# MICROBIAL DIVERSITY IN DENTAL UNIT WATERLINES

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***ABSTRACT***
*Dental health care providers and patients are exposed during ongoing work to contamination by the water used in the dental units, due to accidental swallowing or aspiration of the sprays generated by the high-speed handpiece and the three-way syringe. This study evaluated the quality of water in dental units in the public dental care system of Maceió, Alagoas, Brazil, by conducting analyses of contamination by total coliforms, E.Coli, heterotrophic bacteria and filamentous fungi. We collected 200 mL of water at 5 sites in 6 dental offices of the Department of Health located in different parts of the city. A total 212 isolates and 16 genera of filamentous fungi were identified in the water collected from the dental units. Total coliforms indicated that the water used in dental units was not appropriate for human consumption. The high levels of contamination found in this study showed that water was a potential source of cross-infection.*

***Key words:*** *Biofilms; Dental service Infection control; Water quality*

## DIVERSIDADE MICROBIANA NAS LINHAS D'ÁGUA DAS UNIDADES DENTAIS

***RESUMO***
*Durante a rotina de trabalho, a equipe odontológica e os pacientes ficam expostos a possível contaminação pela água utilizada nas unidades dentais, devido à ingestão acidental da água ou pela aspiração dos sprays gerados pela caneta de alta rotação e seringa tríplice. Este estudo avaliou a qualidade da água usada em consultórios odontológicos da rede pública estadual de Maceió, Alagoas, Brasil, através da análise de contaminação por coliformes totais e E.coli, bactérias heterotróficas e fungos filamentosos. Foram coletados 200 mL de água, em cinco pontos de seis consultórios odontológicos pertencentes à Secretaria Estadual de Saúde, localizados em diferentes bairros da cidade. Um total de 212 isolados e 16 gêneros de fungos filamentosos foram identificados. A presença de coliformes totais indicou que a água utilizada nas unidades dentais era imprópria para consumo humano. O alto índice de contaminação mostrou que as águas estudadas eram uma fonte potencial de infecções cruzadas.*

***Palavras-chave:*** *Biofilme; Controle de infecção; Qualidade da água; Serviços odontológicos.*

## INTRODUCTION

The quality of dental unit water is of considerable importance to patients and dental health care providers because they are exposed to water and aerosols generated from the dental unit during routine practice[1,2].

Microbial concentrations in dental unit waterlines were first reported by Murray and Slack in 1957[3]. Today, the presence of high concentrations of microorganisms in the water of dental units is recognized by the scientific community[4]. This contamination has been an important problem in dentistry for over 50 years[5,6].

In Brazil, there is no specific standard for the microbial quality of water used in dental units, but the Ministry of Health issued Directive # 2914 in December 2011[7], establishing that the quality of potable water supplied to the population by the public distribution systems should be evaluated through monthly bacteriological analyses assessing total coliforms and *Escherichia coli*. Heterotrophic bacteria should be counted in 20% of the samples and the total should not be greater than 500 colony forming units (CFU) per milliliter of water. Similar standards are used in Japan <100 CFU/mL, Europe <200 CFU/mL and the United States <500 CFU/mL for drinking water. Coliform count is also used internationally as an indicator of unsafe drinking water[5,8,9].

This study evaluated the quality of water in dental units in the public dental care system of Maceió, Alagoas, Brazil, by conducting a quantitative analysis of contamination by total coliforms, *E.coli,* heterotrophic bacteria and filamentous fungi.

## MATERIALS AND METHODS

Water samples were collected from six dental clinics of the Department of Health located in different parts of the city, in hermetically closed, sterilized graded wide-mouth bottles containing 0.1mL of 10% sodium thiosulfate solution to neutralize residual chlorine, following the protocol recommended by Standard Methods for the Examination of Water and Wastewater[10]. Water was collected from the following sites: three-way syringe – SYRINGE; high-speed handpiece coupled to tubing – HANDPIECE; tubing of high-speed handpiece without handpiece coupled - TUBING; water reservoir – RESERVOIR; and the site supplying the reservoir – SOURCE.

To disinfect these sites before collection, they were wiped quickly with a piece of gauze soaked in 70% ethyl alcohol. The three-way syringe and high-speed handpiece were turned on and the water allowed to run for 10 seconds before collecting. 200mL of water from each site at the six dental units. The samples were kept cool in ice boxes and processed within four hours of collection.

### Sample Inoculation and Culture

#### *Total coliforms and E. coli*

An enzyme substrate test (Colilert®, IDEXX Laboratories, Westbrook, ME) was used. The water containers were cleaned with a piece of gauze soaked in 70% ethyl alcohol. Then, 100 mL of the collected water were measured with a sterile pipette and placed in a sterile Erlenmeyer flask, and the reagent was added. It was incubated at 35 ± 0.5 °C for 24 hours. Results were read using ultraviolet light. The test was positive for total coliforms if the water was yellow, and for *E.coli*, if it was blue under ultraviolet light. The test was negative if there was no color.

#### *Heterotrophic bacteria*

The methodology used was adapted from the protocol suggested by Mayo et al.[11]. Each sample was diluted to $10^{-1}$, and 0.1 mL of the original sample and of the dilution were plated in duplicate onto Petri dishes containing plate count agar (PCA), to which 50 $mg.L^{-1}$ of ketoconazole was added. The dishes were incubated at 37 °C for 24 to 72 hours.

#### *Filamentous Fungi*

The samples were diluted to $10^{-1}$, and 0.1 mL of the original sample and the dilution were plated in duplicate onto Petri dishes containing Sabouraud dextrose agar, to which 50 $mg.L^{-1}$ of chloramphenicol and 50 $mg.L^{-1}$ ampicillin were added. The dishes were incubated at 28° C for four to six days.

#### *Identification of Filamentous Fungi*

Filamentous fungi were identified according to genus based on macroscopic and microscopic features. Microscopic analysis was conducted using the microculture technique in Lactrimel medium (14 g wheat meal, 14 g dried milk, 7 g honey and 0.4 g chloramphenicol per liter)[12].

## RESULTS

*E. coli* was not detected in any of the water samples analyzed. However, nine of the thirty samples (30%) showed total coliforms (Table 1).

All the dental units had at least three sites at which heterotrophic bacteria exceeded the 500 CFU/mL limit (Table 2).

Filamentous fungi were isolated from 70% of the samples (21/30), totaling 212 isolates grouped in 16 genera. The most frequent genera were *Acremonium* (46.7%), *Exophiala* (14.7%), *Penicillium* (9.4%), *Aspergillus* (8.9%). Other genera had fre-

**Table 1: Total coliforms, quantitative results in 100 mL, according to collection site.**

| Unit | Source | Reservoir | Tubing | Handpiece | Syringe |
|---|---|---|---|---|---|
| 1 | N | N | N | N | N |
| 2 | N | P | P | P | P |
| 3 | N | N | N | N | N |
| 4 | N | N | N | N | N |
| 5 | P | P | P | P | P |
| 6 | N | N | N | N | N |

N = Negative sample P = Positive sample

**Table 2: Heterotrophic bacteria counts in CFU/mL according to collection site.**

| Unit | Source | Reservoir | Tubing | Handpiece | Syringe |
|---|---|---|---|---|---|
| 1 | $2.8 \times 10^4$ | $3.1 \times 10^4$ | $2.5x\ 10^3$ | $6.5 \times 10^2$ | $3.2 \times 10^4$ |
| 2 | $3.5 \times 10^2$ | $7.0 \times 10^3$ | $2.9 \times 10^3$ | $8.6 \times 10^2$ | $6.5 \times 10^2$ |
| 3 | $2.2 \times 10^4$ | $2.3 \times 10^2$ | $1.0 \times 10^3$ | $4.7 \times 10^2$ | $5.3 \times 10^2$ |
| 4 | $1.5 \times 10^2$ | $7.0 \times 10^2$ | $1.1 \times 10^3$ | $2.3x\ 10^2$ | $8.6 \times 10^3$ |
| 5 | $9.3 \times 10^4$ | $8.9 \times 10^4$ | $8.9 \times 10^4$ | $8.6 \times 10^4$ | $1.2 \times 10^5$ |
| 6 | NG | $2.3 \times 10^3$ | $2.4 \times 10^3$ | $1.6 \times 10^3$ | $3.0 \times 10^4$ |

NG = no growth

**Table 3: Number of fungi according to genus isolated and identified in 30 samples from 6 dental units.**

| Isolated fungi | (n°) | (%) |
|---|---|---|
| *Acremonium spp* | 99 | 46.7 |
| *Aspergillus spp* | 19 | 8.9 |
| *Bipolaris spp* | 6 | 2.9 |
| *Cladophialophora spp* | 10 | 4.7 |
| *Cladosporium spp* | 6 | 2.9 |
| *Chrysosporium sp* | 1 | 0.4 |
| *Curvularia spp* | 5 | 2.3 |
| *Exophiala spp* | 31 | 14.7 |
| *Fusarium spp* | 2 | 1 |
| *Mycelia Sterilia* | 6 | 2.9 |
| *Paecilomyces sp* | 1 | 0.4 |
| *Penicillium spp* | 20 | 9.4 |
| *Phoma spp* | 1 | 0.4 |
| *Rhinocladiella spp* | 2 | 1 |
| *Scopulariopri sp* | 1 | 0.4 |
| *Verticilium spp* | 2 | 1 |
| Total | 212 | 100 |

n = total number of isolates
% = percentage according to number of isolates

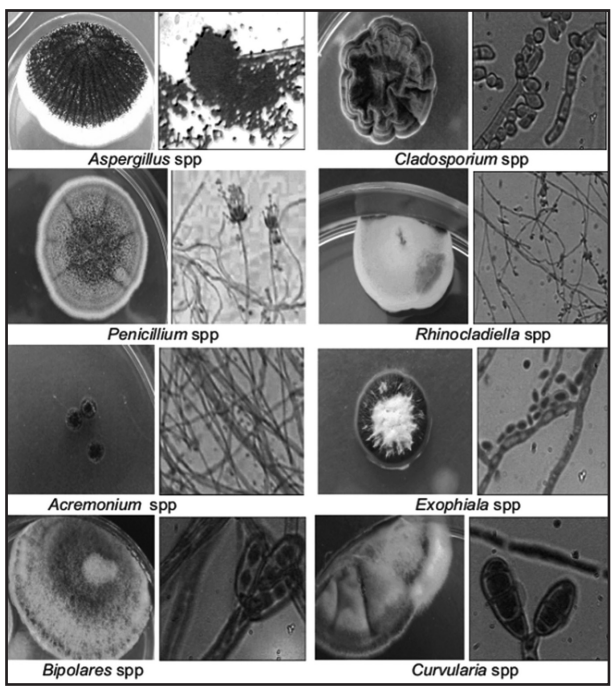


*Fig. 1: Macroscopic and microscopic image of eight of the sixteen genera isolated and identified in 30 samples from 6 dental units.*

quencies below 5%. All the genera isolated include potentially pathogenic species (Table 3 and Fig.1). The highest number of fungi was isolated and identified from tubing, with 108 isolates (50.9%), followed by reservoir, with 43 isolates (20.3%), and handpiece, with 39 isolates (18.4%). The percentages at the remaining sites did not exceed 7% (Table 4).

## DISCUSSION

Although contamination of dental unit water systems was identified over 50 years ago, many dentists nowadays are still unaware of microbiological contamination or its health risk for dental care providers and patients[13].

According to Standard Methods for the Examination of Water and Wastewater, sodium thiosulfate is an adequate dechlorinating agent that neutralizes any residual chlorine and prevents continuation of bactericidal action during sample transport. Thus, the exam will indicate more precisely the true microbial content of the water at the time of sampling[10].

The minimum contamination level of heterotrophic bacteria detected in the water samples collected from the high-speed handpiece was 2.3 x $10^2$ CFU/mL, in agreement with Souza-Gugelmin et al.[14], who found contamination levels of 1.9 x $10^2$ CFU/mL for the same collection site.

The level of bacterial growth from all the water samples collected from the high-speed handpiece, either connected to the tubing (HANDPIECE) or not (TUBING), exceeded acceptable levels, except at dental units 3 and 4 for the HANDPIECE site, for which the results were within the acceptable limits. This finding indicates that bacterial contamination was greater in the tubing than in the handpieces.

According to Watanabe et al[15], water reservoirs should be cleaned regularly with mechanical and

**Table 4: Number of fungi isolated and identified according to collection site.**

| Collection site | Positive Samples | Isolates (n) |
|---|---|---|
| Syringe | 3 | 7 |
| Reservoir | 6 | 43 |
| Handpiece | 3 | 39 |
| Tubing | 5 | 108 |
| Source | 4 | 15 |
| Total | 21 | 212 |

n = number of isolates

chemical methods to remove the biofilm. Our study found that the highest concentration in the reservoirs was $8.9\times10^4$ CFU/mL, which is lower than the result detected in a previous study, in which the concentration was found to be $1.1\times10^5$ CFU/mL[16].

The results of studies conducted by Aprea et al.[17] and Watanabe et al.[15] were negative for bacteria of the coliform group and *E.coli*. However, in our study, nine samples were contaminated with total coliforms.

Opportunistic fungal pathogens, such as *Candida* spp., *Cryptococcus neoformans* and *Aspergillus* spp., usually only cause infections when there are breaks in the protective skin and mucosal barriers or when immune system defects allow their penetration, colonization and reproduction in the host[18]. *Candida* yeasts mixed with traces of saliva may be present in water and aerosols produced by dental handpieces mainly because of dysfunction of anti-retraction valves[19]. Thus, sprays contaminated with yeasts and fungi generated during routine work may be a threat to the health of patients and dental care providers[20].

*Acremonium* spp., *Bipolares* spp., *Cladosporium* spp., *Penicillium* spp., *Paecilomyces* spp. and *Verticillium* spp. may cause corneal infections and be a problem for contact lens wearers[20-26]. In our study the most frequent genus was *Acremonium* spp., which differed from the study conducted by Szymanska[27], in which the most frequent filamentous fungus was *Aspergillus* spp.

*Aspergillus* spp. and some other species of fungi, such as *Curvularia* spp., Mycelia Sterilia, *Phoma* spp. and *Scopulariopsis* spp., have a pathogenic potential to cause allergic reactions and hypersensitivity[27-29]. Infections resulting from inhaling *Aspergillus* spp. spores may cause asthma in patients who are allergic to it[20]. This study showed rates of 8.9%, 2.9%, 2.3%, 0.4% and 0.4% of *Aspergillus* spp., Mycelia Sterilia, *Curvularia* spp., *Phoma* spp. and *Scopulariopsis* spp., respectively.

*Exophiala* spp. and *Chrysosporium* spp. may cause skin lesions and endocarditis[30]. Porteus et al.[31] isolated *Exophiala* sp, which they claim was not often found in dental unit waterlines. *Exophiala* spp. was also found in this study, being the second most frequent genus.

Our study also found *Cladophialophora* spp. (4.7%), *Rhinocladiella* spp. (1.0%) and *Fusarium* spp. (1.0%). The two former are an additional source of concern because they may cause chromoblastomycosis, a chronic, granulomatous infection characterized by verrucous, occasionally ulcerated nodules[32,33]. The latter has been associated to infections in patients after trauma and surgeries, because some of these fungal species may cause ocular and systemic infections, sinusitis, and skin and nail infections[20,34].

According to Mungara et al[35], to maintain the sterility of dental unit waterlines it is essential to have a good water source and an effective disinfectant. In this study, the water delivered to most patients was poor of quality and was considered a potential source of cross infection.

Regular microbiological evaluation of the water used in dental units is extremely important to prevent infections in patients and dental care providers. Standardized procedures to evaluate the water used in dental units should therefore be established. Water should be monitored not only for number of total coliforms, *E.coli* and heterotrophic bacteria, but also for the presence of filamentous fungi.

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