Bacteriological evaluation of biofilm formed in dental units

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ABSTRACT

Contagious/infectious diseases and the possibility of cross-infection between patients and/or health professionals worry physicians and dentists. In Dentistry, microbial adherence to the internal surface of dental tubing and the formation of a highly protective biofilm layer is predictable, given the ideal growth conditions in the tubing. This mechanism has been identified as a cross-contamination risk. In this study, twenty-two small sections of high speed handpiece water line tubing were collected in different dentistry departments of the Craniofacial Anomaly Rehabilitation Hospital (Bauru-São Paulo-Brazil) and submitted to bacteriological evaluation. There was positive microbial growth in all samples with a high frequency of no glucose fermentation bacteria (Gram negative). Strategies to control the contamination are also discussed.

Keywords: biofilm, cross infection, dental units.

INTRODUCTION

For a long time medicine has shown concern with contagious diseases and the possibility of cross infection between patients and/or health personnel. Dentistry, as promoter of oral health and working in a highly contaminated environment, has taken a more clear position on this issue just in the few last decades. This fact is coincident with the discovery and divulgation of AIDS. Despite the obvious delay in terms of time, progress is evident, mostly based in the increasing awareness of users of dentistry services.

Dentists should be aware of a variety of contamination possibilities. Among them, the water supply of the dental unit and the formation of the so-called "biofilm" (Prevost et al., 1995).

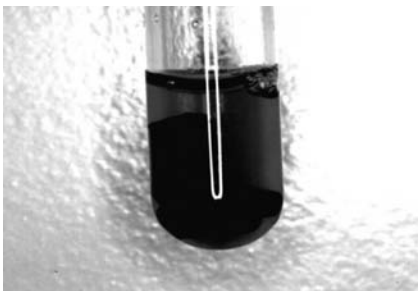
Biofilm is formed when typical planctonic bacteria of the water stick to the inside of the waterline due to the production of a polysaccharide matrix (Fayle & Pollard, 1996). Furthermore, such matrix allows the aggregation of other colonies, function as a nutritional source for microorganisms and, additionally, protect them against antibacterial agents such as surfactants, biocides and antibiotics might be used in water treatment (Peters & Mcgaw, 1996). According to the same authors, the stagnation of water for long periods after working hours, the narrow diameter of tubes and the type of material in its inner surface contribute to an increase in the number microorganisms. Willians & Molinari (1996) made an interesting comparison connecting the process of dental plaque formation, initially firmly attacking the enamel surface and later secreting a polysaccharidic matrix where cooperative specimens grow and reproduce, with the process of stable biofilm formation, rich in heterogeneous microbial flora.

According to Lewis et al. (1992), the biofilm may be responsible for the cross infection detected in any one under dental treatment. When the use of the high-speed turbine is interrupted, there is a backflow of contaminated water from the patient's mouth to the interior of the hand piece and waterline of the dental unit. In this regard, microorganisms (possibly, among them the HIV and B hepatitis virus) may adhere to the biofilm becoming prone to be transferred to the next patient (Mills et al., 1993). According to Goetti & Jardim (1997), the problem of water contamination in dental offices is worsened due to the little attention paid by clinicians to this issue.

In this connection, this study aims to evaluate the bacteriological contamination of biofilm in waterline of dental units in some specialized clinics in the Hospital for Rehabilitation of Craniofacial Anomalies (HRAC-USP) and to discuss methods to minimize such contamination.

MATERIAL AND METHOD

For this study, several 1-cm segments of the dental unit waterline were removed with sterilized surgical scissors from a region 1 cm proximal to the notch of the high–speed handpiece from 22 dental offices at various sectors of the HRAC. Previously, the external aspect of the hose was disinfected with alcohol 70° GL by friction during 5 minutes and rinsed with saline 0.9%. All procedures were made with sterile gloves and scissors. Collected samples were stored in test tubes with bakelite lids containing a brain and heart infusion (BHI – Biobrás) and stored up to 48h in a 37° C stove (FIGURE 1 and 2)



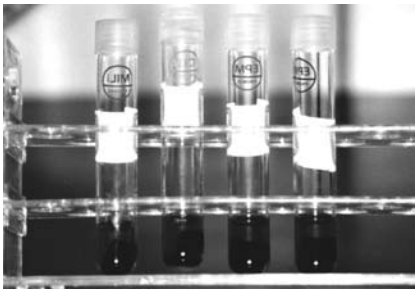


FIGURE 1 and 2 - Segments of high-speed waterline immersed in BHI medium stored in stove for 48h.

At the end of this period, material from test tubes showing turbid medium was transferred to plates with blood agar (Imunoquimica) and MacConkey agar (Oxoid) (FIGURE 3).

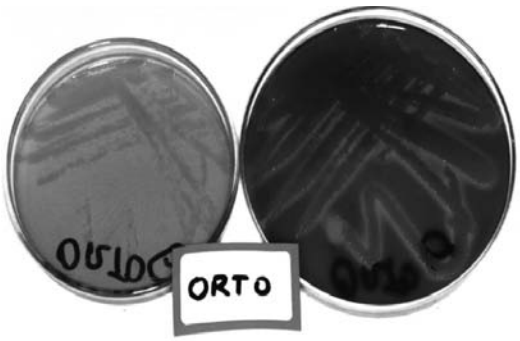


FIGURE 3 - Cultures obtained in MacConckey agar and blood agar from BHI medium of a sample.

The colonies stained with Gram were analyzed from the bacteriological point of view in order to prepare further biochemical analysis which could identify gender and, if possible, specimen of the cultivated microorganisms. Materials used for biochemical analysis were:

1- for gram negative glucose fermentation bacilli: EPM Kit (Escola Paulista de Medicina), MILI (moticity, indol, lysine) from Probac and Simons citrate (Biobrás)

2- for gram-positive non-glucose fermentation bacilli: Bacdent Oxidase (Merck) and NF Kit (Probac)

3- for gram positive cocci: catalase (BHA/Biobrás) and coagulase (Staphytest/Probac)

A sequence of biochemical tests that lead to the identification of bacteria such as *Alcaligenes xylosoxidans ssp denitrificans*, the most frequent, may be seen in FIGURE 4.

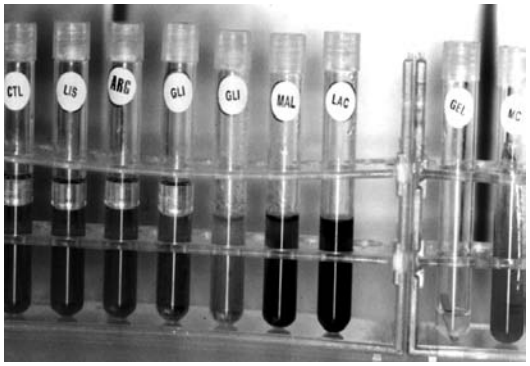


FIGURE 4 - Sequence of biochemical tests for the identification of Gran-negative non-glucose fermentation bacilli.

RESULTS

All fragments of high speed tubing with biofilm produced turbidity of the heart and brain medium in a 24 to 48 h interval. Therefore, in the material of all clinics analyzed there was growth of microorganisms, which were examined from the bacteriological point of view and biochemically tested for identification. TABLE 1 shows the results according to the dental office from where the material was collected.

TABLE 1 - Microorganisms identified by biochemical tests according to the specific site of collection

SPECIALITY	Dental unit	MICROORGANISM
Dentistic	1	*Staphylococcus coagulase negativa* e *Serratia ssp*
	2	*Alcaligenes xylosoxidans ssp denitrificans*
	3	*Pseudomonas pickettii*
	4	*Alcaligenes xylosoxidans ssp denitrificans*
	5	*Klebsiella sp*
	6	*Alcaligenes xylosoxidans ssp denitrificans*
	7	*Alcaligenes xylosoxidans ssp denitrificans*
Endodontics	1	*Alcaligenes xylosoxidans ssp denitrificans*
	2	*Pseudomonas pickettii*
Periodonty	1	*Alcaligenes xylosoxidans ssp denitrificans*
Odontopediatry	1	*Pseudomonas pikettii*
	2	*Alcaligenes xylosoxidans ssp denitrificans*
Ortodontics	1	*Alcaligenes xylosoxidans ssp denitrificans*
	2	*Alcaligenes xylosoxidans ssp denitrificans*
	3	*Alcaligenes xylosoxidans ssp denitrificans*
Prothesis	1	*Alcaligenes xylosoxidans ssp denitrificans*
	2	*Pseudomonas cepacia*

SPECIALITY	Dental unit	MICROORGANISM
Surgery	1	*Alcaligenes xylosoxidans ssp denitrificans*
	2	*Alcaligenes xylosoxidans ssp denitrificans*
	3	*Alcaligenes xylosoxidans ssp denitrificans*
Implantology	1	*Alcaligenes xylosoxidans ssp denitrificans*
	2	*Klebsiella sp*

FIGURE 5 shows examples of some of the identified bacteria.

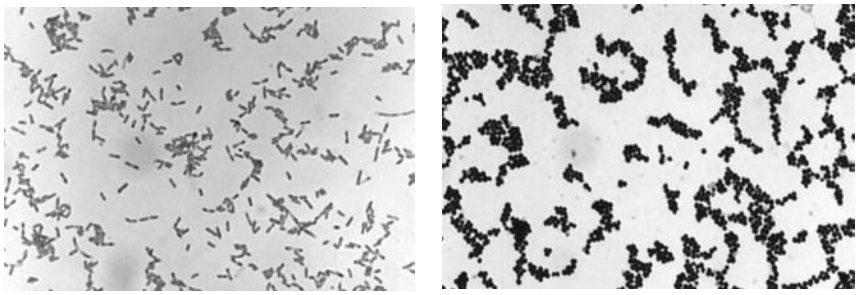


FIGURE 5 - (left) Gram-negative non-glucose fermentation bacilli. Gram positive cocci (right)

DISCUSSION

It was observed that the biofilm present in the water line of all 22 evaluated dental units from different areas of the Hospital for Rehabilitation of Craniofacial Anomalies presented some sort of contamination. Some of the identified microorganisms (*Alcaligenes denitrificans, Klebsiella, Pseudomonas cepacia, Staphylococcus*) were also reported by Miller (1996), Willians & Molinari (1996) and Fayle & Pollard (1996).

The occurrence of the biofilm in the inside of the water hose is well documented since the late 60's when several studies attested its existence and its formation was theorized based in planctonic bacteria in the water supply (Abel et al., 1970). The main concern is that the biofilm, acting as a culture medium, could be contaminated by pathogenic bacteria resulted from the backflow of water of infected individuals. These bacteria could be transmitted to other patients characterizing cross-infection (Peters & Mcgaw, 1996 e Verhagen, 1996).

Microorganisms identified in this study are commonly found in water and ground and show opportunistic characteristics (Bier, 1985). Only organically debilitated and immunodepressed patients, such as those undergoing organ transplant, alcoholics, diabetics, those under renal dialysis, under radio and/or chemotherapy for cancer, AIDS cases, patients with rheumatoid arthritis and those with severe asthma would be in risk

for these agents (Prevost et al., 1995 e Waggoner, 1996). There is controversy on this issue, since there is no documented reports of diseases, infection or death due to the contamination of the biofilm after dental treatment (Waggoner, 1996 e Verhagen, 1996). However, one should admit that these situations are difficult to be proved and divulged.

It was not possible to investigate any relation between the type of microorganism and the specific clinic from were the fragments were obtained (dentistic, endodontics, odontopediatry, surgery, orthodontics, prosthesis and implantology).

The American Dental Association and the American Center for Prevention and Control of Disease have published regulations with directives for the use of saline solutions and sterilized water in surgical procedures for the irrigation and cooling during section of tissues and bone (Waggoner, 1996 e Willians & Andrews, 1996).

The use of disinfectants and antibiotics in reservoirs of water, such as sodium hypochlorite or cloranfenicol, showed to be inefficient and has also produced corrosion of metallic parts decreasing its life span (Murdoch-Kinch et al., 1997). Portable reservoirs with sterile water, autocleaning systems and handpieces with anti-reflux valves are promising innovations that may assure asepsis (Pankhurst & Johnson, 1998), although they are costly to the majority of professionals in Brazil.

We would suggest, based in the literature, some simple daily routine procedures to decrease the possibility of biofilm contamination. Among them: the use of autoclaved handpieces, mostly those used in surgical procedures; friction with alcohol 70^{O}GL of handpieces for 5 minutes before non surgical procedures; use of sterilized or distilled water in the reservoirs; disperse water from the extremity of the handpiece for 30 seconds before each appointment, as well as at the beginning and at the end of the work day, in order to decrease the bacterial flora in the lumen of the water hose; replacement of ground fixed water reservoir for removable plastic ones which can be decontamined and/or sterilized (proxy cost of US$ 25).

These methods are palliative since they do not fully eliminate the possibility of formation, contamination and transmission of microorganism in the biofilm. It remains an unsolved problem that deserve some attention and study by researchers and manufacturers of dental units.

CONCLUSIONS

- All cultures from tubing specimens with biofilm were positive.
- The majority of isolated microorganisms were gram negative non-glucose fermentation.
- Despite the non-pathogenic nature of isolated microorganisms, their opportunistic characteristic may cause problems to immunodepressed patients.

- Quick and low cost measure should be undertaken by professional in order to minimize problems
- New studies should be performed in order to find solutions or effective methods to prevent colonization of the inner aspect of water hose dental units

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BIBLIOGRAPHICAL REFERENCES

ABEL, L. C. et al. Studies on dental aerobiology: IV. Bacterial contamination of water delivered by units. *J. Cent. Res.*, v. 50-2, n. 4, p. 1567-1569, July 1970.

BIER, O. *Microbiologia e imunologia.* 24 ed. São Paulo: Melhoramentos, 1985.

FAYLE, S. A.; POLLARD, M. A. Decontamination of dental unit water systems: a review of current recommendation. *Br. dent. J.*, v. 181, n. 10, p. 78-83, Nov. 1996.

GOETTI, E.; JARDIM, J. R. Contaminação microbiana do sistema de água do equipo odontológico. *Rev. Fac. Odont. Lins*, v. 10, n. 2, p. 32-35, Jul/Dez. 1997.

LEWIS, D. et al. Cross contamination potential with dental equipment. *Lanc.*, v. 340, n. 21, p. 1252-1254, Nov. 1992.

MILLER, C . H. Microbes in dental unit water. *J. Calif. dent. Ass.*, v. 24, n. 1, p. 47-52, Jan., 1996.

MILLS, S. E. et al. Bacteriological analysis of high-speed handpiece turbines. *J. Amer. dent. Ass.*, v. 124, n. 1, p. 59-62, Jan. 1993.

MURDOCH-KINCH, C. et al. Comparison of dental water quality management procedures. *J. Amer. dent. Ass.*, v. 128, n. 9, p. 1235-1243, Sept. 1997.

PANKHURST, C. L.; JOHNSON, N. W. Microbial contamination of dental unit waterlines: the scientific argument. *Int. dent. J.*, v. 48, n. 4, p. 359-368, Apr. 1998.

PETERS, E.; McGAW, W. Dental unit water contamination. *J. Can. dent. Ass.*, v. 62, n. 6, p. 492-495, June 1996.

PREVOST, A. P. et al. Doctor, would you drink water from your dental unit?. *N. Y. St. dent. J.*, v. 61, n. 10, p. 22-28, Dec. 1995.

VERHAGEN, C. M. et al. Backflow prevention: an update. *J. Mich. Dent. Ass.*, v. 78, n. 6, p. 18-20, July-Aug. 1996.

WAGGONER, M. B. The new CDC surgical water recommendation: why they should be implemented and what they require. *Comp. Cont. Ed. dent.*, v. 17, n. 6, p. 612-614, June 1996.

WILLIANS, J. F.; ANDREWS, N. Microbial contamination on dental unit water lines: current preventive measures and emergency options. *Comp. Cont. Ed. dent.*, v. 17, n. 7, p. 691-702, July 1996.

WILLIANS, J. F.; MOLINARI, J. A. Microbial contamination of dental unit waterlines: origins and characteristics. *Comp. Cont. Ed. dent.* , v. 17, n. 6, p. 538-557, June, 1996.

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3.7.2 - For citations of literature inside the text the system author-data, with the initials in capital letters, should be adopted. When there are two authors, link the last names with the "&" sign. When there are three or more authors, mention the last name of the first author followed by the Latin expression **et al**. For works published in the same year, by the same author or the same combination of authors, use letters right after the publication year (NBR 10520 ABNT).

3.7.3 - Abbreviations of the titles of the periodicals should be made according to the MEDLINE standard available at the following BIREME address: http://www.bireme.br/secs/

4 - Presentation of originals

4.1 - Typing

4.1.1 - The author(s) shall present the work printed in triplet, and a floppy disk (3,5') with the full the text in Word 7.0.

4.1.2 - Manuscripts should be printed in double space (in 297mm x 210 mm paper), with margins of 4 cm in the upper and bottom sides and 3 cm on the sides.

4.1.3 - The articles will contain only the necessary information for the understanding of the reader. Should never be more than 15 pages, tables and figures included.

4.1.4 - Articles should contain abbreviations in common use.

4.1.5 - SALUSVITA is a bilingual journal. The author(s) is (are) required to submit a translation of his work into English.

4.2 - Tables

4.2.1 - Shall be numbered consecutively in Arabic numbers and show heading.

4.2.2 - As a general rule, data showed in tables should not be repeated in graphs.

4.2.3 - Tables should follow the norms laid down by NBR 12256/ABNT.

4.2.4 - Footnotes in tables will be restricted to the minimum and will be refereed to by index numbers.

4.3 - Illustrations

4.3.1 - Photos, graphs, drawings, maps, etc., will be named in the text as "Pictures" and numbered sequentially with Arabic numerals.

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