

SI STAMPI 5-2018

N 2862

Presence of *Legionella* spp. in human dental plaque

Presenza di *Legionella* spp. nella placca dentaria umana

Running title: Legionella and dental plaque

M. Tesauro¹, F. Petrelli², A.Lizioli¹, F. Pregliasco³, C. Masia¹, G. Cossellu¹, G. Farronato¹, M. Consonni¹, F. Sisto¹

1. Department of Biomedical Surgical and Dental Sciences, University of Milan, Italy

2. School of Pharmacy, University of Camerino, Italy

3. Department of Biomedical Sciences for Health, University of Milan, Italy

Key words: Dental plaque, *Legionella*, dental unit waterline

Parole chiave: Placca dentaria, *Legionella*, impianto idrico riunito odontoiatrico

Corresponding author: Marina Tesauro, Department of Biomedical Surgical and Dental Sciences, Environmental Hygiene Lab, University of Milan, Via C. Pascal 36, 20133 Milan, Italy

e-mail: marina.tesauro@unimi.it

Phone number: +39 02 50315110

Abstract

Aims: The aim of this research is to verify the presence of *Legionella* in human dental plaque.

Methods: 65 adult patients not treated with systemic or local antibiotics at least 2 months before the time of sample collection were enrolled for plaque collection between September 2015 and December 2016. A brief questionnaire about lifestyle and health risks was administered. *Legionella* spp. detection has been executed by semi-nested PCR.

Results: 8 out of 65 plaque samples (12.3%) were positive for *Legionella* spp. As regards health risks and lifestyle aspects, no relevant difference was observed between patients involved in our study, except for two positive patients who have reported a COPD ongoing and a pneumonia in the past.

Conclusions: This study represents a step forward in the knowledge of reservoirs of the microorganism and richness of oral microbiota.

Introduction

Legionnaires' disease is a sporadic respiratory infection with low notification rates (overall 1.4 per 100 000 inhabitants) in EU/EEA countries in 2014. Five countries (France, Germany, Italy, Portugal and Spain) accounted for 74% of notified cases (1).

1710 cases were reported by the epidemiological surveillance system in Italy in 2016 (2.8 per 100 000 inhabitants). All patients were interviewed on potential exposure to water and/or aerosols including dental treatment 10-15 days before the onset of symptoms. Most of the patients reported that they spent at least one night in different places than their own house (n=177), were hospitalized (n=86) or lived in nursing homes for the elderly (n=35), lived in prisons or attended swimming pools (n=28) and 22 cases received dental care (2).

In Milan (Italy), 485 cases (334 male/151 female) of legionellosis were reported from 2010 to 2014 (unpublished data); for 177 out of 485 cases (36%), environmental and microbiological investigations were carried out in houses or other places normally frequented by the patients. Only 23 (37%) of the 62 samples from the household water systems were found positive for *Legionella pneumophila* (most of them belonged to a different serogroup than the respective cases); instead the samples of water collected from the dental units, where 9 patients received dental treatments 15 days before the onset of the symptoms, were negative for *Legionella pneumophila*. These results could be mainly due to the low sensitivity of the method or the long time between the exposures and the environmental microbiological investigations or a possible reservoir of the microorganism not investigated yet.

The purpose of this work is to verify the presence of *Legionella* in dental plaque, a biofilm present on our teeth, which is potentially able to act as a reservoir of *Legionella*.

Materials and Methods

The study was carried out on patients attending the Maxillofacial Surgery Unit (U.O.C. Chirurgia Maxillo-Facciale ed Odontostomatologica, Fondazione IRCCS Ca' Granda) at Maggiore Policlinico hospital in Milan (Italy) between September 2015 and December 2016.

As inclusion criterion, only patients not treated with systemic or local antibiotics at least 2 months before the time of sample collection were considered.

Supragingival plaque was sampled, after aspiration of saliva, by a single qualified operator from the mandibular molars using a sterile device, then transferred into tubes containing Tris EDTA solution (10mM TrisHCl pH 8,0 1mM EDTA) and immediately stored at -20°C.

All patients were interviewed on their lifestyle (e.g. smoking/number of cigarettes per day), socio-demographic status, oral hygiene habits, water consumption (bottled or tap water), lung/respiratory disease and use of any kind of drugs. Moreover, the presence of metal prosthesis in the mouths of patients was also reported during the medical visits since the metals might affect the presence of *Legionella* in the water environments.

Considering the number and variability of oral bacterial flora, we decided to detect *Legionella* spp., at the genus level. A semi-nested polymerase chain reaction (PCR) was performed according to the method reported by Huang et al. (3), and an amplicon of 421 bp was obtained. In addition, a restriction enzyme analysis (REA) with *BsuRI* enzyme was performed in order to verify the specificity of the amplicon and the expected bands (293 and 128 bp) were obtained.

This study was conducted according to the Good Clinical Practice guidelines and approved by the Human Ethic Committees of the University of Milan (June 3rd, 2015).

Results

65 consecutive patients (38 male/27 female, age range: 27-63 years, median age: 42 years) were enrolled in this study.

8 out of 65 plaque samples analyzed (12.3%), were positive for *Legionella* spp. (Table 1), one patient had a pneumonia in the past and another one suffers from chronic obstructive pulmonary disease (COPD). All these subjects drunk water and brushed their teeth 150 and 220 minutes before sampling, respectively. All the 65 patients used bottled water and only 4 drunk also tap and treated water. Most of the positive (7/8) and part of the negative (40/57) patients for *Legionella* spp. had no metal prosthesis. Moreover, no patient in both groups reported an infectious respiratory diseases at the moment of the sample collection.

As regards the smoking habit, the positive patients are equally distributed (4:4) with a median of 3.5 cigarettes smoked per day. Among the 57 negative patients, 37 (64.9%) were cigarette

smokers (from 2 to 20 cigarettes, median 10). Some of them (n=3) also reported use of cigars and electronic cigarettes (Table 1).

Discussion

The human oral cavity is colonized by a complex microbial community and includes several hundred different species. In the past decades, studies about the composition of the oral microbiota have been performed, especially on healthy adults or on metagenomic sequencing of microorganisms involved in gingivitis and periodontitis (4), but not well defined knowledge on the composition and the richness in species have been evidenced. It is shared opinion that oral microorganisms are in planktonic phase or organized as biofilms/dental plaque (5, 6).

The Legionnaires' disease is a serious problem of public health. Studies on pathogenic mechanisms and way to prevent infections are continuously performed. National and international studies are widely focused on the relationship between dental practice and *Legionella* infections without reaching a final shared opinion (7-9).

Our study highlights the presence of *Legionella* spp. in dental plaque in 12.3% of patients (8/65), with similar oral hygiene habits and risk factors except for sex, presence of past pneumonia and pulmonary chronic illnesses, which are known risk factors for legionellosis and probably influence the immune status of positive patients.

Other studies evidenced the possibility of oropharyngeal colonization by *Legionella* spp., mainly in the immunocompromised and cardiac patients (10), but to the best of our knowledge, this is the first study reporting the presence of *Legionella* spp. in human dental plaque of healthy subjects.

Further investigations are needed in order to evaluate if dental plaque could be confirmed as a new reservoir of the microorganisms. Moreover, future research studies focused on the identification of *Legionella* spp. at species level, the viability of the microorganisms in the dental plaque and the possible transmission of *Legionella pneumophila* from positive plaque patients through environmental monitoring, could clarify the possible role of *Legionella* in the dental plaque on public health aspects.

A special thank to Dr Ettore Amato for his help with the English language revision of the manuscript.

References

1. European Centre for Disease Prevention and Control (ECDC). Legionnaires' disease - Annual Epidemiological Report 2016 [2014 data]. Available from: <https://ecdc.europa.eu/en/publications-data/legionnaires-disease-annual-epidemiological-report-2016-2014-data> [Last accessed 2018 Jan 16].
2. Rota MC, Caporali MG, Bella A et al. Rapporto annuale sulle legionellosi in Italia nel 2016. Not Ist Super Sanita 2017; **30**(9): 3-8.
3. Huang SW, Hsu BM, Chen NH, et al. Isolation and identification of *Legionella* and their host amoeba from weak alkaline carbonate spring water using a culture method combined with PCR. Parasitol Res 2011; **109**: 1233-41.
4. Xie G, Chain PS, Lo CC, et al. Community and gene composition of a human dental plaque microbiota obtained by metagenomic sequencing. Mol Oral Microbiol 2010; **25**(6): 391-405.
5. Arweiler NB, Netuschil L. The Oral Microbiota. Adv Exp Med Biol. 2016; **902**: 45-60.
6. Wade W, Thompson H, Rybalka A, Vartoukian S. Uncultured Members of the Oral Microbiome. J Calif Dent Assoc 2016; **44**(7): 447-56,
7. Petti S. Did a patient acquire *Legionella pneumophila* from the cup filler of a dental unit or did a patient infected with *L.pneumophila* contaminate the cup filler? J Hosp Infect 2017; **96**: 201-2.
8. Pasquarella C, Veronesi L, Napoli C, et al. SItI Working Group Hygiene in Dentistry. Microbial environmental contamination in Italian dental clinics: A multicenter study yielding recommendations for standardized sampling methods and threshold values. Sci Total Environ 2012; **420**: 289-99.
9. Tesauro M, Bollani M, Cesaria M, et al. Analisi delle problematiche sanitarie delle attività odontoiatriche monospecialistiche (AOM): studio pilota nel territorio milanese. Dent Cadmos 2015; **83**(3): 176-86.
10. Jaresova M, Hlozaneck I, Striz I et al. *Legionella* detection in oropharyngeal aspirates of transplant patients prior to surgery. Eur J Clin Microbiol Infect Dis 2006; **25**: 63-4.

Conflicts of interest: none

Funding: None

Competing interests: None declared

Ethical approval: the research has been approved by the Human Ethic Committees of the University of Milan (3 June 2015)