234 CFU/mL to 65 CFU/mL, corresponding to a 72.2% reduction relative to baseline. BE2 decreased from 136 CFU/mL to 48 CFU/mL, corresponding to a 64.7% reduction.

Importantly, both units transitioned from non-compliant to compliant status at T1, achieving heterotrophic plate counts below the RKI threshold. This early response suggests rapid suppression of planktonic bacterial populations following continuous HOCl activation.

Sustained microbiological suppression (T2)

At ≥4 months of uninterrupted continuous HOCl operation, heterotrophic plate counts in both units were reduced below the laboratory detection threshold (<1 CFU/mL).

For conservative quantitative estimation, post-intervention values were calculated using 1 CFU/mL. The cumulative reductions from baseline (T0→T2) were:

• BE1: ≥99.57% reduction, corresponding to ≥2.37 $\log_{10}$ reduction
• BE2: ≥99.26% reduction, corresponding to ≥2.13 $\log_{10}$ reduction

The trajectory of microbiological decline across T0, T1, and T2 demonstrates a progressive and monotonic reduction pattern without evidence of rebound. The longitudinal evolution relative to the regulatory threshold is illustrated in Figure 1.

Legionella spp. remained undetectable at all three timepoints.

Regulatory compliance evolution

Regulatory classification changed sequentially over time. At T0, both dental units were classified as non-compliant. Following system activation, compliance was restored at T1 and maintained at T2, with bacterial counts falling below detection limits.

Regulatory status across timepoints is summarized in Table 1. The intervention therefore resulted not only in restoration of compliance but also in sustained maintenance over the longitudinal observation period.

Table 1. Regulatory classification of dental unit water quality according to Robert Koch Institute (RKI) criteria across three sampling timepoints.

Parameter	RKI Threshold	BE1 (T0)	BE1 (T1)	BE1 (T2)	BE2 (T0)	BE2 (T1)	BE2 (T2)
Heterotrophic plate count (CFU/mL, 36°C)	<100 CFU/mL	234	65	<1	136	48	<1
Legionella spp.	Not detectable	ND	ND	ND	ND	ND	ND
Compliance status	—	No	Yes	Yes	No	Yes	Yes

ND: not detected (<1 CFU/mL); CFU: colony-forming units; RKI: Robert Koch Institute; T0: pre-intervention baseline; T1: early follow-up; T2: late follow-up after ≥4 months of uninterrupted continuous hypochlorous acid operation.

DISCUSSION

The present longitudinal real-world evaluation demonstrated progressive and sustained microbiological suppression following implementation of continuous hypochlorous acid (HOCl) disinfection in dental unit waterlines (DUWLs). At baseline (T0), both evaluated units exhibited heterotrophic plate counts exceeding the regulatory threshold established by the Robert Koch Institute (RKI). Following system activation, a marked early reduction was observed at three weeks (T1), with complete suppression below detection limits achieved at four months (T2). This pattern corresponded to ≥ 2 $\log_{10}$ cumulative reduction and full restoration and maintenance of regulatory compliance.

Importantly, the inclusion of an intermediate timepoint allowed assessment of microbiological trajectory rather than a simple binary pre–post comparison. The progressive decline observed across T0, T1, and T2 suggests gradual destabilization of biofilm architecture rather than isolated suppression of planktonic organisms.

Magnitude and trajectory of microbiological reduction

The cumulative ≥ 2 $\log_{10}$ reduction observed in both units exceeds reductions frequently reported for intermittent shock disinfection protocols. Previous investigations evaluating sodium hypochlorite, hydrogen peroxide, or chlorine dioxide shock treatments have documented temporary reductions ranging between 1–1.5 $\log_{10}$, often followed by recolonization within weeks due to residual biofilm matrix persistence [15–19].

Biofilm matrices in DUWL systems exhibit structural heterogeneity, extracellular polymeric substance (EPS) protection, and diffusion-limited antimicrobial penetration [4, 20]. As described by Flemming and Wingender [4], the biofilm matrix acts as a physicochemical barrier that impairs antimicrobial diffusion and enhances microbial resilience. Consequently, short-duration chemical exposure may reduce planktonic bacterial load without fully destabilizing established biofilm structures.

Continuous HOCl dosing, in contrast, provides sustained oxidative pressure within the waterline microenvironment. HOCl exists predominantly in its non-ionized form at physiological pH, enabling enhanced membrane permeability and rapid oxidative interaction with microbial cellular components [21, 22]. Sustained exposure may interfere with EPS stabilization and inhibit recolonization cycles, thereby preventing re-establishment of structured biofilms.

Recent experimental investigations (2022–2025) have demonstrated superior biofilm penetration and more persistent microbial suppression with continuous HOCl application compared with episodic shock strategies [23, 24]. However, many of these studies were conducted under laboratory or simulated conditions. The present study extends these findings to a regulated real-world clinical environment.

Regulatory implications and compliance restoration

A notable strength of the present investigation is the evaluation of regulatory compliance as a primary outcome rather than CFU reduction alone. While quantitative reductions are frequently reported in the literature, categorical restoration of compliance under national hygiene standards has rarely been emphasized.

In Germany, failure to comply with RKI-recommended thresholds (< 100 CFU/mL) may result in mandatory corrective measures and increased regulatory oversight. The transition observed in this study, rom non-compliance at T0 to compliance at T1 and sustained compliance at T2, represents a clinically and operationally meaningful outcome beyond numerical microbial reduction.

This compliance restoration is particularly relevant given recent surveillance reports documenting persistent contamination of DUWL systems in European settings despite routine maintenance protocols [10].

Stability over time and absence of rebound

The longitudinal design allowed evaluation of microbiological stability over a four-month interval. Importantly, no rebound phenomenon was observed between T1 and T2. Previous studies have reported rapid recolonization following episodic disinfection events, often attributed to survival of residual biofilm communities [18, 19].

The absence of rebound in the present study suggests ecological stabilization of the DUWL environment under continuous HOCl exposure. Rather than transient suppression, the progressive decline across timepoints supports the hypothesis of sustained biofilm disruption and inhibition of recolonization cycles.

Such stability is particularly relevant in high-use clinical environments where intermittent shock strategies may fail to maintain consistent microbiological control.

Clinical and occupational considerations

Although severe infections directly attributable to DUWL contamination are uncommon in immunocompetent individuals, aerosolization of contaminated water during high-speed instrumentation and ultrasonic procedures remains a documented occupational concern [12, 13]. Zemouri et al. [12] have highlighted the potential for bioaerosol dissemination in dental settings, emphasizing the importance of environmental microbial control.

Continuous maintenance of low heterotrophic counts may therefore contribute to improved environmental hygiene and occupational safety, particularly in pediatric clinics where aerosol-generating procedures are frequent. Pediatric populations may also include medically compromised patients for whom precautionary infection prevention measures are especially important.

While this study did not assess clinical infection outcomes, sustained microbiological stabilization aligns with established infection control principles and precautionary hygiene frameworks.

Comparison with recent literature

Recent publications between 2022 and 2025 have reported promising outcomes with continuous disinfectant systems in DUWL management [23–25]. However, many of these investigations were limited to short observation intervals or lacked evaluation within a regulatory compliance framework.

The present study contributes longitudinal real-world evidence under regulated European clinical conditions, incorporating ISO 17025-accredited laboratory analysis and DIN EN ISO 11731 methodology. Although limited in sample size, the consistency of response across all timepoints strengthens internal validity.

Limitations

The principal limitation of this study remains the limited number of evaluated dental units (n = 2), which precludes inferential statistical testing and broader generalization. Additionally, structural biofilm imaging or molecular quantification techniques were not performed, preventing direct visualization of biofilm matrix disruption.

Although three timepoints were included, longer-term follow-up would further strengthen conclusions regarding sustained ecological stability. Future multicenter investigations incorporating molecular biofilm analysis, metagenomic profiling, and extended monitoring intervals are warranted.

Strengths

Despite these limitations, several methodological strengths support the robustness of findings:

- Real-world clinical setting under regulated hygiene standards
- Longitudinal design with intermediate and late follow-up
- Independent ISO 17025-accredited laboratory analysis
- DIN EN ISO 11731 methodology

- Evaluation of regulatory compliance as primary outcome
- Conservative statistical estimation approach

Overall interpretation

Within the limitations of this observational longitudinal design, continuous hypochlorous acid disinfection demonstrated progressive and sustained suppression of heterotrophic bacterial load and achieved restoration and maintenance of regulatory compliance in two previously non-compliant dental units.

These findings support continuous antimicrobial dosing as a potentially reliable long-term DUWL management strategy in regulated healthcare environments.

CONCLUSION

Within the limitations of this longitudinal real-world evaluation, continuous hypochlorous acid disinfection was associated with progressive and sustained suppression of heterotrophic bacterial load in dental unit waterlines, resulting in restoration and maintenance of regulatory compliance over a four-month observation period.

The temporal stabilization observed suggests that continuous antimicrobial dosing may represent a reliable long-term strategy for DUWL management in regulated clinical environments.

Declarations

Ethics approval and consent to participate

This study involved environmental water sampling only and did not include human participants, patient data, or biological specimens. According to applicable German regulations, formal ethical approval and informed consent were not required.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Thiago Costa de Oliveira Galiza conceptualized and designed the study, coordinated data acquisition, performed data analysis, interpreted the results, and drafted the manuscript.

Julia de Jesus Capez contributed to data collection, assisted in data organization, and participated in manuscript drafting.

Guilherme Pires de Campos Cardoso contributed to data interpretation and critically revised the manuscript for important intellectual content.

Isabel Cristina Oliveira Fernandes contributed to data organization and assisted in manuscript preparation.

Felipe Augusto Vieira contributed to data analysis support and manuscript review.

Gabriele Emiliano Verità supervised the study implementation in Germany, contributed to methodological refinement, and critically revised the manuscript.

Cristina Verità contributed to study supervision, regulatory guidance, and manuscript revision.

Elcio Magdalena Giovani supervised the study design, provided academic oversight in Brazil, and critically revised the manuscript.

All authors read and approved the final manuscript.

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Figures

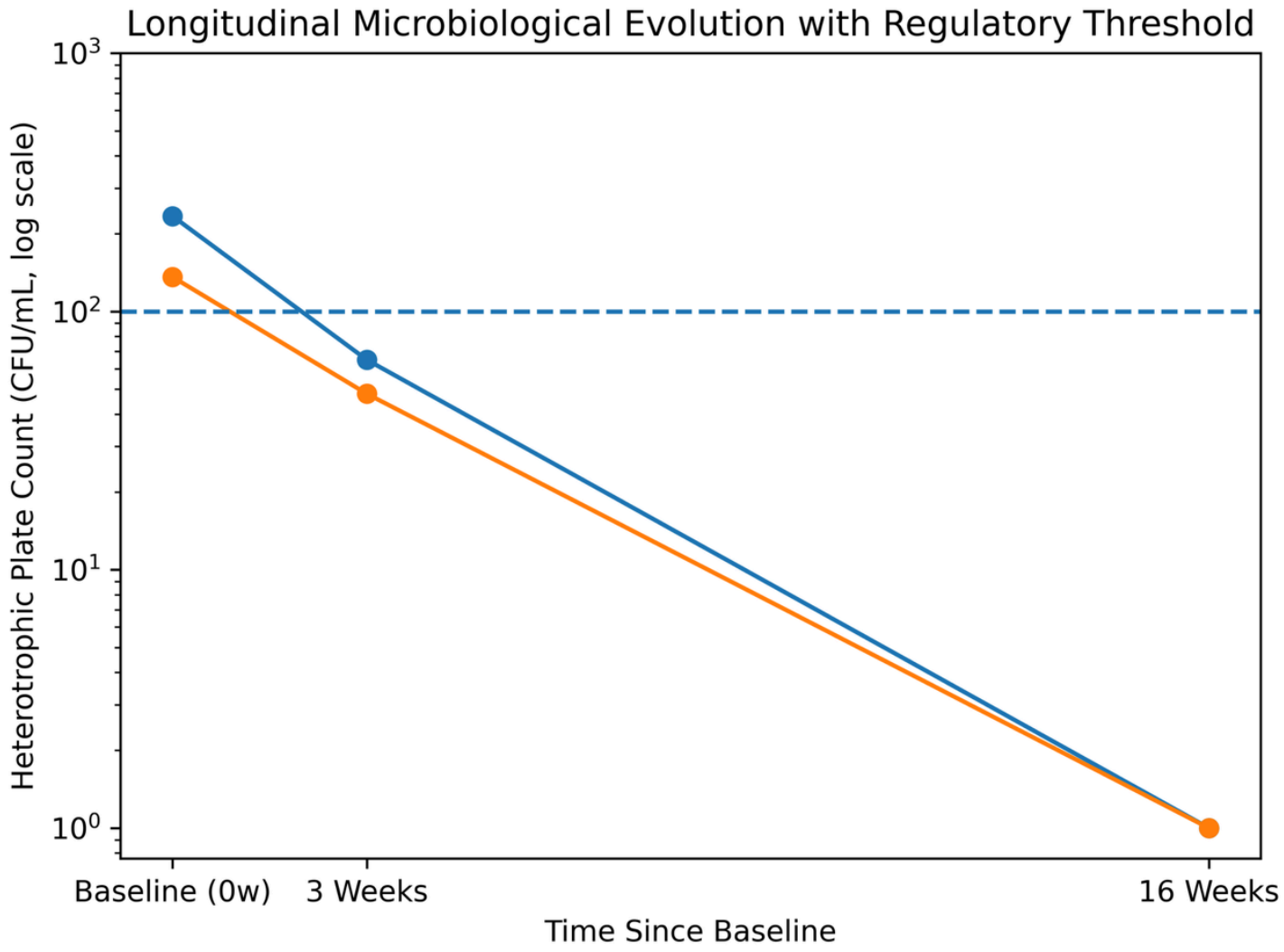


Figure 1

Longitudinal evolution of heterotrophic plate counts (CFU/mL, log scale) in dental units BE1 and BE2 across three sampling timepoints (T0: baseline; T1: 3 weeks; T2: 16 weeks). The dashed horizontal line represents the regulatory threshold established by the Robert Koch Institute (<100 CFU/mL).