Diversidade microbiana nas linhas d'água das unidades dentais

Guacyra M. Lisboa1, Yves R.M. Lisboa2, Telma M.L. Pinheiro2, Roberto C. Stegun3, Eurípedes A. da Silva-Filho1

1 Department of Biological Sciences, Federal University of Alagoas, Brazil
2 Department of Microbiology, Central Laboratory of Public Health of Alagoas, Brazil
3 Department of Dental Prosthesis, School of Dentistry, University of São Paulo, Brazil

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 of 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.

Keywords: Biofilm; Dental service infection control; Water quality

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

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, 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.

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. 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. 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 chloramphenicol and 50 mg.L⁻¹ ampicillin were 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.

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-
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 \(2.3 \times 10^2\) CFU/mL, in agreement with Souza-Gugelmin et al.\(^{14}\), who found contamination levels of \(1.9 \times 10^2\) 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 3: Number of fungi according to genus isolated and identified in 30 samples from 6 dental units.**

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>(n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acremonium spp</td>
<td>99</td>
<td>46.7</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>19</td>
<td>8.9</td>
</tr>
<tr>
<td>Bipolaris spp</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>Cladosporium/pilosa spp</td>
<td>10</td>
<td>4.7</td>
</tr>
<tr>
<td>Cladosporium spp</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>Chrysosporium sp</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Curvularia spp</td>
<td>52</td>
<td>9.4</td>
</tr>
<tr>
<td>Exophiala spp</td>
<td>31</td>
<td>14.7</td>
</tr>
<tr>
<td>Fusarium spp</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Mucor spp</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>Paecilomyces sp</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>20</td>
<td>9.4</td>
</tr>
<tr>
<td>Phoma spp</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Rhinocladiella spp</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Scopularia sp</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Venturillium spp</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td>100</td>
</tr>
</tbody>
</table>

\(n\) = total number of isolates  
\(\%\) = percentage according to number of isolates

---

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

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Positive Samples</th>
<th>Isolates (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Reservoir</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>Handpiece</td>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>Tubing</td>
<td>5</td>
<td>108</td>
</tr>
<tr>
<td>Source</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>212</td>
</tr>
</tbody>
</table>

\(n\) = 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.
chemical methods to remove the biofilm. Our study found that the highest concentration in the reservoirs was $8 \times 10^5$ CFU/mL, which is lower than the result detected in a previous study, in which the concentration was found to be $1.1 \times 10^6$ CFU/mL. The results of studies conducted by Aprea et al. and Watanabe et al. 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. Candida yeasts mixed with traces of saliva may be present in water and aerosols produced by dental handpieces mainly because of dysfunction of anti-refraction valves. Thus, sprays contaminated with yeasts and fungi generated during routine work may be a threat to the health of patients and dental care providers.

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. Infections resulting from inhaling Aspergillus spp. spores may cause asthma in patients who are allergic to it. 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 Chrysosporum spp. may cause skin lesions and endocarditis. Porteus et al. 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 Cladophialaphora 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. 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.

According to Mungara et al., 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 coliforms, E. coli and heterotrophic bacteria, but also for the presence of filamentous fungi.

ACKNOWLEDGEMENTS
We thank Elaine Santos, Mirella Vieira, Rosana Santos and Iara Fonseca from UFAL for their valuable assistance in identifying fungi.

REFERENCES

CORRESPONDENCE
Eurípedes A. da Silva-Filho
Av. Lourival Melo Mota, s/n, Cidade Universitária
57072-900 – Maceió, AL, Brazil
alvesfl@gmail.com


