Research Article

Plant-Based versus Chlorine-Based Disinfection: Short-Term Efficacy of *Aloe vera* and NaDCC Against Microbial Contamination in Dental Unit Waterlines

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Abstract | Dental unit waterlines (DUWLs) can develop persistent microbial biofilms due to narrow-bore tubing, low water flow, and repeated exposure to oral contaminants, making effective decontamination an important infection-control measure. This study aimed to compare the short-term reduction of bacterial and fungal contamination in dental unit water bottles and air/water syringe outlets after treatment with 0.1% sodium dichloroisocyanurate (NaDCC) or *Aloe vera* prepared as a 1:1 (v/v) gel-to-dist.-water solution. Twenty-eight dental units (14 per group) were sampled at the water bottle and air/water syringe before and after a standardized 120-min. contact period, yielding 112 water samples. Samples were processed by culture-based methods, and bacterial and fungal counts (cfu/ml) were compared using Wilcoxon signed-rank and Mann–Whitney U tests. In bottle water, mean bacterial counts fell from 454,000 ± 176,974.93 to 80,000 ± 41,557.00 cfu/ml after NaDCC and from 838,000 ± 398,226.07 to 90,000 ± 44,221.66 cfu/ml after *Aloe vera*. In syringe water, counts fell from 66,000 ± 33,096.30 to 18,000 ± 15,156.33 cfu/ml after NaDCC and from 144,000 ± 87,320.61 to 18,000 ± 12,000.00 cfu/ml after *Aloe vera*. No culturable fungi were detected post-treatment, but residual bacterial contamination remained after both treatments. Both interventions produced substantial short-term reductions in cultivable microbial contamination. The larger absolute bacterial reductions with *Aloe vera* reflected higher baseline counts in that group and should not be taken as evidence of superiority. *Aloe vera* warrants further controlled evaluation with standardized preparation and assessment of biofilm eradication and safety.

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Introduction

Dental unit waterlines (DUWLs) are important but often overlooked components of the dental infection prevention (Blake, 1963; Pankhurst *et al.*, 1998). Their narrow-bore tubing, high surface-area-to-volume ratio, intermittent water flow, and periods of stagnation create favorable conditions for microbial attachment and biofilm development (Spagnolo *et al.*, 2020). Once established, DUWL biofilms can act as persistent reservoirs from where the microorganisms are released into the flowing water, making the contamination difficult to control by flushing alone (Suleyman *et al.*, 2018; Volgenant and Persoon, 2019).

Microorganisms may enter with the incoming water supply, while the dental procedures may facilitate contamination through aspiration or backflow when the appropriate anti-retraction mechanisms are inadequate (Alkhulaifi *et al.*, 2020). Bacteria recovered from DUWLs may include environmental Gram-negative bacteria, Gram-positive bacteria, oral *streptococci*, and other opportunistic microorganisms such as fungi have also been reported (Tall *et al.*, 1995; Fan *et al.*, 2021). The control strategies include routine flushing, independent water reservoirs, filtration, ultraviolet treatment, and continuous or periodic chemical disinfection (Linger *et al.*, 2001; Watanabe *et al.*, 2008; Scott, 2019), but their effectiveness depends on the agent, its concentration, contact time, organic load, and compatibility with the dental unit (Bansal *et al.*, 2011; Li and Xin, 2025). The Centers for Disease Control and Prevention (CDC) recommends that the routine dental water treatment meets a heterotrophic bacterial quality target of no more than 500 cfu/ml (Kohn *et al.*, 2003; CDC, 2024). Therefore, a large percentage reduction in microbial counts should not by itself be interpreted as achievement of acceptable dental water quality if substantial residual contamination remains.

Among the chemical approaches, chlorine-releasing agents are widely used because of their broad antimicrobial activity and practical application in waterline disinfection (Zhang *et al.*, 2023; Yue *et al.*, 2024). Sodium dichloroisocyanurate (NaDCC) is a chlorine-releasing compound that provides available chlorine in aqueous solution. Its antimicrobial performance is affected by the available chlorine concentration, organic burden, contact time, pH, and the presence and maturity of biofilms (Linger *et al.*, 2001; Zheng *et al.*, 2025). However, the chlorine-based disinfectants have recognized drawbacks, where at high concentrations they may corrode the metal components of the dental units and form undesirable by-products, and their activity declines in the presence of organic matter and mature biofilms (Yue *et al.*, 2024; Zheng *et al.*, 2025). These limitations, together with the growing interest in ecological, less toxic, and less corrosive agents that are compatible with the dental equipment, have encouraged the search for natural alternatives. In the present study, NaDCC was evaluated at 0.1% (1000 µg/ml available chlorine) with a standardized 120-min. contact period (Alkhulaifi *et al.*, 2020; Petchphayaprai *et al.*, 2023).

Aloe vera, in contrast, is a natural product with a long history of use in dentistry (Neena *et al.*, 2015; Abu-Seida and Seif, 2023), which makes it an attractive candidate as a potentially less corrosive and less toxic alternative. Experimental studies have reported the antibacterial and antifungal activity of *Aloe vera* preparations against a range of microorganisms, including the oral and clinically relevant species (Jain *et al.*, 2016; Habibi *et al.*, 2018; Danish *et al.*, 2020). The proposed antimicrobial mechanisms of these preparations include interference with the microbial adhesion and biofilm-associated processes, in addition to their effects on the microbial membranes and cellular metabolism (Fani and Kohanteb, 2012; Xiang *et al.*, 2017). However, the biological activity of *Aloe vera* is strongly influenced by the plant material, processing, stabilization, storage, and formulation; therefore, the results obtained with one preparation cannot automatically be extrapolated to another (Sims and Zimmerman, 1971; Athiban *et al.*, 2012; Karim *et al.*, 2014). An available DUWL's study conducted by Pareek *et al.* (2013) demonstrated the antimicrobial potential of *Aloe vera* in an *in-vitro* waterline model, while several other studies have mainly investigated the *Aloe vera* activity against the oral microorganisms (Athiban *et al.*, 2012; Karim *et al.*, 2014; Jain *et al.*, 2016). Evidence on DUWL decontamination with *Aloe vera* therefore remains limited, and the effectiveness of its specific preparation under clinical dental-unit conditions has not been established.

Therefore, the objectives of the present study were to compare the short-term effect of 0.1% NaDCC and *Aloe vera* prepared as a 1:1 (v/v) gel-to-dist.-water solution on the cultivable bacterial and fungal contamination in DUWL at two sampling locations before and after a standardized 120-min. treatment period, and determine whether either intervention reduced the contamination towards the recommended dental water-quality target.

Materials and Methods

Study design and setting

This comparative experimental study was conducted at the Faculty of Oral and Dental Medicine, Ahram Canadian University (ACU), Egypt. Twenty-eight dental units were included. The units were allocated equally between the two intervention groups: NaDCC (n = 14) and *Aloe vera* (1:1, v/v) (n = 14). The units were allocated to the treatment groups by simple randomization. Each dental unit was assessed at two sampling locations: the dental unit water bottle and the air/water syringe outlet. Both locations were sampled before treatment and after treatment. Accordingly, each dental unit contributed four observations, yielding 112 water samples in total. The dental unit, rather than the individual water sample, was considered the experimental unit because the repeated samples originated from the same physical unit.

Treatment groups

NaDCC preparation and application

Effervescent sodium dichloroisocyanurate (NaDCC) tablets (Haz-Tabs; Guest Medical Ltd., Essex, United Kingdom) were used. Each tablet weighed 2.65 g and provided 1.5 g (1500 mg) of available active chlorine.

A 0.1% solution equivalent to 1000 µg/ml available chlorine was prepared by dissolving one complete 2.65-g tablet in 1.5 l (1500 ml) of dist. water. The concentration was calculated from the available chlorine content: 1.5 g/1500 ml = 1 mg/ml = 1000 mg/l = 1000 µg/ml, equivalent to 0.1% w/v available chlorine. The concentration and contact time were selected on the basis of previously reported waterline decontamination protocols (Alkhulaifi *et al.*, 2020; Petchphayaprai *et al.*, 2023; Zheng *et al.*, 2025). The complete tablet was dissolved rather than manually divided to reduce the dosing variability. The solution was allowed to dissolve completely before application. The dental unit water bottle was pre-cleaned according to the study protocol, rinsed, and filled with the prepared NaDCC solution. A 265-ml aliquot was used for treatment. The solution was allowed to remain in the waterline for 120 min. After the contact period, the treatment solution was evacuated and the system was flushed according to the study protocol before post-treatment sampling.

Aloe vera preparation and application

Aloe vera treatment used a pure inner-leaf *Aloe vera* gel prepared as a 1:1 (v/v) *Aloe vera* gel-to-dist.-water solution. The gel was extracted from fresh leaves of cultivated plants obtained from a local flower and plant nursery (Egypt). The leaves were washed with water, the thick outer epidermis was removed, and the inner mucilaginous gel was collected in a sterile container (Jain *et al.*, 2016). The use of *Aloe vera* for waterline decontamination was based on the in-vitro model of Pareek *et al.* (2013). For each preparation, 132.5 ml of *Aloe vera* gel were combined with 132.5 ml of dist. water to obtain a final volume of 265 ml. The mixture was homogenized before application. The prepared *Aloe vera* (1:1, v/v) solution was introduced into the dental unit water reservoir and allowed to contact the waterline for the same standardized 120-min. period. After treatment, the solution was evacuated and the system was flushed according to the study protocol before post-treatment sampling. The 1:1 ratio describes the preparation of the test solution; it should not be interpreted as a chemically standardized concentration of a specific *Aloe vera* antimicrobial constituent.

Sampling protocol

The samples were collected aseptically from the dental unit water bottle and the air/water syringe outlet. A 20-ml water sample was collected from each sampling location into a sterile labeled 50-ml tube containing 0.1 ml of 10% sodium thiosulfate, which was used to neutralize the residual chlorine-containing disinfectant activity during sample handling (ASTM, 2008; Rice *et al.*, 2017). Because the same sampling approach was used for both

intervention groups, any potential interaction between the neutralizer and *Aloe vera* should be considered when interpreting the protocol and should ideally be addressed by validation experiments. The pre-treatment samples represented the baseline microbial burden immediately before application of the assigned intervention. Post-treatment samples were collected after completion of the 120-min. contact period and the standardized flushing procedure. Sample identifiers were maintained throughout the transport and laboratory processing.

Transportation and laboratory processing

The samples were placed in an insulated transport container maintained at 4 ± 2°C and transported to the microbiology laboratory within 2 h. On receipt, each sample was mixed to obtain a homogeneous suspension. Decimal serial dilutions from 10^{-1} to 10^{-3} were prepared using a sterile diluent, and 100 µl of each appropriate dilution was spread with a sterile L-shaped glass spreader onto the relevant culture media (Rice *et al.*, 2017).

Culture media and incubation

Blood agar (BA) Petri plates were used for recovery and enumeration of the heterotrophic bacteria and incubated aerobically at 37°C for 48 h. MacConkey agar plates were used for selective recovery of the Gram-negative bacilli and differentiation according to lactose fermentation, followed by incubation at 37°C for 24–48 h. Mitis Salivarius agar plates were used for recovery of the presumptive oral streptococci and incubated at 37°C in 5% CO_2 for 48 h. Sabouraud dextrose agar (SDA) plates supplemented with chloramphenicol were used for recovery of the fungi/yeasts and incubated at 25–28°C for 5–7 d (Forbes *et al.*, 2007; Rice *et al.*, 2017).

The media were selected to cover the main microbial groups reported in DUWL water: aerobic heterotrophic bacteria, environmental Gram-negative bacilli, oral streptococci, and fungi (Tall *et al.*, 1995; Walker *et al.*, 2004; Damasceno *et al.*, 2017; Fan *et al.*, 2021; Omran *et al.*, 2021). Blood agar is a non-selective enriched medium that supports a broad range of aerobic and facultative heterotrophic bacteria, including the fastidious microorganisms of oral origin. MacConkey agar contains bile salts and crystal violet that inhibit the Gram-positive bacteria, allowing selective recovery of the Gram-negative bacilli with differentiation by lactose fermentation. Mitis Salivarius agar is selective for the oral streptococci, which may enter the waterlines through retraction of the oral fluids. SDA, with its acidic pH and supplemented with chloramphenicol to suppress the bacterial overgrowth and favors the recovery of yeasts and molds (Forbes *et al.*, 2007).

Because the study used culture-based recovery, growth on the selective media was interpreted as presumptive rather than a definitive species identification unless confirmatory identification was performed. In particular, growth on Mitis Salivarius agar alone does not establish species-level identification of the *Streptococcus mutans* or other streptococci, MacConkey agar does not by itself identify *Pseudomonas* species, and fungal growth on SDA does not by itself establish *Candida* species.

Microbial enumeration

After incubation, the countable individual colonies were enumerated using a digital colony counter (Model SC6 Plus; Stuart, UK). The obtained results were expressed as colony-forming units/ ml (cfu/ml). The plates with no recoverable colonies were reported as no culturable growth under the relevant assay conditions, where log transformation of zero value should only be performed after applying a prespecified assay-specific lower limit of detection. Each sample was plated in triplicate (three replicate plates per dilution); the dental unit (n = 14 per treatment group) was the unit of analysis.

Outcome measures

The main microbiological outcomes were bacterial cfu/ml and fungal/yeast cfu/ml at each sampling location before and after treatment. Absolute reduction was calculated descriptively as pre-treatment cfu/ml minus post-treatment cfu/ml. Because the treatment groups differed in baseline bacterial burden, absolute reduction was not considered sufficient by itself to establish a treatment superiority. Each outcome was determined for n = 14 dental units per treatment group at each sampling location, using one pre-

treatment and one post-treatment sample per location.

To account for a baseline microbial variation among the groups, baseline-adjusted relative changes and log_{10} reductions were calculated for each experimental unit alongside raw absolute differences (Vickers and Altman, 2001). Continuous variables were expressed as mean ± standard deviation (±SD) and log-transformed values (log_{10} cfu/ml). Within the groups pre- and post-treatment, comparisons were evaluated using the Wilcoxon signed-rank test, whereas among the groups, comparisons of the mean log reductions were analyzed using the Mann–Whitney U test (Wilcoxon, 1945; Mann and Whitney, 1947).

Statistical analysis

Descriptive statistics were expressed as mean ± standard deviation (±SD). Within the groups, pre- versus post-treatment comparisons were assessed using the Wilcoxon signed-rank test. Meanwhile, among the groups, comparisons of the mean reduction in cfu/ml were assessed using the Mann–Whitney U test. Comparisons between the dental unit water bottle and the air/water syringe sampling locations were also performed using the Mann–Whitney U test. A p value < 0.05 was considered statistically significant. A priori sample size calculation was performed using the G*Power software (version 3.1.9.7) (Faul *et al.*, 2007). The analysis was based on a significance level (α) of 0.05, statistical power of 80% (1 − β = 0.80), and an assumed correlation of 0.5 among the repeated measurements. A medium-to-large effect size (f = 0.30) was specified, and the resulting target sample size was 28 dental units, which were allocated equally between the two intervention groups (14 unit/ group). This calculation assumed a parametric repeated-measures framework and should be regarded as an approximate guide, since the non-parametric tests ultimately applied (Wilcoxon signed-rank, Mann–Whitney U) do not share the same power properties.

Results

Study sample and sampling structure

Twenty-eight dental units were evaluated, including 14 units in the NaDCC group and 14 units in the *Aloe vera* (1:1, v/v) group. Each unit contributed four water samples (water bottle before treatment, water bottle after treatment, air/water syringe before treatment, and air/water syringe after treatment), resulting in 112 processed water samples. Microbial contamination was present at the baseline at both sampling locations. Bacterial counts were consistently higher than fungal counts. The baseline bacterial burden was also higher in the *Aloe vera* group at both sites; therefore, absolute reductions were interpreted together with relative and logarithmic changes.

Microbial burden before and after treatment

Within-group comparisons of bacterial and fungal counts (cfu/ml) before and after treatment at each sampling site, using the Wilcoxon signed-rank test have been presented in Table 1 and Figure 1.

For the fungal cfu, no culturable fungi were detected in the reported post-treatment samples at either sampling location, and all comparisons reached a statistical significance ($p \leq 0.04$), except for *Aloe vera* at the air/water syringe outlet, where the reduction from 4,000 ± 6,909.85 to 0 ± 0 cfu/ml did not reach a statistical significance ($p = 0.06$), most likely attributable to the high baseline variability relative to the small sample size. The representative pre-treatment fungal culture plates from the four dental-unit sites are shown in Figure 2, where the corresponding post-treatment plates are not shown, as no culturable fungal growth was observed following either intervention.

Where; $^{a}p < 0.05$ by Wilcoxon signed-rank test. NaDCC: sodium dichloroisocyanurate; SD: standard deviation. Results are mean ± SD of n = 14 dental units per group. Each sample was plated in triplicate.

For the bacterial cfu, the reductions were substantial and statistically significant across all the site treatment combinations ($p = 0.01$ for all). At the water bottle, the bacterial counts fell from 454,000 to 80,000 cfu/ml after NaDCC and from 838,000 to 90,000 cfu/ml after *Aloe vera* treatment. At the air/water syringe outlet, the counts fell from 66,000 to 18,000 cfu/ml after NaDCC and from 144,000 to 18,000 cfu/ml after *Aloe vera*. Despite these marked reductions, the residual bacterial contamination

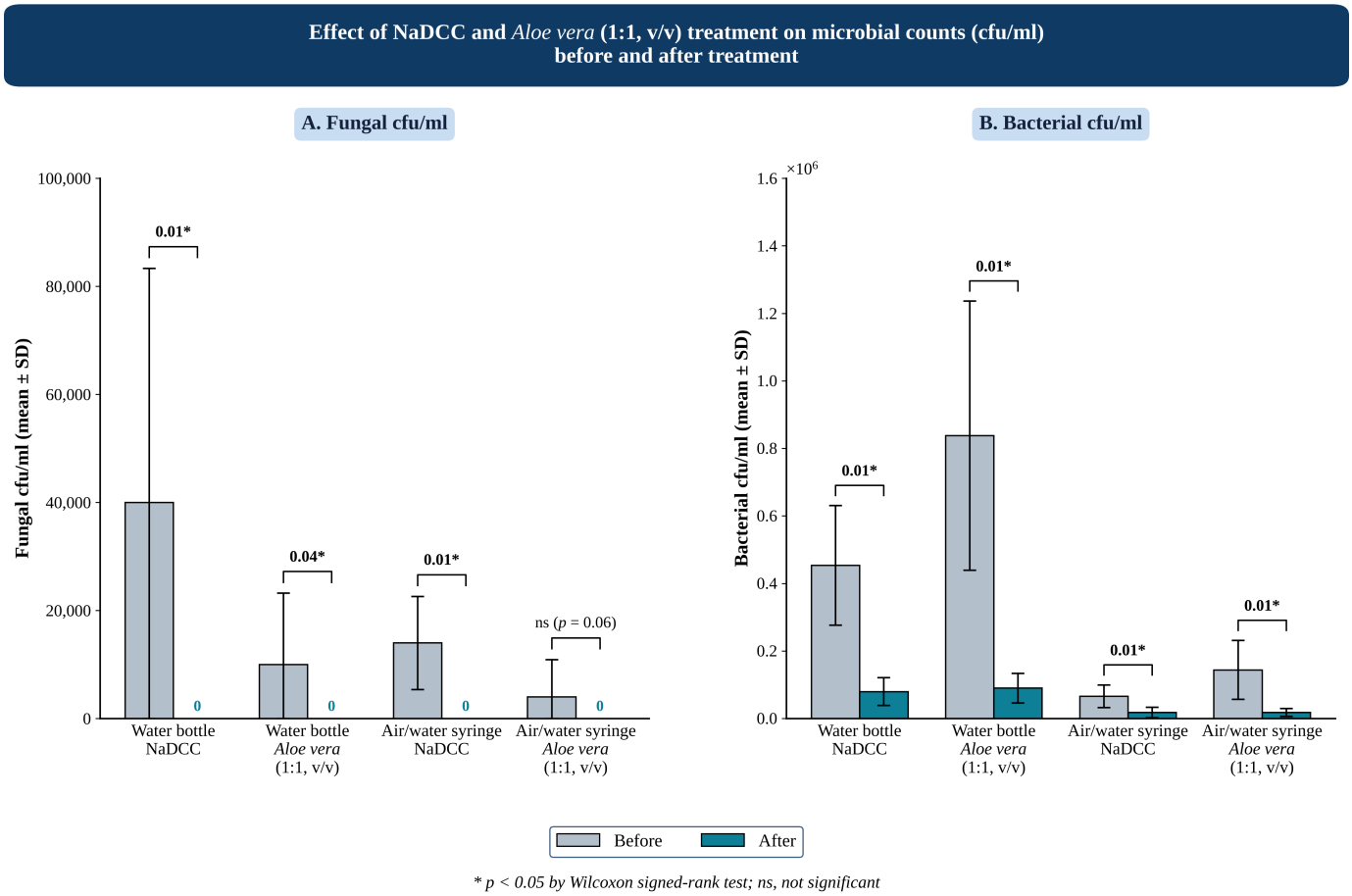


Figure 1: *Effect of sodium dichloroisocyanurate and Aloe vera (1:1, volume/volume) treatment on microbial counts (cfu/ml) before and after treatment. (A) Fungal counts. (B) Bacterial counts. Bars show mean ± standard deviation; asterisks indicate p < 0.05 (Wilcoxon signed-rank test); ns, not significant. Post-treatment fungal counts were 0 cfu/ml in all groups.*

remained detectable after both treatments at both sites, indicating that neither intervention achieved complete bacterial elimination. The representative culture plates illustrating this reduction in bacterial colony growth before and after treatment are shown in Figure 3.

Between-group comparison of the observed microbial count reductions

Between-group comparison of observed reductions in fungal and bacterial cfu/ml after treatment with NaDCC versus Aloe vera (1:1, v/v), stratified by sampling site (Table 2 and Figure 4). At the dental unit water bottle, *Aloe vera* produced a smaller fungal reduction than NaDCC (10,000 vs. 40,000 cfu/ml), and this difference was statistically significant (p = 0.015). Conversely, for bacterial reduction at the same site, *Aloe vera* outperformed the NaDCC recording 748,000 vs. 374,000 cfu/ml; p = 0.010.

At the air/water syringe outlet, the same pattern was observed: NaDCC achieved a greater fungal reduction than *Aloe vera* (14,000 vs. 4,000 cfu/ml; p = 0.020), while *Aloe vera* achieved a greater bacterial reduction than NaDCC (126,000 vs. 48,000 cfu/ml; p = 0.015). Because baseline bacterial burden was higher in the *Aloe vera* group, these absolute differences do not by themselves establish treatment superiority.

Where; [a] p < 0.05 by Mann–Whitney U test. NaDCC: sodium dichloroisocyanurate; SD: standard deviation. Results are mean ± SD of n = 14 dental units per group. Each sample was plated in triplicate.

Comparison by the sampling location

The results in Table 3 and Figure 5 show that reduction in both the fungal and bacterial counts

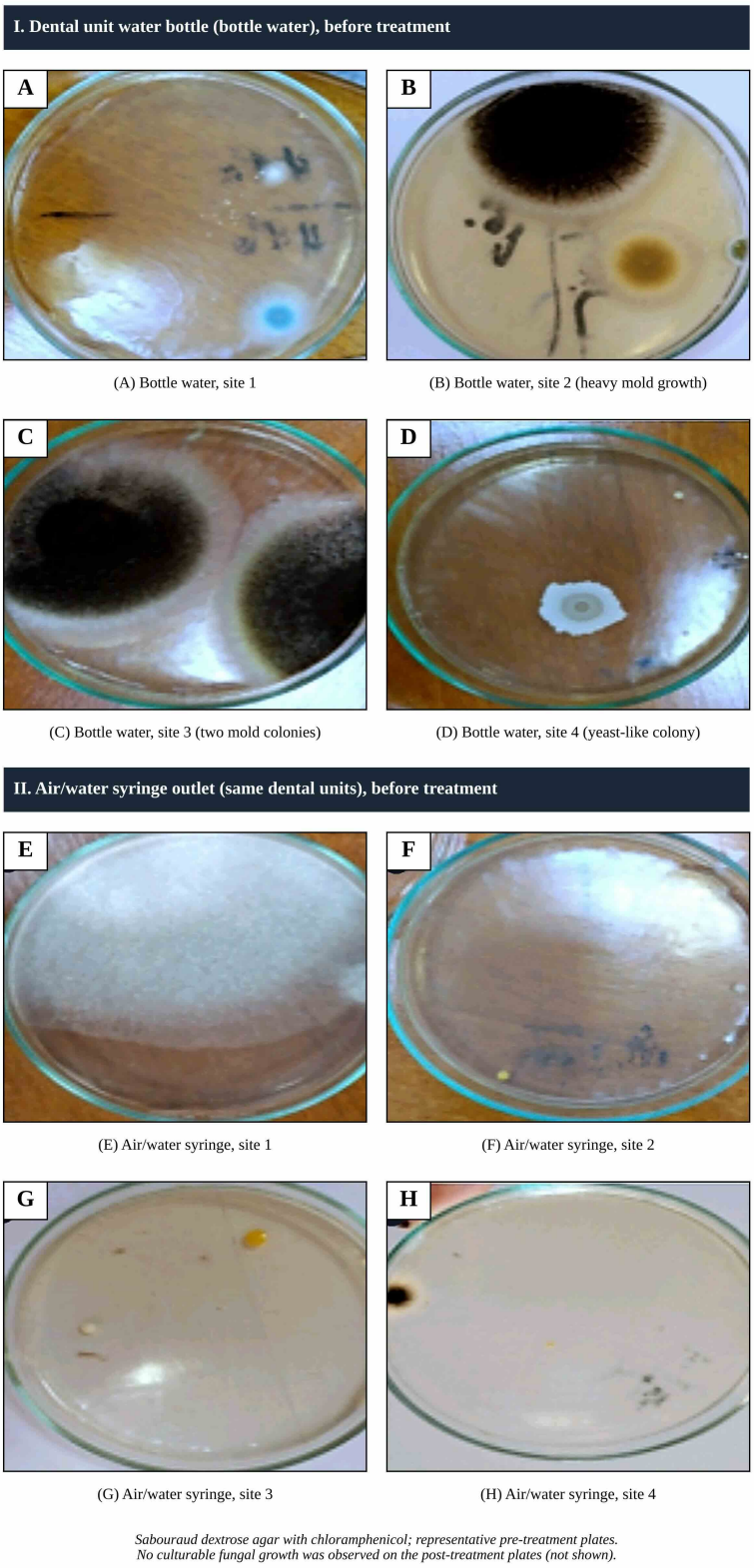


Figure 2: *Representative pre-treatment fungal culture plates (Sabouraud dextrose agar supplemented with chloramphenicol). Section I, panels A–D: bottle-water samples from four sampling sites, namely (A) site 1, (B) site 2 with heavy mold growth, (C) site 3 with two mold colonies, and (D) site 4 with a yeast-like colony. Section II, panels E–H: the corresponding air/water syringe samples from the same sites (sites 1–4). Post-treatment plates are not shown because no culturable fungal growth was observed after either treatment. The images provide qualitative visual documentation of colony growth, where the presumptive colony morphology was interpreted without a species-level identification unless confirmed by additional testing.*

Table 1. *Mean of the bacterial and fungal counts (cfu/ml) before and after treatment, observed absolute reduction, and within-group p value.*

Sampling site	Outcome (cfu/ml)	Treatment	Before, (cfu/ml)	After, cfu/ml	Observed reduction cfu/ml	*p* value
Water bottle	Fungal	NaDCC	40,000 ± 43,351.64	0 ± 0	40,000	0.01[a]
		Aloe vera (1:1, v/v)	10,000 ± 13,213.75	0 ± 0	10,000	0.04[a]
	Bacterial	NaDCC	454,000 ± 176,974.93	80,000 ± 41,557.00	374,000	0.01[a]
		Aloe vera (1:1, v/v)	838,000 ± 398,226.07	90,000 ± 44,221.66	748,000	0.01[a]
Air/water syringe	Fungal	NaDCC	14,000 ± 8,626.28	0 ± 0	14,000	0.01[a]
		Aloe vera (1:1, v/v)	4,000 ± 6,909.85	0 ± 0	4,000	0.06
	Bacterial	NaDCC	66,000 ± 33,096.30	18,000 ± 15,156.33	48,000	0.01[a]
		Aloe vera (1:1, v/v)	144,000 ± 87,320.61	18,000 ± 12,000.00	126,000	0.01[a]

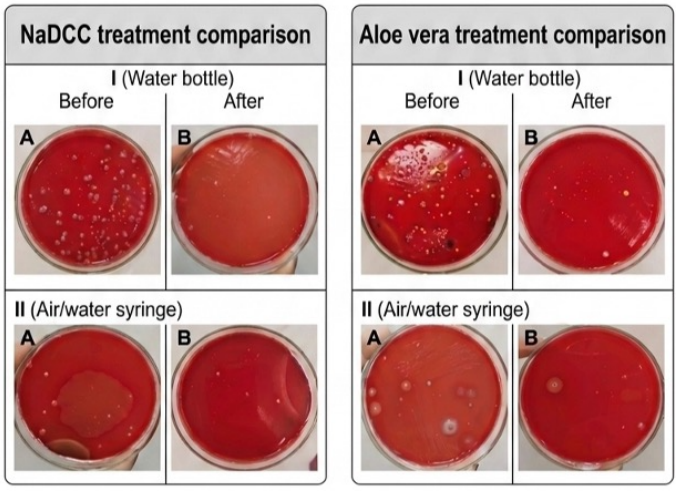


Figure 3: *Representative blood agar for water-bottle and air/water syringe samples before and after treatment with sodium dichloroisocyanurate and Aloe vera (1:1, volume/volume). The panels provide qualitative visual documentation and are not a substitute for quantitative measurements of counts(cfu/ml).*

was consistently greater when measured in the bottle-water samples compared to the air/water syringe outlet, across both interventions (NaDCC and *Aloe vera*).

Where; [a] $p < 0.05$ by Mann–Whitney U test. NaDCC: sodium dichloroisocyanurate; SD: standard deviation. Results are mean ± SD of n = 14 dental units per group. Each sample was plated in triplicate.

For fungal cfu, although the descriptive trend favored greater reduction in the bottle water (40,000 for NaDCC and 10,000 for *Aloe vera*) compared to the syringe (14,000 and 4,000, respectively), these differences did not reach a statistical significance ($p = 0.110$ and 0.320, respectively). This is likely attributable to the high variability in the data (relatively large SDs) combined with the small sample size.

For bacterial cfu, however, the difference between the sampling locations was large and statistically significant for both the NaDCC (374,000 *vs.* 48,000; $p = 0.0001$) and the *Aloe vera* (748,000 *vs.* 126,000; $p = 0.003$). This suggests that the sampling location (*i.e.*, bottle-water *vs.* syringe outlet) has a substantial effect on the magnitude of the observed bacterial reduction, independent of the disinfectant used.

For both interventions, absolute bacterial reduction was greater in the bottle water than in the air/water syringe outlet. The fungal reductions showed the same descriptive direction, but the reported fungal location comparisons were not statistically significant.

Log_{10} reduction and compliance with the recommended water quality limit

Bacterial burden expressed on a Log_{10} scale before and after the 120-min. treatment period, along with the corresponding mean Log_{10} and descriptive percentage reductions, allowing direct comparison

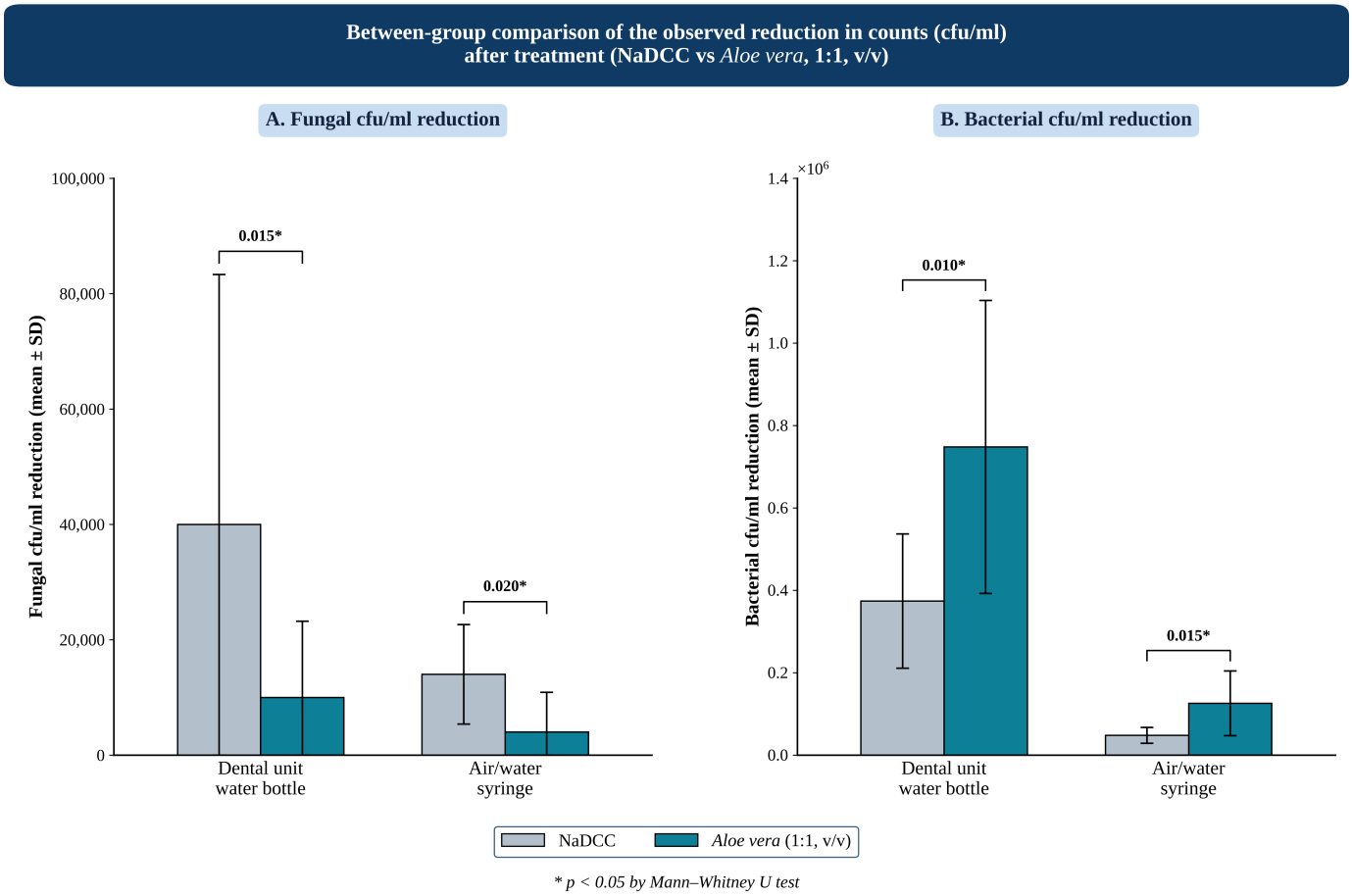


Figure 4: *Between-group comparison of the observed reduction in counts (cfu/ml) after treatment (sodium dichloroisocyanurate versus Aloe vera, 1:1 volume/volume). (A) Fungal reduction. (B) Bacterial reduction. Asterisks indicate p < 0.05 (Mann–Whitney U test).*

Table 2. *Reduction in bacterial and fungal counts (cfu/ml) after treatment, with the between-group comparison.*

Sampling site	Outcome (cfu/ml)	NaDCC reduction,	*Aloe vera* (1:1, v/v) reduction	*p* value
Dental unit water bottle	Fungal	40,000 ± 43,351.64	10,000 ± 13,213.75	0.015[a]
	Bacterial	374,000 ± 163,247.21	748,000 ± 355,386.49	0.010[a]
Air/water syringe	Fungal	14,000 ± 8,626.28	4,000 ± 6,909.85	0.020[a]
	Bacterial	48,000 ± 19,037.14	126,000 ± 78,413.64	0.015[a]

of the relative treatment efficacy independent of the baseline burden differences (Table 4 and Figure 6).

At the water bottle, *Aloe vera* achieved a slightly greater mean Log_{10} reduction than NaDCC (0.97 *vs.* 0.76 Log_{10} cfu/ml), corresponding to a higher percentage reduction (89.3% *vs.* 82.4%). A similar pattern was observed at the air/water syringe outlet, where *Aloe vera* again outperformed the NaDCC in relative terms (0.90 *vs.* 0.56 Log_{10} cfu/ml; 87.5% *vs.* 72.7%). When expressed on the Log_{10} scale, the relative efficacy of both disinfectants was fairly consistent across the sampling sites, in contrast to the absolute cfu/ml reductions reported in Tables 1–3, which were strongly influenced by the higher baseline microbial burden in the *Aloe vera* group. This supports the earlier point that the percentage/logarithmic reduction, rather than the absolute reduction alone, provides a more balanced measure of the comparative treatment efficacy between NaDCC and *Aloe vera*. Overall, both disinfectants achieved a remarkable reduction in the bacterial

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burden (>70% at both sites), though neither achieved complete elimination within the 120-min. treatment period, consistent with the residual contamination noted in Table 1.

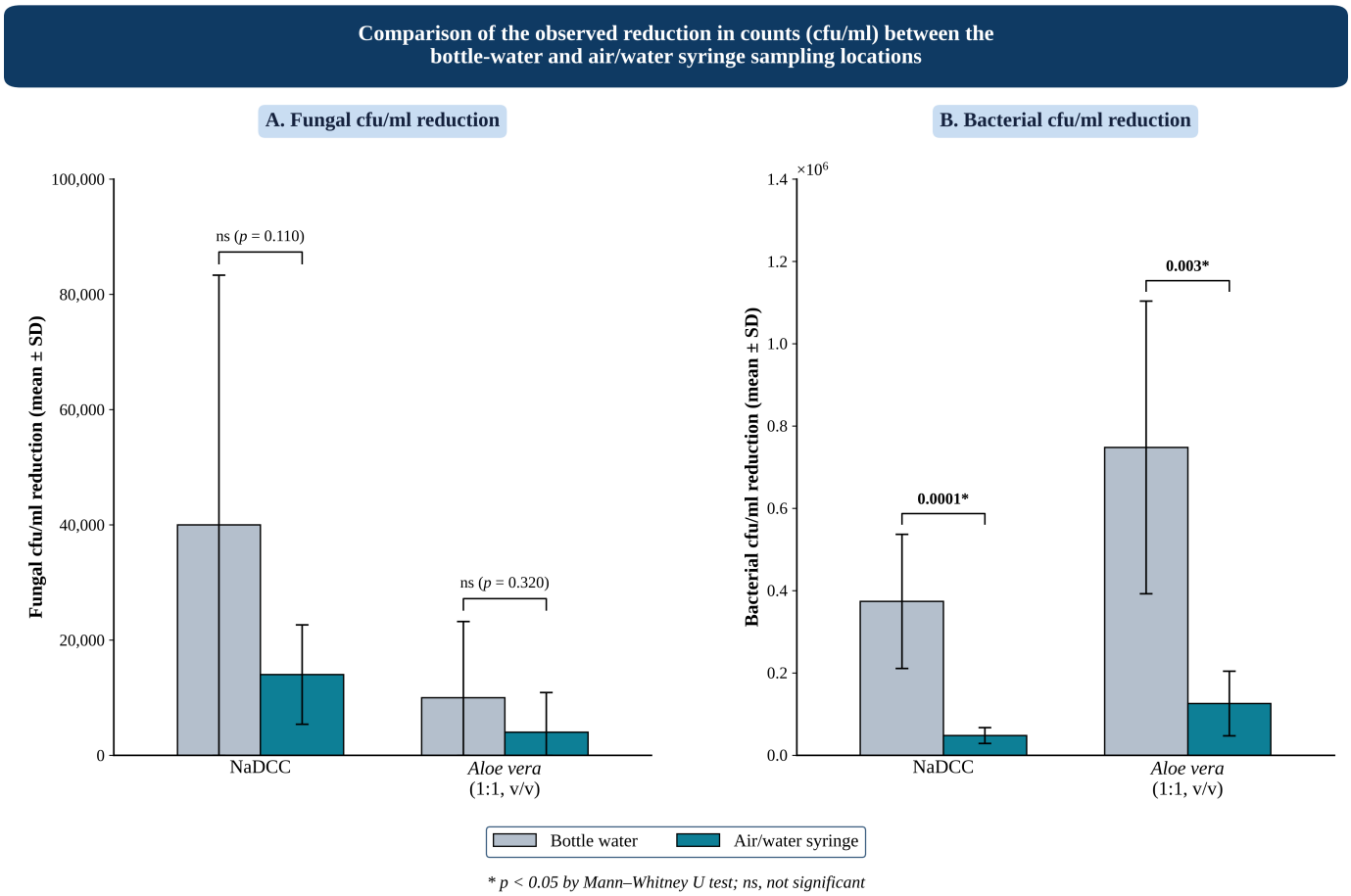


Figure 5: *Comparison of observed reduction in counts (cfu/ml) between the bottle-water and air/water syringe sampling locations. (A) Fungal reduction. (B) Bacterial reduction. Asterisks indicate p < 0.05 (Mann–Whitney U test); ns, not significant.*

Table 3. *Reduction of the bacterial and fungal count (cfu/ml) by the sampling location, with the comparison between bottle-water and air/water syringe measurements.*

Outcome (cfu/ml)	Treatment	Bottle-water reduction	Air/water syringe reduction	*p* value
Fungal	NaDCC	40,000 ± 43,351.64	14,000 ± 8,626.28	0.110
	Aloe vera (1:1, v/v)	10,000 ± 13,213.75	4,000 ± 6,909.85	0.320
Bacterial	NaDCC	374,000 ± 163,247.21	48,000 ± 19,037.14	0.0001[a]
	Aloe vera (1:1, v/v)	748,000 ± 355,386.49	126,000 ± 78,413.64	0.003[a]

Where; NaDCC: sodium dichloroisocyanurate; SD: standard deviation. Values are mean ± SD of n = 14 dental units per group. Each sample was plated in triplicate. Percentage reduction = (mean before − mean after)/mean before × 100, using the mean counts in Table 1.

No culturable fungi were detected in the reported post-treatment samples. However, residual bacterial counts remained substantially above the recommended water-quality target of ≤ 500 cfu/ml (≈2.70 Log_{10} cfu/ml); therefore, neither single 120-min. treatment reached the recommended

microbiological limit under the conditions of this study. Microscopic examination of the recovered isolates revealed a range of bacterial and fungal morphologies, including Gram-positive bacilli, streptococci, staphylococci, and *Candida* (Figure 7), consistent with a typical dental unit waterline flora.

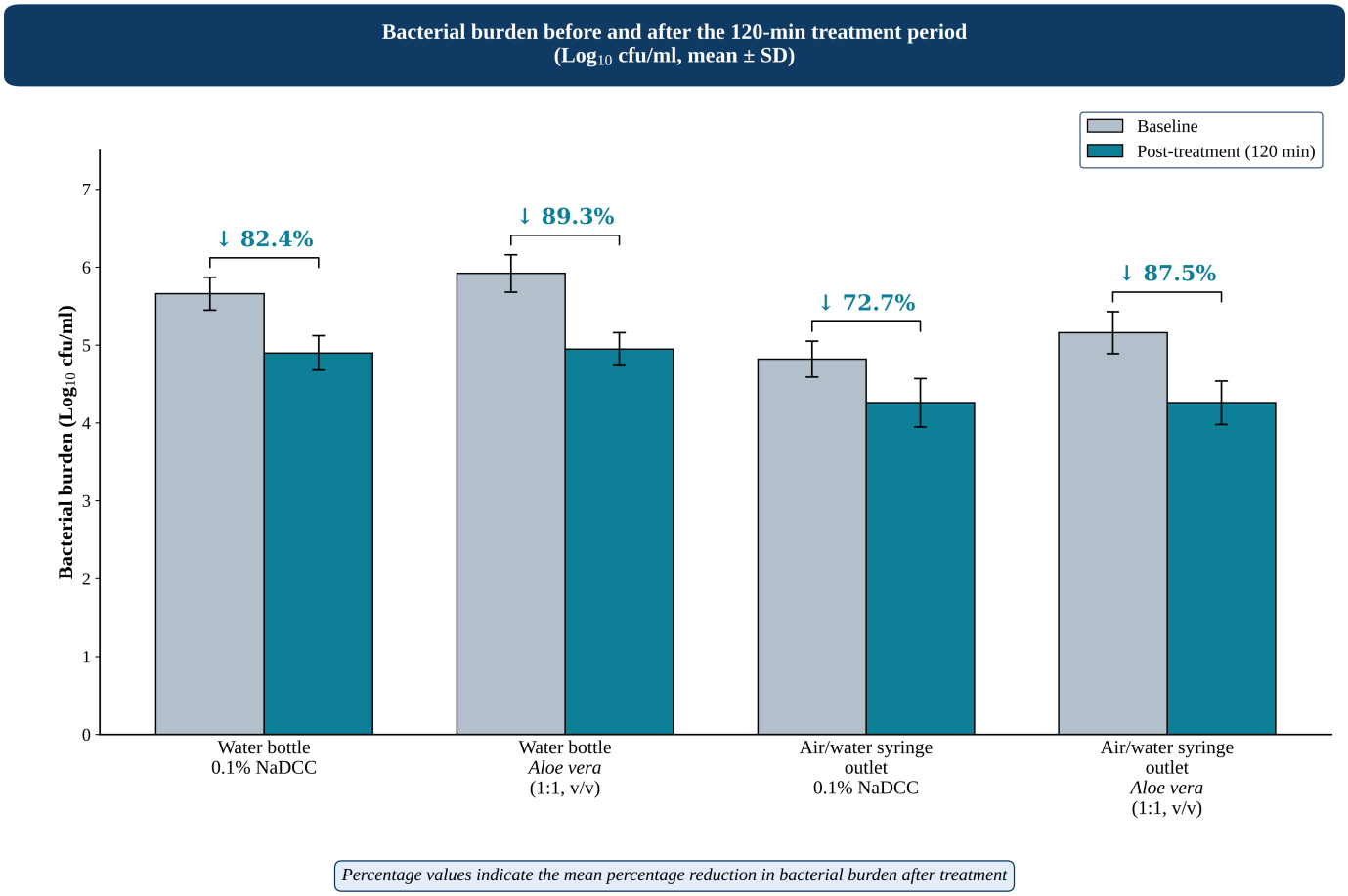


Figure 6: *Mean bacterial burden before and after the 120-min. treatment period with 0.1% sodium dichloroisocyanurate and Aloe vera (1:1, volume/volume) at the dental unit water bottle and air/water syringe outlets. Bars represent mean Log₁₀ cfu/ml ± standard deviation (n = 14 units/group). Percentages indicate the mean percentage reduction in the bacterial burden after treatment.*

Table 4. *Bacterial burden before and after the 120-min. treatment period expressed as Log₁₀ cfu/ml, with a descriptive percentage reduction.*

Sampling site	Treatment group	Baseline (Log_{10} cfu/ml)	Post-treatment (Log_{10} cfu/ml)	Mean Log_{10} reduction	Percentage reduction (%)
Water bottle	0.1% NaDCC	5.66 ± 0.21	4.90 ± 0.22	0.76	82.4%
	Aloe vera (1:1, v/v)	5.92 ± 0.24	4.95 ± 0.21	0.97	89.3%
Air/water syringe outlet	0.1% NaDCC	4.82 ± 0.23	4.26 ± 0.31	0.56	72.7%
	Aloe vera (1:1, v/v)	5.16 ± 0.27	4.26 ± 0.28	0.90	87.5%

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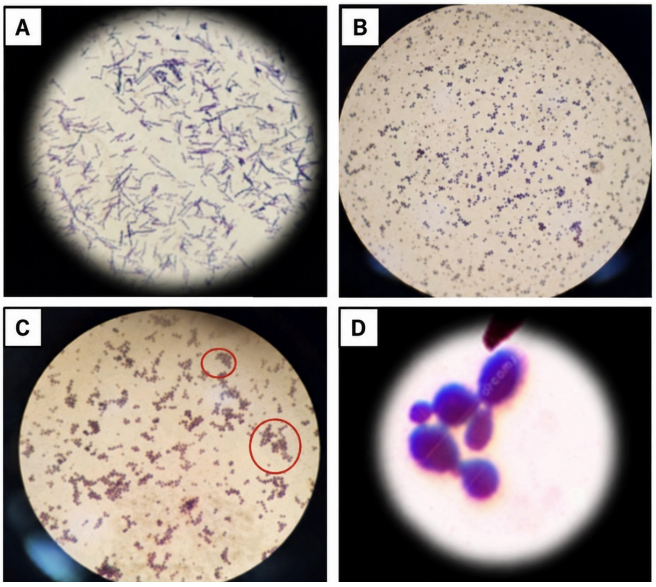


Figure 7: *Representative bacterial Gram-stained isolates and fungal microscopic findings obtained from the culture assessment. Panels show: (A) Gram-positive bacilli, (B) Gram-positive streptococci, (C) Gram-positive staphylococci, and (D) Candida sp. These images illustrate the recovered microbial morphology and do not establish a species-level identification without confirmatory testing.*

Discussion

Both 0.1% NaDCC and *Aloe vera* (1:1, v/v) produced marked short-term reductions in the bacterial and fungal contamination at the water bottle and the air/water syringe outlets. No culturable fungi were recovered after treatment with either agent, whereas a residual bacterial contamination remained detectable in both groups. The absolute bacterial reduction was numerically larger with *Aloe vera*; however, because the baseline bacterial counts were also higher in that group, this observation cannot be taken as evidence of superior antimicrobial efficacy without accounting first for the baseline imbalance, as discussed below.

The study has several strengths. First, the dental unit was treated as the experimental unit rather than treating individual samples as independent observations. Second, each unit was evaluated before and after intervention, providing an internal temporal comparison. Third, two sampling locations were assessed, allowing the distribution of microbial contamination within the water delivery system to be described. Fourth, both bacterial and fungal culture outcomes were evaluated. Finally, the NaDCC formulation was explicitly quantified by available chlorine concentration, using one 2.65-g tablet containing 1.5 g available chlorine dissolved in 1.5 l of dist. water to produce 1000 µg/ml available chlorine, which is a clearer definition than simply referring to a "chlorine tablet", and the *Aloe vera* preparation was defined by a reproducible 1:1 volumetric ratio. Nonetheless, both the NaDCC concentration and the 120-min. exposure period represent the experimental protocol and should not be generalized to the other formulations or clinical schedules without a compatibility and efficacy testing.

The baseline bacterial burden was higher in the *Aloe vera* group at both sampling sites (Table 1) (ADA, 1996; Fiorillo, 2020; Petchphayaprai *et al.*, 2023). This pattern is consistent with the established biology of DUWLs, where the stagnant water bottles and the associated tubing provided favorable conditions for microbial attachment and biofilm development (Volgenant and Persoon, 2019; Alkhulaifi *et al.*, 2020; Spagnolo *et al.*, 2020), so that the microbial load can become substantially greater than that of the source water (Walker *et al.*, 2004; Nikaeen *et al.*, 2009; Blaszczyk *et al.*, 2022). Because a group starting with a higher microbial burden has a greater numerical scope for reduction even when the proportional antimicrobial effect is similar, the larger absolute bacterial reduction observed with *Aloe vera* should not, by itself, be interpreted as evidence that it is intrinsically more effective than NaDCC (Watanabe *et al.*, 2016; Suleyman *et al.*, 2018; Fan *et al.*, 2021). When the same data were re-expressed as Log_{10} reduction and percentage reduction (Table 4), the relative efficacy of the two agents was more consistent across the sampling sites than the absolute reductions suggested, although *Aloe vera* still showed a slightly higher percentage/Log_{10} reduction at both locations. This finding is compatible with, rather than proof of, comparable proportional antimicrobial activity, since a higher initial microbial load mathematically permits a larger logarithmic decay window (Vickers and Altman, 2001; Barnett *et al.*, 2005). Analysis of covariance adjusting for baseline is generally preferred to the analysis of change scores when the groups differ at baseline (Vickers and Altman, 2001) and should be adopted in larger future studies.

An additional interpretive limitation concerns the

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distinction between the planktonic microbial counts and biofilm eradication. The cfu/ml measured in the collected water primarily reflected that microorganisms were released into the flowing water but it did not directly quantify the biomass attached to the internal tubing wall; a treatment that may markedly reduce the planktonic counts (Walker *et al.*, 2004; Watanabe *et al.*, 2016), while leaving an established biofilm capable of subsequent recolonization (Li and Xin, 2025). This distinction is particularly relevant here, since the planktonic sampling only was performed in the present protocol (Fiorillo, 2020).

The stronger numerical fungal reduction observed with NaDCC is biologically compatible with the broad oxidative activity expected from the chlorine-releasing chemistry, but because the present study did not directly assess the chemical mechanism, biofilm penetration, or fungal species identity, such an explanation should be regarded as a hypothesis rather than an established conclusion. The fungal findings also differed from the bacterial findings in an informative way: the fungal counts were lower than the bacterial counts at baseline, and no culturable fungi were recovered after treatment with either intervention. NaDCC showed a numerically larger absolute fungal reduction, driven mainly by higher baseline fungal counts in the NaDCC group at both locations rather than by a demonstrably larger proportional effect. Fungal counts in DUWLs are usually lower than bacterial counts, and fungi, particularly *Candida* spp., can persist within biofilms (Damasceno *et al.*, 2017; Omran *et al.*, 2021); moreover, the dependence of absolute change on the baseline level applies equally to the fungal data (Barnett *et al.*, 2005). This outcome is best described as "no culturable fungi detected after treatment" rather than a complete fungal eradication, since culture-based absence of growth does not exclude those microorganisms below the detection limit, viable-but-nonculturable cells, or fungi not recovered under the incubation conditions used (Oliver, 2010; Damasceno *et al.*, 2017); growth on SDA should likewise be interpreted as fungal/yeast recovery rather than a species-level identification.

Two methodological points warrant emphasis. First, sodium thiosulfate was used to neutralize the residual chlorine activity during sample transport for both intervention groups; because this neutralizer was applied uniformly, future work should formally validate that it does not itself interfere with the microbial recovery following *Aloe vera* exposure (ASTM, 2008; Rice *et al.*, 2017). Second, absolute bacterial reductions were consistently larger in the bottle water than in the syringe outlet for both interventions, paralleling the higher baseline bacterial counts in the bottle water; this does not establish that the bottle location is intrinsically more responsive to treatment, since stagnation in the reservoir favors higher microbial loads (Walker *et al.*, 2004; Alkhulaifi *et al.*, 2020) and the absolute change depends on the baseline level (Barnett *et al.*, 2005). Because the bottle and syringe samples were obtained from the same dental units, they represent paired rather than independent observations, and a paired longitudinal model would be needed to formally assess the location effects and treatment-by-location interactions (Liang and Zeger, 1986; Fitzmaurice *et al.*, 2011).

Findings from previous comparative studies of DUWL disinfectants have not uniformly favored the biologically derived agents, where several have favored the conventional chemical disinfectants, underscoring that an agent's apparent performance depends on the baseline microbial burden, biofilm maturity, exposure conditions, neutralization, sampling technique, and microbiological recovery method, rather than on the intrinsic antimicrobial activity alone (Sunil Raj *et al.*, 2017; Yoon and Lee, 2018; Li and Xin, 2025). Within the *Aloe vera* literature specifically, Pareek *et al.* (2013) reported the antimicrobial activity of *Aloe vera* in an *in-vitro* DUWL model, while several other dental studies have demonstrated the *Aloe vera*'s activity against the oral microorganisms (Athiban *et al.*, 2012; Fani and Kohanteb, 2012; Jain *et al.*, 2016), and have explored its applications in the periodontal, endodontic, and prosthodontic contexts (Karim *et al.*, 2014; Trivedi *et al.*, 2019). However, differences in the experimental models, exposure periods, concentrations, and endpoints make direct quantitative comparison among these studies and the present results inappropriate; thus, the present findings should therefore be viewed as comparative evidence under one defined protocol rather than as a universal ranking of NaDCC and *Aloe vera*.

The present *Aloe vera* protocol used a 1:1 (v/v)

mixture of gel and dist. water, which improved the reproducibility of the dilution procedure but did not define the concentration of any single active antimicrobial constituent. *Aloe vera* gel contains a complex and variable mixture of polysaccharides, phenolic compounds, anthraquinone-related constituents such as aloe-emodin, enzymes, and other bioactive substances (Jain *et al.*, 2016; Habibi *et al.*, 2018; Danish *et al.*, 2020), and its biological activity is strongly influenced by the plant source, extraction method, stabilization, and storage (Athiban *et al.*, 2012; Kahramanoglu *et al.*, 2019; Sanchez *et al.*, 2020). The individual Aloe-derived compounds have shown antimicrobial and antibiofilm activity in several *in vitro* studies (Lawrence *et al.*, 2009; Xiang *et al.*, 2017; Li *et al.*, 2021), which provides biological plausibility for the reduction observed here, but does not establish that all *Aloe vera* products have equivalent activity.

Aloe vera nonetheless remains a promising candidate for further investigation as a biologically derived adjunct to conventional DUWL disinfection. Future studies should report the botanical source, extraction or stabilization method, product lot, and storage conditions, and should establish standardized chemical characterization, optimal concentration and contact time, long-term efficacy, direct biofilm activity, microbial identification, neutralization validity, and compatibility with the dental-unit materials. Until such evidence is available, *Aloe vera* should be regarded as an investigational or adjunctive approach rather than a validated stand-alone replacement for the established DUWL disinfection and water-quality management.

Irrespective of the metric used, the post-treatment bacterial concentrations remained well above the Centers for Disease Control and Prevention (CDC)/American Dental Association (ADA) water-quality target of ≤ 500 cfu/ml (≈2.70 Log_{10} cfu/ml) at both sampling sites (ADA, 1996; Kohn *et al.*, 2003; CDC, 2024), indicating that a large relative reduction does not equate to achieving acceptable dental water quality. The obtained mean post-treatment bacterial counts of approximately 80,000–90,000 cfu/ml in bottle water and 18,000 cfu/ml in syringe water confirm that the culturable bacterial burden was reduced but not eliminated by either agent. This finding is compatible with the biology of a mature DUWL biofilm (ADA, 1996; Fiorillo, 2020), in which the attached microorganisms may be protected by the extracellular polymeric substances and the physical structure of the tubing (Tall *et al.*, 1995; ADA, 1996; Fiorillo, 2020).

These results support the concept of a microbial-load reduction but do not support the use of either NaDCC or *Aloe vera* alone as a substitute for an established DUWL water-quality management program. A single treatment event should not be considered equivalent to a validated long-term maintenance protocol, where routine management requires an integrated approach that considers the source-water quality, minimization of stagnation, routine flushing, regular disinfection, equipment design, and compliance with the manufacturer instructions and infection-control guidance (Watanabe *et al.*, 2008; Samaranayake *et al.*, 2024). The present study evaluated a single treatment event and therefore cannot establish the optimal frequency of application or the duration of protection against recolonization.

Conclusions and Recommendations

Under the conditions of this study (n = 14 dental units per group; a single 120-min. contact period), both 0.1% NaDCC and *Aloe vera* (1:1, v/v) markedly reduced the cultivable bacterial contamination (p = 0.01 at both sampling sites). In bottle water, mean bacterial counts fell from 454,000 to 80,000 cfu/ml with NaDCC (82.4%; 0.76 Log_{10}) and from 838,000 to 90,000 cfu/ml with *Aloe vera* (89.3%; 0.97 Log_{10}); meanwhile in syringe water, they fell from 66,000 to 18,000 cfu/ml (72.7%; 0.56 Log_{10}) and from 144,000 to 18,000 cfu/ml (87.5%; 0.90 Log_{10}), respectively. No culturable fungi were detected after either treatment, although the *Aloe vera* reduction at the syringe outlet (4,000 to 0 cfu/ml) did not reach a statistical significance (p = 0.06). The larger absolute reductions with *Aloe vera* reflected its higher baseline counts but did not demonstrate superiority, whereas the comparable relative reductions suggest a broadly similar short-term activity. However, the residual bacterial counts (80,000–90,000 cfu/ml in bottle water and 18,000 cfu/ml in syringe water) remained far above the 500 cfu/ml target (≈2.70 Log_{10} cfu/ml); therefore, neither single treatment achieved acceptable dental water quality. *Aloe vera*

can thus be regarded as a promising natural candidate, which may be attractive because natural products are generally expected to be less corrosive and less toxic (a premise not tested here); however, based on the present evidence, it is an investigational adjunct rather than a validated alternative to NaDCC.

The authors recommend that: (1) Future studies should use a fully characterized *Aloe vera* product (*i.e.*, botanical source, lot, extraction or stabilization method, and storage) and define the optimal concentration and contact time, (2) Neutralizer validation should be performed for both agents, (3) Biofilms should be assessed directly (e.g., tubing sections, ATP bioluminescence, or microscopy) together with recolonization after repeated treatment cycles and species-level identification of the residual flora, (4) Compatibility with the dental-unit materials (corrosion) and safety (cytotoxicity) of *Aloe vera* should be compared directly to NaDCC, (5) Larger, adequately powered studies with baseline-adjusted analysis (*i.e.*, ANCOVA or mixed models) are needed, and (6) Until such evidence is available, the dental clinics should continue applying an integrated DUWL management program (*i.e.*, source-water control, flushing, validated disinfection, and routine monitoring against the 500 cfu/ml target).

Novelty Statement

In the previous studies, evidence on *Aloe vera* for DUWL decontamination has been limited to an *in-vitro* waterline model. The novelty of the present study lies in comparing a defined 1:1 (v/v) *Aloe vera* preparation with 0.1% NaDCC in functioning dental units at two outlets, reporting both bacterial and fungal outcomes, and using baseline-aware metrics (Log_{10} and percentage reduction), which were benchmarked against the 500 cfu/ml water-quality target, revealing that large relative reductions can still fall short of acceptable water quality.

Authors' Contributions

HAA: Conceptualization; Methodology; Validation; Writing – review and editing; Supervision. **SSMS:** Conceptualization; Methodology; Validation; Writing – original draft; Visualization

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Ethical approval

This study was conducted according to the institutional ethical requirements. Ethical approval was obtained from the Institutional Review Board of the Faculty of Oral and Dental Medicine, Ahram Canadian University (IRB No. IRB00012891#151). Permission to conduct the study was obtained from the relevant faculty authorities. No human participants were directly enrolled; the study involved dental-unit water sampling and laboratory microbiological assessment.

AI Use Disclosure

Generative artificial intelligence (AI) was used to assist with language editing of portions of the manuscript. The authors reviewed and verified all AI-assisted content and remain fully responsible for the final manuscript.

Data availability statement

All data used in the current study are available from the corresponding author on reasonable request.

Conflict of interests

All authors declare no conflicts of interest.

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