

Original Article

Study of some risk factors for fungal contamination of dental unit waterlines in Alexandria, EgyptEman Abdel Hamid Omran¹, Aleya Abdel Gawad Abbass¹, Amani Farouk Abaza¹, Eman Moftah Elzouki²¹ Department of Microbiology, High Institute of Public Health, Alexandria University, Alexandria, Egypt² Faculty of Medical Technology, University of Darnah, Darnah, Libya**Abstract**

Introduction: Monitoring the microbial quality of water in dental unit waterlines is an important part of infection control measures carried out in dental clinics. Fungal contamination of such waterlines has not been extensively studied, compared with bacterial contamination. This study aimed at assessing the magnitude and risk factors for fungal contamination of dental unit waterlines.

Methodology: This cross-sectional study included 82 dental units, randomly collected from 3 private clinics and 8 governmental hospitals in Alexandria, Egypt. A total of 204 water samples from dental unit waterlines output were membrane-filtered and cultured for fungal enumeration and species identification. The biofilm forming-ability was assessed for the most prevalent fungal species. The acceptability of samples was determined according to the Swedish drinking water guidelines.

Results: The acceptability of samples was 89.7%. The most common mould was *Aspergillus flavus*, while *Candida* spp. was the most common yeast (10 isolates), with unusual predominance of *Candida dubliniensis* (9 isolates). All isolates of *Aspergillus flavus* and *Candida dubliniensis* were biofilm-formers. The risk factors for fungal contamination of dental unit waterlines included: dental specialty ($p = 0.042$), time of sample collection ($p < 0.001$), older age of dental unit ($p < 0.001$) and use of 5-15% of sodium hypochlorite.

Conclusions: The presence of biofilm-forming fungi in dental unit waterlines is a potential hazard, even when samples have acceptable levels of fungal counts. Risk factors for contamination are numerous and should be addressed.

Key words: *Aspergillus flavus*; *Candida dubliniensis*; dental unit waterlines; hydrogen peroxide.

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Introduction

Dental unit waterlines (DUWLs) supply water to the dental unit (DU). Such water is required for operating several dental tools, including air-water syringes, cup-fillers, and handpieces [1]. Water inside DUWLs may be contaminated with microorganisms. These pathogens may reach the DUWLs either directly from the municipal water supplies, or from sources inside the DU itself [2].

Due to the overall features of water flow in DU pipelines (laminar flow, water stagnation, and high surface/volume ratio), biofilm formation might form in their interior if proper infection control measures were not considered [3,4]. Contaminated DUWLs may lead to the production of microorganisms-containing aerosols, which are then dispersed in the clinical environment, leading to diseases among dental staff and patients [5,6].

Fungi such as *Alternaria*, *Aspergillus* and *Penicillium* may cause allergies, hypersensitivity pneumonitis, and skin irritation [7]. In more severe conditions, fungal pneumonia and systemic infections

(by moulds and *Candida* spp.) may occur among immunocompromised patients. These infections have a high mortality rate, (50.0% - 100.0%) and they are increasing worldwide, owing to the growing numbers of susceptible individuals. Fungi in DUWLs may cause diseases among patients and dental teams. Despite the growing awareness on the pathogenic ability of fungi, they remain largely overlooked in the regulations of water quality. Since dental water might be accidentally swallowed, the regulations for drinking water might apply as an alternative standard for DUWLs water quality. The Swedish drinking water regulations include fungi as an indicator parameter, with a maximum limit of 100 CFU/100 mL [8].

Output water from DUWLs of dental clinics may be a potential source of infection for both dental healthcare workers (HCWs) and patients, thus its mycological quality was evaluated in this study. Water samples from different parts of the DUWLs (high-speed hand-piece, cup- filler samples and air/water syringe) were studied for their total fungal counts (expressed as colony-forming units), as well as their percentage acceptability

(compared to the Swedish standards for drinking water). The isolated fungal species were identified and their abilities to form biofilms were determined. Possible risk factors for fungal contamination of DUWLs were also assessed, including the age of the dental unit, dental specialty, the type and frequency of disinfectant used in their reservoirs, and the time of water sample collection (beginning/end or working shift).

Methodology

This cross-sectional study was carried out during a sixteen-month period, from the first of April 2017 until the end of July 2018. Based on a previous study [9] where the prevalence of mycological contamination of DUWLs was found to be 63%, accordingly, a sample size of 200 water samples collected from outlets of the DUWLs was required for the present study to achieve the same aim (with precision of 6% using alpha error = 0.05.). The sample size was calculated using Epi-Info™ 7- (CDC, Atlanta, GA, USA) by Windows software.

Sampling

Samples were randomly collected from 82 DUs in health-care facilities (HCFs) (62 DUs from 9 governmental hospitals, and 20 DUs from 3 private dental clinics). Administrative approvals were taken from the hospitals/ clinics in which the selected dental units were located. A total of 204 water samples from DUWLs outlets were collected as follows: 71 samples from high speed handpieces, 66 samples from air/water syringes, and 67 samples from cup fillers. All DUs had an attached water reservoir bottle, inside which, a disinfectant was applied on a weekly/daily basis.

Water samples from outlets of the DUWLs were collected on two separate occasions; at the beginning of the work shift and at its end. Samples were aseptically collected after alcohol disinfection of faucets into sterile ground-glass containers (250 mL capacity) containing 3.0% sodium thiosulfate to deactivate any residual chlorine [10]. Samples were clearly labeled, transported, and delivered in an ice box to the laboratory to be analyzed within 1-2 hours of collection. Each sample was accompanied by a sheet including the following information: frequency of DUWLs disinfection, type and concentration of disinfectant used, source of water to the dental reservoir, and age of the DU.

Laboratory processing of samples

All samples were subjected to membrane filtration technique followed by determination of total fungal

counts (FCs), acceptability, and species identification. Biofilm forming-ability was determined for the most prevalent mould (*Aspergillus flavus*) and yeast (*Candida* spp.) in DUWLs output water samples.

a) Membrane filtration, fungal enumeration and identification

Membrane filtration was performed using sterile cellulose acetate membrane filters (0.45µm pore size and 47mm diameter) (Metricel® S-Pack, PALL, USA). Membrane filters were then cultured on Rose bengal-chloramphenicol (RBCh) (Oxoid Ltd, Basingstoke, UK) plates and incubated aerobically at 25 °C for up to 7 days. Identification of fungal colonies from DUWLs samples was done macroscopically and microscopically according to standard procedures [10,11]. *Candida* colonies were smooth, convex and creamy-white and showed oval, budding yeast cells on microscopic examination. *Aspergillus* isolates were examined macroscopically for colony growth rates, color, texture, surface and pigmentation. Microscopic examination was done to visualize the size and shape of conidiophore and phialides as well as the arrangement of spores. Colonies with rapid growth rate, downy to powdery texture, yellow/ green/dark colours, with pale reverse, and microscopically had conidial heads with uniseriate / biseriate phialides were identified as *Aspergillus* and the species determined according to morphology of spores, phialides and conidiophores. Fungal colonies were counted and recorded as colony forming-units (CFUs)/100mL.

Candida spp. isolates were subjected to API 20 *Candida* system (BioMe'rieux- France) for species identification. Yeast isolates that failed to be identified by the API 20 *Candida* system were further identified using matrix-associated laser desorption ionization–time of flight mass spectrometry (MALDI TOF MS) (Bruker Daltonics GmbH, Germany). This step was done because API 20 *Candida* system identifies only 15 common *Candida* species [12].

b) Measuring biofilm-forming ability of *Candida* spp. and *A. flavus*

This procedure was done for isolates of *Candida* spp. and *A. flavus* (the most prevalent yeast and mould respectively, in DUWLs samples). Isolates were grown on 96-well microtiter plates and stained by crystal violet (CV), in order to measure the ability of isolates to produce biofilm. Steps for biofilm formation and crystal violet staining were done as described elsewhere [13,14]. Absorbance values were measured with a microtiter plate reader (DIAREADER Univ.,

ELX800G:236726, Neudorf, Austria) at 540 nm. Isolates were classified according to Stepanovic *et al.* as poor, weak, moderate, and strong biofilm producers [13].

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percentage. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using mean and standard deviation. Significance of the obtained results was judged at the 5% level [15,16].

Results

Out of the 204 DUWLs output water samples, 49 samples (24.0%) yielded positive fungal cultures. The majority of isolated fungi were moulds (79.6%) with predominance of *A. flavus* (17 isolates, representing 34.7% of all fungal isolates). Only 20.4% of fungal isolates were yeasts, with predominance of *C. dubliniensis* (9 isolates, representing 18.4% of all isolates).

According to the Swedish standards for drinking water [8], 89.7% of the DUWLs samples were acceptable (95.8% of the high-speed hand-piece

samples, 86.8% of the cup filler samples and 86.4 % of the air/water syringe samples, ($p = 0.002$) (Table 1).

All private dental clinics, in addition to one hospital, had 100.0% acceptable DUWLs output water samples, while 8 hospitals had variable degrees of acceptability, with fungal contamination of their DUWLs ranging between 5.9 - 29.4% ($p = 0.017$) (data not shown). Mean values of FCs varied significantly among the 7 dental specialty clinics ($p = 0.042$), with a total mean value of FC of 133.62 CFUs/100ml. The highest acceptability was recorded from samples from the implant clinics (100.0%) (Table 2). The highest prevalence of *A. flavus* was isolated from minor operation clinics (30.4 %), followed by oral surgery clinics (15.2%), ($\chi^2 = 18.840, p = 0.001$). *C. dubliniensis* isolates came from periodontics clinics (42.9%), fixed prosthodontics clinics (6.6%), and pediatric dentistry clinics (3.4%), ($\chi^2 = 24.460, p < 0.001^*$) (Table 3).

There was a decrease in FC mean values for the samples collected at the end of shift compared to those collected at the beginning of the work shift (Table 4). At the beginning of the shift, the acceptability of output water samples from high speed handpiece, air/water syringe and cup fillers were 91.4%, 72.7%, and 88.2%, respectively.

Table 1. Distribution of the 204 examined DUWL water samples from different water sources according to their FC mean values and acceptability.

Water source	Examined water samples (204)	FCs (CFU/100 mL)				H	p value
		Mean ± SD	Median	0-100 (Acceptable)			
				No.	%		
DUWLs (n=204)							
High-speed handpiece	71	39.5 ± 97.0	0.0	68	95.8	0.261	0.878
Air/water syringe	66	38.8± 97.6	0.0	57	86.4		
Cup filler	67	17.2 ± 48.5	0.0	58	86.8		
Total DUWLs	204	31.5 ± 83.9	0.0	183	89.7		

H: H for Kruskal Wallis test; * Statistically significant at $p \leq 0.05$.

Table 2. Mean values of FC and acceptability of the 204 examined DUWL output water samples according to clinic specialty.

Clinic specialty	Examined water samples	FCs (CFU/100 mL)			H	p value
		Mean ± SD	(Acceptable) 0-100			
			No.	%		
Pediatric dentistry	59	120.4 ± 100.6	54	91.5	10.392*	0.042*
Conservative treatment	50	133.3 ± 114.7	47	94.0		
Oral surgery	33	64.3 ± 80.5	28	84.8		
Minor operations	23	200.0 ± 173.2	20	87.0		
Fixed prosthodontics	15	180.0 ± 113.4	12	80.0		
Periodontics	14	148.0± 183.7	12	85.7		
Implants	10	0.00	10	100.0		
Total	204	133.62 ± 130.4	183	89.7		

H: H for Kruskal Wallis test; * Statistically significant at $p \leq 0.0$.

Table 3. Distribution of the 204 examined DUWL output water samples collected from different clinic specialties according to the isolated fungal species.

Clinic specialty	Examined water samples	<i>A. flavus</i>		<i>A. niger</i>		<i>Penicillium spp.</i>		<i>C. dubliniensis</i>		<i>C. albicans</i>	
		No.	%	No.	%	No.	%	No.	%	No.	%
Pediatric dentistry	59	4	6.8	5	8.5	2	3.4	2	3.4	0	0.0
Conservative treatment	50	0	0.0	0	0.0	3	6.0	0	0.0	0	0.0
Oral surgery	33	5	15.2	2	0.6	3	9.1	0	0.0	0	0.0
Minor operations	23	7	30.4	5	8.5	0	0.0	0	0.0	0	0.0
Fixed prosthodontics	15	1	6.6	0	0.0	2	13.3	1	6.6	0	0.0
Periodontics	14	0	0.0	0	0.0	0	0.0	6	42.9	1	7.1
Implants	10	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	204	17	8.3	12	5.8	10	4.9	9	4.4	1	0.5
χ^2 (MCp)		18.840 (0.001*)		12.145 (0.018*)		4.947 (0.440)		24.460 (< 0.001*)		9.316 (0.112)	

χ^2 : Chi square test; MC: Monte Carlo; p: p value for association between different categories; * Statistically significant at $p \leq 0.05$.

Table 4. Mean FC values and acceptability of the 204 examined water samples from different sources in relation to the collection shift.

Water source	Collection shift	Examined water samples (204)	FCs (CFU100/ml)		Z	p value
			Mean \pm SD	(Acceptable) 0-100		
				No.	%	
High speed handpiece	Beginning of shift	35	17.2 \pm 48.5	32	91.4	3.624*
	End of shift	35	3.0 \pm 0.0	35	100	
Air/water syringe	Beginning of shift	33	38.8 \pm 97.6	24	72.7	3.415*
	End of shift	33	3.2 \pm 17.6	33	100	
Cup filler	Beginning of shift	34	39.5 \pm 97.0	30	88.2	3.623*
	End of shift	34	5.1 \pm 29.6	29	85.3	

Z: Wilcoxon signed ranks test; * Statistically significant at $p \leq 0.05$.

Table 5. Results of the 204 DUWL samples in relation to the type, concentration and frequency of disinfectant application in DUs and FC acceptability.

Disinfectant in DUs	FCs CFU/100 mL				Total (n = 204)		χ^2	FEp
	0-100 (Acceptable)		> 100 (Unacceptable)		No.	%		
	No.	%	No.	%				
Type of disinfectant								
Sodium hypochlorite	165	88.7	21	11.3	186	100.0	2.265	0.226
Hydrogen peroxide	18	100.0	0	0.0	18	100.0		
Concentration of disinfectant								
<i>Hydrogen peroxide</i>								
0.2%	18	100.0	0	0.0	18	100.0	59.911*	< 0.001*
<i>Sodium hypochlorite</i>								
5.0%	21	13.5	31	63.3	52	100.0		
10.0%	41	80.8	10	19.2	51	100.0		
15.0%	47	92.2	4	7.8	51	100.0		
20.0%	31	96.9	1	3.1	32	100.0		
Frequency of disinfectant application								
Daily	86	92.5	7	7.5	93	100.0	1.417	0.234
Weekly	97	87.4	14	12.6	111	100.0		

χ^2 , p: χ^2 and p values for Chi square test; ^{MC}p: p value for Monte Carlo for Chi square test.

These values increased at the end of the shift to be as follows: 100%, 100% and 85.3%, respectively. Differences between the beginning and end of shift regarding the FC mean values and acceptability of samples was statistically highly significant among all water sources ($p < 0.001$).

At the beginning of the shift, 37 mould isolates were recovered from cultures, while no yeasts were detected. On the other hand, at the end of the shift, only 2 mould isolates were detected in addition to 10 yeast isolates (9 *C. dubliniensis* isolates) and a single *C. albicans* isolate). However, there was no statistically significant association between fungal species and the time of work shifts (Supplementary Table 1).

DUWLs with age more than 10 years yielded a lower percentage of FC acceptable samples (76.3%) compared to those built within the last 10 years (95.2% acceptability), ($\chi^2 = 16.224$, $p < 0.001$). Species distribution did not differ markedly with age of the DU, except for *A. flavus*, which showed a statistically significant association with the increase of the age of the DUWLs ($\chi^2 = 9.810$, $p = 0.006$).

Most of the samples ($n = 141$) were from DUs using distilled water in their reservoirs while only 63 samples were from DUs using tap water. DUWLs samples that were from DUs using distilled water in their reservoirs had a higher FC acceptability than those filled with tap water (91.5% *versus* 36.5%, respectively), ($\chi^2 = 21.966$, $p < 0.001$).

The majority of samples ($n = 186$) were from DUs that used sodium hypochlorite as a disinfectant in their reservoirs (at variable concentrations; 5.0%, 10.0%, 15.0% and 20.0%) while only 18 samples (8.8%) were from DUs using 0.2% % H_2O_2 (Table 5). The frequency of disinfectant application (daily *versus* weekly) was not of statistical significance in the fungal contamination of water samples. Samples from DUs using 0.2 % H_2O_2 and 20.0% sodium hypochlorite had acceptability rates for their samples of 100.0% and 96.9% respectively, in contrast to samples from DUs that used 5% sodium hypochlorite (13.5% acceptability) (Table 5). It is noteworthy to mention that the 49 samples showing fungal growth were all from DUs using sodium hypochlorite as a disinfectant (48 samples were from DUs using it at concentrations 5% - 15%, and only 1 sample was from a DU using it at 20% concentration).

Biofilm-forming ability of A. flavus and Candida spp. isolates

All *A. flavus* isolates were biofilm-producers with varying degrees, the majority of which were weak

producers (53.0%). Similarly, all *Candida* spp. isolates were biofilm-producers (50.0% of them were moderate producers). As an indicator of fungal virulence and potential health hazard, biofilm-forming ability of dominant fungal species was assessed.

Discussion

Many factors may contribute to the contamination of DUWLs output water by fungi. The FC acceptability of samples reflects the condition of DUWLs, level of disinfection, as well as practices performed by the dental staff. The dark, damp, and warm interior of DUWLs may serve as an ideal incubator for microbial proliferation [17, 18].

In our study, the mean FC acceptability of all DUWL output water samples was 89.7% with a mean value of 31.5 CFU/100 mL. A nearly similar result (35.02 CFU/100mL) was reported by Nikaeen *et al.* [19] in Iran, while Ali *et al.* [20] reported a much lower result (12.63 CFU/100mL) in Alexandria, Egypt. This was in contrast to findings of Kadaifciler *et al.*, who reported significantly higher FCs, denoting proliferation of fungi inside DUWLs and improper disinfection [9]. Differences between studies are attributed to several risk factors, including those investigated in this study.

A significantly higher acceptability was revealed from samples of DUWLs whose age was less than 10 years compared to those 10 years of age or more. The reason behind this is that, as the age of the DUWLs progresses, it becomes a more suitable environment for biofilm formation and thus further fungal contamination. This might explain why all private dental clinics had 100.0% acceptable samples. Those HCFs were newly constructed (3-6 years), while most of the governmental hospitals were of older age and were found to have unacceptable samples ranging between 5.9 - 29.4% ($p = 0.017$). On the contrary, a study on the bacterial contamination of DUWLs revealed no statistical correlation between the age of a DU and the bacterial counts in output water [21]. Structural differences between fungi and bacteria might lead to the more prolonged persistence of fungal biofilms and their relative better adherence to surfaces. This is attributed to the resilient hyphal structure that makes fungi more capable of attachment over time [22]. This was further emphasized by a finding in our study, where *A. flavus* (mould) showed a statistically significant association with the increase in DUWLs age ($\chi^2 = 9.810$, $p = 0.006$). On the contrary, our study found no similar significant increase in *Candida* (yeast) colonization among DUWLs of older age. We speculate

that differences in structure and adhesion ability between bacteria, moulds, and yeasts, as well as inter-species differences might contribute to differences between studies.

Regarding dental clinic specialty, water samples from the minor operation dental clinics had the highest mean value of FCs (200.0 CFU/100mL), while samples from the implant dental clinic showed no fungal contamination at all. In contrast, Al-Hiyasat *et al.* reported that the conservative dentistry clinics had higher contamination levels in comparison to periodontics and prosthodontics clinics [23]. They attributed this finding to the more invasive nature of dental procedures performed in the former specialty, which might introduce blood and pus by back-suction into the DUWLs. Szymanska *et al.* reported that the level of contamination of DUWLs in different dental specialties may be related to the kind of procedures performed and to the degree of using the handpiece, air/water syringe, and cup-fillers [17].

The time of sample collection was a further contributor to the FC of the DUWL output water samples. There was a significant decrease in FC mean values in water samples collected at the end of shift compared to those collected at the beginning of the shift [high speed handpiece: (17.2, 3.0 CFU/100mL), air/water syringe: (38.8, 3.2 CFU/100 mL), cup filler: (39.5, 5.1 CFU/100 mL, respectively)] ($p < 0.001$). This was in concordance with findings of Al-Hiyasat *et al.*, who reported that the total microbial count in output water from handpiece, air/water syringe, and cup-fillers obtained at the beginning of the working day was significantly higher than that obtained at mid-day [23]. This might be attributed to the more frequent flushing occurring during the day and between treatment sessions, with subsequent washing-off of biofilm parts from DUWLs and thus lower FCs at the end of the shift. A study reported an increase in the mean microbial count with the increase in number of work shifts in the DUs, where no flushing between patients was performed [20].

Another factor that might affect the level of fungal contamination of DUWLs is the source of water. A study reported that high-speed handpiece lines had significantly higher counts than air/water syringe lines, and they attributed their findings to the fact that the air/water syringe was used more frequently than the high-speed line, as well as differences in water flow rates [26]. In contrast, a study found no statistically significant difference between counts in air/water syringe, handpieces, and cup filler water samples [9]. This was in concordance with our study.

Moulds isolated in the present study were comprised of *A. flavus* (8.3%), *A. niger* (5.9%), while *Penicillium* spp. had a 4.9% prevalence. Similar to these results, Göksay *et al.* [18] reported that *A. flavus* and *Penicillium* spp. were the two predominant fungal contaminants of DUWLs. On the contrary, Szymanska *et al.* reported in their study that yeasts prevailed in DUWLs, and constituted of *C. albicans*, *C. curvata* and *Geotrichum candidum* [17]. Kadaifciler *et al.* isolated different yeast species from DUWLs which were: *C. famata*, *C. guilliermondii*, and *Cryptococcus laurentii* [9]. Studies concluded that *Candida* spp. found in DUWLs were most likely derived from back-suction of patients' saliva, especially in earlier-generation dental equipment without anti-reaction valves [19].

To the best of the authors' knowledge, *C. dubliniensis* has not been isolated previously from water samples. This finding is important owing to the high oral carriage rate of *C. dubliniensis*, as well as its ability to become invasive in immunocompromised individuals. Another reason for the significance of this species, is the fact that it is very similar phenotypically to *C. albicans*, so that it is often microbiologically misidentified as *C. albicans* [25]. In our study, the use of API 20 *Candida* system (BioMe'rieux- France) was not useful for identification (except for a single isolate of *C. albicans*) because *C. dubliniensis* is not among its identification panel. The nine *C. dubliniensis* isolates were identified using the MALDI-TOF MS tool.

The distribution of different fungal species was also studied in relation to risk factors for mycological contamination of in DUWLs. Regarding the dental specialty, minor operations and surgery clinics had a predominance of *A. flavus* (30.4 % and 15.2%, respectively). Surgical clinics had the highest mean FCs, probably due to the more aggressive manipulations that encourage water splashing of contaminated water. A study reported that the most frequently isolated fungal genera were *Aspergillus* spp. (42.8%) and *Penicillium* spp. (41.4%) while 42.9% of *C. dubliniensis* isolates were from periodontics clinics ($\chi^2 = 24.460$, $p < 0.001$). *Candida* spp. contamination might denote back-suction from patients' oral cavities or cross-contamination during handling of water reservoirs [27].

An additional factor that affected the fungal species was the time of sample collection. At the beginning of the shift, all isolates were comprised of moulds, while at the end of the shift, moulds decreased significantly and 8.9% of samples were contaminated by *Candida* spp. These results may suggest the predominance of moulds at the beginning of the work shift (due to

overnight stagnation), and they probably become washed away from DUWLs by time. However, at the end of the shift, more oral microorganisms, including *Candida* spp., contaminate the DUWLs due to back-splashing.

In the present study, the majority of *A. flavus* isolates were weak biofilm-producers while most *Candida* spp. isolates produced it moderately, denoting stronger biofilm production by *Candida* spp. isolates. This finding indicates the potential health hazard of fungi in water due to their ability to form biofilms. Patients with candidemia caused by biofilm-forming strains have a worse outcome than those infected by non-biofilm-forming strains [26].

In our study, the majority of samples (186/204, 91.1%) were from DUs that used 5% - 20% sodium hypochlorite as disinfectants in their DU reservoirs. Contrary to what might be expected, the frequency of disinfectant use (daily *versus* weekly) was not a significant determinant of mycological contamination of DUWL output water (Table 5). The stability of sodium hypochlorite recorded by some studies might explain this finding [28,29]. On the other hand, the concentration of disinfectant used was an important determinant of mycological contamination. It was found that 20% sodium hypochlorite was more effective than 5% sodium hypochlorite in eradicating fungi from DUWLs. Lin *et al.* [30] recommended the use of 0.05% H₂O₂ periodically due to its ability to reduce biofilm formation and planktonic contamination in DUWLs.

Conclusions

A. flavus was the most prevailing mould contaminant in DUWL outlet water samples while *C. dubliniensis* was the most commonly isolated yeast. Some factors contributed to the fungal contamination of DUWLs such as: age of DUs, dental specialty, time of sample collection (beginning/end of shift), water source and type and frequency of disinfectant used. H₂O₂ was a more effective antifungal disinfectant compared to 5%-15% sodium hypochlorite, and it was almost as equally effective as 20% sodium hypochlorite.

Authors' contributions

All authors contributed equally to this manuscript at all its stages of production.

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Annex – Supplementary Items

Supplementary Table 1. Distribution of the 204 examined DUWL output water samples according to the collection shift and the isolated fungal species.

Fungal spp.	Collection shift				Total		χ^2	MCp
	Beginning of shift (n = 102)		End of shift (n = 102)		(n = 204)			
	No.	%	No.	%	No.	%		
No fungal growth	65	63.7	90	88.2	155	76.0	16.787*	< 0.001*
<i>A. flavus</i>	16	15.7	1	0.1	17	8.3	2.074	0.355
<i>A. niger</i>	11	10.8	1	0.1	12	5.9	1.960	0.369
<i>Penicillium</i> spp.	10	9.8	0	0.0	10	4.9	0.347	0.887
<i>C.dublinsiensis</i>	0	0.0	9	8.8	9	4.4	0.613	0.914
<i>C.albicans</i>	0	0.0	1	0.1	1	0.5	1.742	1.000