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EVALUATION OF THE PRESENCE OF PSEUDOMONAS AERUGINOSA BIOFILMS IN DENTAL UNIT WATER LINES

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Abstract: Introduction: Bacterial biofilm in dental unit water lines (LAUD) is a widespread problem, and poses a potentially significant risk of infection to dental staff and patients. **Goal:** To evaluate the hygienic-sanitary conditions of the LAUD in a Dental Specialty Center. **Methods:** Ninety-three mineral water samples were analyzed at thirteen LAUD. Fifteen samples were taken from the water supply, thirty-nine samples from the LAUD reservoir and thirty-nine samples from water after passing through the triple syringe. The procedures were performed for the detection of Pseudomonas aeruginosa bacteria, coliforms and heterotrophic bacteria. **Results:** Pseudomonas aeruginosa was detected in 43.01% of the samples and 13.98% showed contamination from the coliform group. 77.41% of the samples showed amounts of heterotrophic bacteria above 500 colony forming units per milliliter (CFU/mL). Among the samples that were positive for Pseudomonas aeruginosa, 30% were from the reservoir, 70% were after passing through the triple syringe and none of the supply water samples tested tested positive. 53.5% of the water samples that were positive for Pseudomonas aeruginosa after passing through the triple syringe were negative in the water supply and LAUD reservoir. It was found that after passing through the triple syringe, 66.6% of the samples had heterotrophic bacteria, 30% more than the amount detected in the LAUD reservoir. The presence of Pseudomonas aeruginosa only in the triple syringe, the increase in heterotrophic bacteria and the fact that the bacteria remained even after cleaning, suggest that the biofilm was formed by Pseudomonas aeruginosa in the analyzed LAUD.

Keywords: Pseudomonas aeruginosa, biofilm, Dental Unit Waterline

INTRODUCTION

Most dental procedures require the use of large amounts of water for irrigation, cooling and washing of oral instruments during patient care1. However, the water coming out of the dental unit's water lines (lauds) can be a potential source of infection for both oral health professionals and their patients.[2]

In general, cross-infection results from the transfer of the causative agent of a transmitted disease between patients and professionals in the clinical environment. [3,4]. However, the highest rate of cross-infection is associated with immunocompromised patients, including the elderly, transplant patients, HIV patients, cancer patients, and those on immunosuppressive therapy.[5-8]

Several studies have reported concerns about the high level of microbial contamination in dental unit water lines. (DUWL) [9-12]. In addition to the risk of cross-infection, DUWL contamination can alert dentists and researchers to the fact that there are microorganisms capable of colonizing the inner surface of piping systems.[13-14]

During dental treatment/surgery, several microorganisms from the oral cavity are sucked and deposited inside the tubing. [14], where they can be colonized by bacteria, fungi or protozoa. Cross-infection can occur as a result of aerosol produced during dental treatment, for example, water contaminated by pathogenic microorganisms that can cause disease transmission to both staff and clinic visitors.[14]. These systems provide an environment conducive to the formation of bacterial biofilms on the inner surfaces of water pipes.[11-14]

Biofilms have a heterogeneous composition, consisting of a complex structure of micro colonies and channels that allow the flow of fluids and nutrients. [14]. The presence of biofilm-forming bacteria inside the pipes of dental equipment increases the risk of contamination for the patient. Cells may remain viable even after surfaces have been cleaned and disinfected, affecting safety standards and procedures.[2,15]

There has been considerable progress in the field of environmental microbiology in explaining the microbial interactions involved in biofilm formation. These include the genetic exchange of virulence factors, cell-cell signaling or quorum sensing, and bacterial succession, all of which have broad implications for the prevention and management of biofilms in DUWL.[16]

In view of the potential risk of patient contamination and biofilm formation in the DUWL, the objective of this study was to analyze the microbiological quality of the Water Lines of the Dental Unit of a Specialized Dental Center (SDC), by conducting research on coliforms, Pseudomonas aeruginosa and heterotrophic bacteria.

METHODOLOGY

Ninety-three samples of mineral water were analyzed in thirteen DUWLs from February to November 2020. Aliquots were taken from the feeding device (fifteen samples), from the reservoir (thirty-nine) and after passing through the same tubing through the syringe triple (thirty-nine samples).

In the extraction of the samples, several bottles of mineral water were used to supply the equipment and for the consumption of SDC by the patients. Approximately 100 ml of each sample was collected in sterile sampling bags. The date, time and place of collection were recorded and the samples transported to the laboratory for analysis. The samples were submitted to microbiological analysis (looking for P. aeruginosa, heterotrophic bacteria, total and fecal coliforms), and analyzed at the Laboratory of Microbiology, Food Science and Water Analysis, located on Campus II of ASCES Unita.

DETECTION OF PSEUDOMONAS AERUGINOSA AND COLIFORMS

The multiple tube technique was performed as recommended by the Standard methods for the examination of water and effluents (APHA)[16] for detection of P. aeruginosa and coliforms. The culture conditions for P. aeruginosa testing were asparagine broth (presumptive test) and acetamide broth (confirmatory test) incubated at 35°C/48 h. The detection of coliforms involved the Lactose Broth (presumptive test) and Brilliant Green Bile Lactose Broth (confirmatory test) tests incubated at 35ºC/48h. Bacteria quantification was performed in 3 sets of five tubes that gave the Most Likely Number (MPN/100mL)[16].

DETECTION OF HETEROTROPHIC BACTERIA

The plate count of aerobic heterotrophic bacteria used the pour plate technique where the samples were inoculated in triplicate in two sets of three Petri dishes containing sterile samples of 1ml and 0.1ml, following the methodology described by Domingues et al.[13.]

ETHICAL CONSIDERATIONS

This study was approved by the Scientific Committee and the Research Ethics Committee (CEP) of Faculdade ASCES, under process number: 206/2011.

RESULTS

The bacteriological analysis of the water showed that of the thirteen DUWL analyzed, 11 were positive for one or more of the bacteria studied. The analysis of the water used to supply this equipment proved to be positive for coliforms in 7.6% of the samples, while in the water reservoir 13.3% of the samples were detected as positive for the group and 15.3% after passing through the triple syringe.

Pseudomonas aeruginosa was not found in the supply water, although it was present in 30.7% of the samples collected in reservoirs and in 63.7% of the water samples after passing through the triple syringe. The values found in the Most Likely Number (MPN/100ml) increased from 6 to 70 after the triple syringe.

The amount of heterotrophic bacteria is shown in Table I.

DISCUSSION

A wide variety of bacteria, protozoa and fungi were recovered from DUWL [14]. The presence of large numbers of microorganisms, including opportunistic pathogens such as Pseudomonas aeruginosa, which are transported by DUWL water, is of concern because of the increased risk of cross-infection, especially in immunocompromised patients.[5-7] Occupational asthma in dentists. Several studies have shown that water emitted by dental unit waterline systems can be heavily contaminated.[9-12] There are two major concerns regarding DUWL contamination. The first is the importance of the presence of bacteria in the DUWL in terms of public health and risks of cross-infection, and the second is that many researchers and dentists are primarily concerned with the microorganisms present in the aqueous phase and not those that colonize the inner surface of the DUWL. pipe[11]. Microorganisms found in the large aqueous phase have been associated with their exposure to endotoxin in aerosols caused by DUWL[12] highly contaminated. These cases highlight the potential risks of propagating microorganisms from DUWL, such as those from an aerosol.[11]

Drinking water must be free of pathogenic microorganisms and must not be contaminated by fecal indicator bacteria. According to Eaton [10], the presence of

Teams	Supply (Average±DP**)	Reservoir (Average ±DP**)	Triple syringe (Average ±DP**)
Team 1	793±91	1022±105	1557±147
Team 2	305±37	762±72	1420±137
Team 3	701±34	775±74	1052±104
Team 4	331±21	834±81	911±87
Team 5	305±27	510±43	1789±113
Team 6	327±35	392±35	833±85
Team 7	274±24	468±45	1089±96
Team 8	105±16	476±29	618±46
Team 9	698±59	975±8	1973±178
Team 10	95±7	944±91	1114±101
Team 11	52±4	510±44	959±78
Team 12	542±35	625±64	1045±98
Team 13	128±9	210±19	321±22

*CV: Coefficient of variation;

**DP: Standard deviation.

Table I. Evaluation of the presence of heterotrophic bacteria in Water Lines of Dental Units (CFU/mL). CV* = 10.1± 2.5.

coliforms in water indicates the possibility of fecal contamination, since these microorganisms are commonly found in the environment. However, the presence of fecal coliforms determines the origin of the risk of contamination and evidences the presence of other pathogenic microorganisms, while their absence indicates that the water is microbiologically potable. In drinking water, the amount of coliforms present must be ≤ 1.8 NMP/100mL. However, coliforms are not usually found in DUWL, except in unusual circumstances, such as, for example, due to errors in handling reservoir bottles, such as non-compliance with hygiene standards by professionals.[6].

Contamination of DUWL by opportunistic pathogens such as Pseudomonas aeruginosa poses a serious health risk to staff and patients. Pankhurst and Coulter [16] reviewed studies that suggest a screening system for microbial virulence factors in DUWL to obtain a more accurate estimate of the risks involved. These virulence factors include pathogen colonization, host tissue invasion, and cytopathic effects. Regarding virulence factors, it was demonstrated that cytolysis was observed in 79% of the teeth and was strongly correlated with the presence of P. aeruginosa. Based on the European Union (EU) guidelines for drinking water, it is clear that the number of CFU/ml released by the DUWL is much higher than the number found at the water source. This inherent multiplication factor suggests that microorganisms are proliferating in the DUWL. The main reservoir of microbes in DUWL is the large number of cells that attach to the sides of the pipe in a biological film called a biofilm from which organisms can be released into the aqueous phase.[13].

Obtaining the conditions for biofilm

formation is a precursor step in the development of biofilm on surfaces where it has contact with water. This film is formed by the adsorption of organic molecules dissolved in the aqueous medium on the surface and leads to the displacement of microorganisms and particles to the solid surface. As a result, adhesion depends heavily on factors involving characteristics of the microorganism when adhering to the surface and environmental conditions.[5,8,10]. Current scientific works have performed a quantitative analysis of P. aeruginosa to assess the quality of water used for dental equipment. In this study, we observed an increase from 30.7% in the number of bacteria in the water reservoir to 71.7% in the water syringe.

Counting heterotrophic bacteria, which are generically defined as microorganisms that require organic carbon as a source of nutrients, provides valuable information about the bacteriological quality of water. The test involves determining whether non-specific bacteria, or bacterial spores, are of fecal origin, and whether the natural components are derived from water or biofilm formation in the delivery system. It thus serves as a complementary indicator of water quality and reveals possible failures in disinfection, colonization and biofilm formation in the distribution system[14].

These studies were performed to assess the level of microbial contamination of DUWL in general dental practice. In 1996, the American Dental Association (ADA) (through its Scientific Affairs Council and Board of Directors) set a goal of less than 200 CFU/mL in water output from dental unit water lines for all dental procedures., based on their standards for dialysis, although this has been superseded by the CDC guidelines issued in 2003. In the EU, there are no current guidelines for DUWL, but some countries use the drinking water standard of <100 CFU/L as a reference [1-7]. In the United States of America (USA) the acceptable value must be <500 heterotrophic aerobics/mL, and this also applies to Brazil where the same standards were established by the Ministry of Health (MS) ordinance 54/2000[6].

DUWL contamination can occur as a result of the presence of an anti-retraction valve in dental equipment. This means that in the slow pedal of the dental mechanism, there is a risk of a reflux of microorganisms produced by the oral microbiotic to the tube either by diffusion and convection, gravity or Brunean movement, as is the case with laminar flow or stagnation. Where the flow is increased, for example, and dental equipment instruments are being used, bacteria are activated that have a transport capacity due to planktonic motility by flagella. In DUWL, the flow is more likely to be laminar (60–100mL/min compared to the flow of a 0.5 in. (25mm) diameter copper pipe of about 5,000 mL/min) with long run times. stagnation, e.g. overnight (16 h) and weekend (64 h), which would lead to microbial colonization of surfaces[15].

The most common laboratory methods used to study the development of biofilms on surfaces are based on the removal of adhered cells from surfaces and subsequent quantification by conventional plate counts. These are employed in this experiment and are indirect methods of studying bacterial biofilm formation on surfaces. However, these techniques are also abrasive to the attached cells and can result in injury, which can in turn result in viable but non-culturable bacteria. Thus, most of these techniques incorporate a resuscitation stage lasting several minutes to allow for cellular recovery. Methods that have previously been used to remove adherent bacteria from surfaces for further enumeration by plate counting include the following: surface scraping of stainless steel coupons[3]; silicone tube vortex [4], polystyrene

and colon tissue; sonication of polyvinyl chloride and polycarbonate coupons, sheets, enamel discs, silicone catheter sections and geotite particles; and flipping stainless steel or glass wool coupons with beads. Although these methods allow calculating the number of bacteria adhering to surfaces by colony counts, they do not reveal the in situ structure of a biofilm. [10,15].

DUWL contamination can be evidenced by the presence of microorganisms as indicators of sanitary conditions. The considerable increase in the same passage through DUWL, and the isolation of Pseudomonas aeruginosa, suggest the possible formation of bacterial biofilms inside the pipes and apparatus, as well as a possible failure in the colonization and disinfection of the water distribution system. However, the results of this study must be investigated in more detail, performing more strain research to confirm whether molecular methods by cell-to-cell signaling or quorum sensing can be employed for biofilm production[2].

There is also a need to examine bacterial cells within a population, as well as their density and number, through molecular signals that diffuse freely across cell membranes and between cells. In general, the quorum-sensing system in Gram-negative bacteria is based on acyl homoserine lactones, while that of Gram-positive cells is based on peptide molecules[11]. Studies have shown that quorum sensing can be an integral part of biofilm development. For example, P. aeruginosa cells supposedly produce the quorum sensing signal (3OC12-HSL), which is necessary for both cell-to-cell communication and stable biofilm formation. [13].

CONCLUSION

The presence of Pseudomonas aeruginosa only in the triple syringe, the increase in heterotrophic bacteria and the fact that bacteria remained even after cleaning suggest that the biofilm was formed by Pseudomonas aeruginosa in the analyzed DUWL.

CONFLICT OF INTERESTS

All authors report that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) this work.

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