RISK ASSESSMENT OF COLONIZATION OF LEGIONELLA SPP. IN DENTAL UNIT WATERLINES

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SUMMARY
The aerosol produced during the use of dental instruments can spread pathogens potentially harmful to health. Most of the pathogens found in hydraulic system are Gram-negative aerobic heterotrophic environmental bacterial species exhibiting very low pathogenicity, although they may be of concern in the treatment of vulnerable patients, such as immunocompromised, medically compromised individuals and dental team. Dental team can be exposed to pathogenic microorganisms including cytomegalovirus, hepatitis B virus, hepatitis C virus, herpes simplex virus and Legionella spp. Legionella spp. are ubiquitous in hydraulic system, in fact surveys have shown that the percentage of samples taken at different dental chairs that were positive for Legionella spp. and ranged from 0 to 100%. The concentration of Legionella spp. in hydraulic system may reach 1000 organisms per ml. The primary route of Legionella spp. transmission is inhalation or aspiration of environmentally contaminated aerosols. All dentists are required to conduct a statutory risk assessment of their hydraulic system, in fact to comply with their legal duties, dentists must identify and assess the sources of risk and prepare a scheme for preventing and controlling risks. Moreover, they must monitor the quality of their hydraulic system at least annually to ensure that they are "legionellae free".

Key words: legionella, oral pathogens, dental chair, dental infection control, biofilm.

Introduction

The aerosol produced during the use of dental instruments can spread pathogens potentially harmful to health. In addiction, a dental unit is furnished of a hydraulic system (HS), which delivers water to the different hand pieces, air/water syringes, and ultrasonic scalers.

Contamination can occur when oral pathogens enter the HS through back siphonage when the handpiece is turned off. The entrapped pathogens can then be ejected with the water through the HS, increasing the potential for cross-infection from patient to patient. To prevent HS from being contaminated, the handpieces are manufactured with a retraction mechanism. This mechanism can actively "suck-back" contaminants from the oral cavity. The HSs are equipped with anti-retraction valves to prevent suck-back of contaminants from the oral cavity and are designed to give a short "terminal flush" of water to push out any suck-back. In the *in vitro* and *in vivo* experimental studies, even new and unused anti-retraction valves were shown to be quite unreliable, leading to microbial suck-back into the HS from the patient (1).

HS can also become contaminated from the main water supply, which, although potable, still carries pathogens. Some studies have reported that the primary colonizers of HS are not oral microorganisms, but rather the bacteria that are normally found in potable water (*Moraxella* spp., *Flavobacterium* spp., *Legionella* spp., *Pseudomonas* spp., *Klebsiella pneumoniae*, *Acinetobacter* spp., *Mycobacterium avium*) (2,

3). These pathogens colonize and replicate on the inner surfaces of the HS biofilm. The biofilm protects the pathogens from desiccation, chemical insult and predation, and it serves as a reservoir that significantly changes the number of free-floating microorganisms in the HS. Pathogens on the surfaces are continuously released from the biofilm into the HS, so that the biofilm becomes the primary reservoir for continued contamination of the HS.

Factors associated with biofilm formation in HS could include the following: long periods of stagnation on weekends and evenings, nutrients in the water that promote microbial survival, mineral content and hardness of water that promote coating of the lumen, microbial quality of the water of HS, and failure of anti-retraction valves leading to contamination from the oral cavity of patients.

Pathogens in water lines

Most of the pathogens found in HS output water are Gram-negative aerobic heterotrophic environmental bacterial species that exhibit very low pathogenicity, although they may be of concern in the treatment of vulnerable patients, such as immunocompromised and medically compromised individuals and dental team. Some bacterial pathogens such as *Pseudomonas aeruginosa*, *Legionella pneumophila* and non-tuberculosis *Mycobacterium* species were found in HS (4). Only a few instances of cross-infection related to HS and associated biofilms have been reported in the literature (5). However, it is still possible that infections caused by HS have gone undetected or unreported because of the failure to associate exposure to HS and aerosols generated from this water with the development of specific infections. Sporadic infections not requiring hospital admission are also less likely to be investigated. Because of these contaminants, it is important to establish control methods for cleaning and disinfecting the HS and for providing quality irrigant/dental treatment water. Different dental chair manufacturers recommend specific products to disinfect their HS. Due to issues of material compatibility, practitioners should consult the manufacturer of their HS prior to introducing any chemical agent, as this may otherwise invalidate their warranty. Depending on the nature of various germicidal agents and the various devices or systems provided with the HS, chemical treatment protocols could be used intermittently as a “shock” treatment (higher concentration) and/or continuously introduced into waterlines in small quantities. Although the disinfectants of HS are more efficacious at high concentrations, these levels are limited by the degree of risk to personnel, surfaces or equipment; overall, the continuously applied products performed better than those applied intermittently. This protocol requires having an independent reservoir system that can accommodate the solution of choice.

Infection risk for patients and dental team

Dental team (DT) and patients can be exposed to pathogenic microorganisms including cytomegalovirus, hepatitis B virus, hepatitis C virus, and herpes simplex virus. These pathogens can be transmitted in through direct contact with blood, oral fluids, or other patient materials; contact of conjunctival, nasal, or oral mucosa with droplets containing microorganisms generated from an infected person and propelled a short distance; inhalation of airborne microorganisms that can remain suspended in the air for long periods; and indirect contact with contaminated objects.

Due to the nature of their profession DT is at higher risk to acquire pathogenic microorganisms. DT risk of exposure is in line with the infectious nature of their patients, interventions or instruments that produce bio-aerosols. DT working in wards with patients suffering from pneumonia, who produce high virulence bio-aerosols, or DT exposed to bio-aerosol sources in dental

practices, is at higher risk for developing disease or allergies. According to a risk assessment study, conducted in a hospital with DT exposed to high-risk procedures, a risk ratio (RR) of 2.5 was found for acquiring viral or bacterial infection. Thus, the risk of acquiring pathogenic agents by HS may be a hazard to both healthy and immunosuppressed patients as well as to DT (5).

Legionella spp. are ubiquitous in HS, in fact surveys have shown that the percentage of samples taken at different dental sites that were positive for Legionella spp. was highly variable and ranged from 0 to 100% (6). The concentration of Legionella spp. in HS may reach 1000 organisms per ml (6). The primary route of Legionella spp. transmission is inhalation or aspiration of environmentally contaminated aerosols (7).

The presumed natural reservoirs for Legionella spp. are amoebae that Legionella cells can invade and in which they can replicate, in fact amoebae are the natural hosts of legionellae in the environment; the relationship between these organisms is unique in that, amoebae which generally use other bacteria as food, are parasitized by legionellae (8). Exposure to HS containing free-living amoebae may cause infections for pathogens such as Legionella; in fact freshwater amoebae have been detected in HS (8). The presence of Legionella in HS leads to the widespread creation of potentially pathogenic aerosols. Because the primary route of infection by Legionella pneumophila is inhalation, these aerosols could represent a serious health problem for both patients and DT, but there is no published evidence of clusters or outbreaks of legionellosis linked to dental care. Considering that DT experience daily cumulative exposure to aerosols created by HS and that there has been only one proven case of Legionnaires' disease caused by exposure to HS, the occupational risk due to exposure to legionellae appears to be very low. The infection risk category for immunocompetent healthcare workers ranges from 1 to 5 for low levels of Legionella pneumophila in HS. Annual reports of Legionella in HS, have described only a few cases in which dental treatment has been reported as the only risk factor for the disease, in addiction the 2015 Italian guidelines for the prevention of Legionnaires' disease included for the first time safety recommendations for dental surgeries (8).

Like Legionella pneumophila periodontal and peri-implantitis (9-15) pathogens may be present in HS biofilm and represent a risk of contamination. In fact peri-implantitis and periodontal disease spring from bacterial infection (16). So, every factor favouring oral biofilm formation (poor oral hygiene), host defence capability (smoking habit, excessive alcohol consumption, genetic traits, history of periodontitis, oral mucosal lesions and prosthetics) (17-26), might favour developing of peri-implantitis and periodontal disease, which pathogens may contaminate dental unit water-lines (26-33).

Conclusions

All dentists are required to conduct a statutory risk assessment of their HS, in fact to comply with their legal duties, employers must identify and assess the sources of risk and prepare a scheme for preventing and controlling risks. Moreover, they must monitor the quality of their HS at least annually to ensure that the HS are "legionellae free".

Appropriate procedures (Table 1) to decontaminate HS, including autoclaving and handpiece replacement between patients, have been developed and implemented in dental practices. These procedures are aimed at reducing the likelihood of dissemination of pathogens within HS, which can lead to infections. However, decontamination of handpieces (such as high-speed drills and syringes) does not eliminate potential exposures to pathogens that originate from the HS.

Various products have been developed to treat the water used in HS so as to reduce the number of bacteria delivered to the patient.

Because disinfectants for registration are tested *in vitro* and the construction characteristics of the HS vary widely, the field tests and disinfec-

Table 1 - Prevention of contamination of dental unit Hydraulic System (HS).

Indipendent water supply	Distilled or sterile water coming from independent tanks (sterilizable or disposable) can solve the problem of contamination of the dental unit but does not solve the problem of retrograde contamination.
Filters at the dispensing taps	0.22 micron filters at taps do not avoid retrograde contamination, compromise the efficiency of the dental unit, resulting in drips and loss of flow.
Flushing	Forced rinsing for few minutes (30 "-2 ') after each patient; forced rinsing at the beginning and at the end of the day; both procedures do not remove the biofilm.
UV Ray	They can reduce the contamination of water origin at the entrance but it is difficult to guarantee its effectiveness.
Electrolictic treatment of water	Biocidal oxidizing radicals such as chlorine derivatives, can be released into the water. However they have a corrosive action on water tubes of the dentist unit.
Continuous treatment with disinfectants	The use of a low concentration of disinfectant in the water is not harmful for patients or for operators but have modest effectiveness and select resistant microorganisms.
Discontinuous treatment with disinfectants	Use of medium or high concentration of disinfectants added to water between one patient and another at night or once a week overnight involves particular attention to the possible deterioration of the implant (valves, tubes, connections) of the dental unit. Different types of treatment have been tried: peracetic acid, sodium hypochlorite at different concentrations (1500-5000 ppm), chlorine dioxide, glutaraldehyde, hydrogen peroxide, phenol, povidone iodine, isopropanol, chlorhexidine, ethanol and chlorhexidine, phosphoric acid, ozone. Sperimentations were carried out on pilot dental unit with the objective of reducing the bacterial charge and reducing or eliminating the biofilm. The results are encouraging on the reduction of bacterial charge, but not on the elimination of the biofilm; the most important problem seems to be the maintenance of the bacterial reduction.In no case the experiments are sufficiently conclusive, although the use of peracetic acid seems to be among the most encouraging.
Antireflux valves	Prevent retro-aspiration but may contaminate and accumulate biofilms or incrustations that make them less efficient. They involve periodic maintenance and replacement.

tion protocols are tailored to the individual devices. It is not usual to find a disinfection protocol that is effective for all. The artificially contaminated simulation device made it possible to measure, under controlled conditions, the performance of the HS disinfection, in the absence of variables such as biofilms, flow intensity, other microbial species, temperature, and chemical characteristics of the water.

Germicidals are safe for patient contact, so they could be used as a preventive option, and may be useful in HS disinfection, alone or coupled with a daily or periodic shock treatment. Because previous studies have shown that aerobic heterotrophic bacteria are the predominant organisms present in HS, this review could offer a stimulus to monitoring the total heterotrophic bacteria contamination in HS.

References

1. Ma'ayeh SY, Al-Hiyasat AS, Hindiyeh MY, et al. Legionella pneumophila contamination of a dental unit water line system in a dental teaching centre. Int J Dent Hyg. 2008;6:48-55.
2. O'Donnell MJ, Boyle MA, Russell RJ, et al. Management of dental unit waterline biofilms in the 21st century. Future Microbiol. 2011;6:1209-1226.

3. Mungara J, Dilna NC, Joseph E, et al. Evaluation of microbial profile in dental unit waterlines and assessment of antimicrobial efficacy of two treating agents. J Clin Pediatr Dent. 2013;37:367-371.
4. Leoni E, Dallolio L, Stagni F, et al. Impact of a risk management plan on Legionella contamination of dental unit water. Int J Environ Res Public Health. 2015;12:2344-2358.
5. Dutil S, Veillette M, Meriaux A, et al. Aerosolization of mycobacteria and legionellae during dental treatment: low exposure despite dental unit contamination. Environ Microbiol. 2007;9:2836-2843.
6. Singh T, Coogan MM. Isolation of pathogenic Legionella species and legionella-laden amoebae in dental unit waterlines. J Hosp Infect. 2005;61:257-262.
7. Trabelsi H, Sellami A, Dendena F, et al. Free-living Amoebae (FLA): morphological and molecular identification of Acanthamoeba in dental unit water. Parasite. 2010;17:67-70.
8. Pankhurst CL, Coulter W, Philpott-Howard JJ, et al. Prevalence of legionella waterline contamination and Legionella pneumophila antibodies in general dental practitioners in London and rural Northern Ireland. Br Dent J. 2003;195:591-594; discussion 581.
9. Danza M, Paracchini L, Carinci F. Tridimensional finite element analysis to detect stress distribution in implants. Dental Cadmos. 2012;80:598-602.
10. Danza M, Grecchi F, Zollino I, et al. Spiral implants bearing full-arch rehabilitation: analysis of clinical outcome. J Oral Implantol
11. Danza M, Zollino I, Avantaggiato A, et al. Distance between implants has a potential impact of crestal bone resorption. Saudi Dental Journal. 2011;23:129-133.
12. Carinci F, Danza M. Clinical outcome of implants inserted in piezo split alveolar ridges: A pilot study. In: eds. Perspectives on Clinical Dentistry. 2011:29-30.
13. Danza M, Zollino I, Guidi R, et al. Computer planned implantology: Analysis of a case series. In: eds. Perspectives on Clinical Dentistry. 2011;287-300.
14. Danza M, Carinci F. Flapless surgery and immediately loaded implants: a retrospective comparison between implantation with and without computer-assisted planned surgical stent. Stomatologija. 2010;12:35-41.
15. Danza M, Quaranta A, Carinci F, et al. Biomechanical evaluation of dental implants in D1 and D4 bone by finite element analysis. Minerva Stomatol. 59:305-313.
16. Danza M, Riccardo G, Carinci F. Bone platform switching: a retrospective study on the slope of reverse conical neck. Quintessence Int. 2010;41:35-40.
17. Scapoli L, Girardi A, Palmieri A, et al. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents. 2015;29:101-110.
18. Lauritano D, Martinelli M, Mucchi D, et al. Bacterial load of periodontal pathogens among Italian patients with chronic periodontitis: A comparative study of three different areas. Journal of Biological Regulators and Homeostatic Agents. 2016;30:149-154.
19. Lauritano D, Muzio LLO, Gaudio RM, et al. The ecological catastrophe of oral diseases: A possible link between periodontitis and protozoa. Journal of Biological Regulators and Homeostatic Agents. 2016;30:143-147.
20. Lauritano D, Muzio LL, Gaudio RM, et al. Why should patients with systemic disease and tobacco smokers go to the dentist? Journal of Biological Regulators and Homeostatic Agents. 2016;30:135-141.
21. Lauritano D, Scapoli L, Mucchi D, et al. Infectogenomics: Lack of association between vdr, il6, il10 polymorphisms and "red Complex" bacterial load in a group of Italian adults with chronic periodontal disease. Journal of Biological Regulators and Homeostatic Agents. 2016;30:155-160.
22. Lauritano D, Pazzi D, Iapichino A, et al. Evaluation of the efficacy of a new oral gel containing carvacrol and thymol for home oral care in the management of chronic periodontitis using PCR analysis: a microbiological pilot study. J Biol Regul Homeost Agents. 2016;30:129-134.
23. Lauritano D, Bignozzi CA, Pazzi D, et al. Evaluation of the efficacy of a new oral gel as an adjunct to home oral hygiene in the management of chronic periodontitis. A microbiological study using PCR analysis. Journal of Biological Regulators and Homeostatic Agents. 2016;30:123-128.
24. Lauritano D, Cura F, Candotto V, et al. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents. 2015;29:127-130.
25. Lauritano D, Cura F, Gaudio RM, et al. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents. 2015;29:131-135.
26. Lauritano D, Bignozzi CA, Pazzi D, et al. Efficacy of a new coating of implantabutment connections in reducing bacterial loading: An in vitro study. ORAL and Implantology. 2017;10:1-10.
27. Lopez MA, Andreasi Bassi M, Confalone L, et al. Clinical outcome of 215 transmucosal implants with a conical connection: a retrospective study after 5-year follow-up. J Biol Regul Homeost Agents. 2016;30:55-60.
28. Andreasi Bassi M, Lopez MA, Confalone L, et al. Clinical outcome of a two-piece implant system with an internal hexagonal connection: a prospective study. J Biol Regul Homeost Agents. 2016;30:7-12.
29. Andreasi Bassi M, Serra S, Andrisani C, et al. Polymerization shrinkage and spherical glass mega fillers: Effects on cuspal deflection. ORAL and Implantology. 2016;9:71-79.

30. Lopez MA, Andreasi Bassi M, Confalone L, et al. Retrospective study on bone-level and soft-tissue-level cylindrical implants. J Biol Regul Homeost Agents. 2016;30:43-48.
31. Lopez MA, Andreasi Bassi M, Confalone L, et al. The influence of conical plus octagonal internal connection on implant survival and success rate: a retrospective study of 66 fixtures. J Biol Regul Homeost Agents. 2016;30:49-54.
32. Andreasi Bassi M, Andrisani C, Lopez MA, et al. Endoscopically controlled hydraulic sinus lift in combination with rotary instruments: one-year follow-up of a case series. J Biol Regul Homeost Agents. 2016;30 (S2):21-28.
33. Carinci F, Lauritano D, Cura F, et al. Prevention of bacterial leakage at implant-Abutment connection level: An in vitro study of the efficacy of three different implant systems. Journal of Biological Regulators and Homeostatic Agents. 2016;30:69-73.

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