ORIGINAL ARTICLE

Environmental microbial contamination in dental setting: a local experience

M. GUIDA, F. GALLÉ[*], V. DI ONOFRIO[*], R.A. NASTRO, M. BATTISTA[**], R. LIGUORI[*], F. BATTISTA[**], G. LIGUORI[*]
Department of Structural and Functional Biology, University of Naples "Federico II"; [*] Chair of Hygiene and Epidemiology, Department of Studies of Institutions and Territorial Systems, University of Naples "Parthenope"; [**] Complex Operating Unit of Odontostomatology and Maxillo-Facial Surgery, Pellegrini Hospital, Naples, Italy

Key words

Microbial contamination • Dental clinic • Environmental microbiological monitoring

Summary

Introduction. *Patients and operators are exposed during dental practice to an infective risk, which derives especially from microorganisms suspended in aerosols. Environmental microbiological monitoring in dental settings represents a good instrument to detect critical situations.*
Methods. *In order to investigate environmental microbial contamination level in a local reality, we analyzed water, air and surfaces samples of a community-based dental facility by using protocol and threshold values proposed in a recent multicenter study carried out by the Italian Society of Hygiene, Preventive Medicine and Public Health (S.It.I.) working group "Hygiene in Dentistry". Microbial contamination was assessed in the same room for 4 non-consecutive weeks during all the five working days, before and at the end of the daily activity. Air was sampled also during clinical activity, through both active and passive sampling systems.*
Results. *Contamination of water showed a decrease during activities, while a decrease in air contamination was registered only at the end of the day. Passive sampling values resulted more often above threshold values adopted. At the same time, surfaces contamination increases at the end of the activity. It seems that in the dental clinic analyzed microbial buildup represents the higher critical element. No differences have been registered among the different days of the week.*
Discussion. *Our study highlights the need to improve disinfection procedures and air treatment systems in the considered environment. Microbiological monitoring could represent an important element to detect the presence of risk factors and to adopt control measures.*

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Introduction

Patients and staff of dental clinics are frequently at risk for infections. They are regularly exposed to water from aerosols generated during work, therefore the quality of dental unit water is of great importance, and Dental Unit Water Line (DUWL) contamination has become alarming [1]. The presence of opportunistic and pathogenic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Legionella pneumophila* in dental units water has been reported in several studies [2-7].
The presence of bacterial biofilms on the inside of DUWLs has been well documented and recognized as an undisputed source of contamination for water used in patient dental treatment [8, 9]. The risk of acquiring infections through Dental Unit Water Systems (DUWS) supplies is known to be not very uncommon. Often potential pathogenesis can spread through surgical procedures, local mucosal contact, ingestion and inhalation [10].
In particular, aerosols containing biological material (saliva, blood, and dental plaque) and microorganisms are produced by high-velocity rotating instruments and air-water sprays or ultrasonic scalers [11, 12]. Splatter and aerosols from dental procedures may infect health care personnel [13].
Smaller particles (<5 μm) can float in the air and have the potential to penetrate in the small passages of the lungs, while larger ones settle easily onto environmental surfaces which can become contaminated during patient care [12, 14-18].
Certain surfaces, especially clinical contact surfaces that are frequently touched (e.g. dental unit switches, light handles, drawer knobs), can act as reservoirs of microorganisms. When these surfaces are touched, microorganisms can be transferred to instruments, other environmental surfaces, or to the nose, mouth or eyes of healthcare workers or patients [19].
Despite everything, data on microbial contamination in the dental clinic environment are not exhaustive [11, 12, 15, 16, 18-26].
Moreover, while the evaluation of water contamination is based on generally accepted and standardized sampling and processing protocols and well defined threshold values [27, 28], methods for air and surface sampling are still debated [28, 29] and no recommended levels of contamination for dental clinics have been established.
Since its founding, the working group "Hygiene in Dentistry" of the Italian Society of Hygiene, Preventive Medicine and Public Health (S.It.I.) analyzes the aspects

of microbial environmental contamination in dental clinics [30-33].
Recently, in order to establish a benchmark model for dental units microbial contamination, and considering the critical issues that have been observed in a previous pilot study, the working group carried out a wider multicentre study to assess water, air and surfaces microbial contamination using a standardized protocol [34, 35].
In this study, we analyzed the water, air and surfaces microbial contamination level of a community-based dental facility in the Hospital "Vecchio Pellegrini" of Naples by applying that protocol and by using threshold values proposed in the multicenter study.
The purposes of this study were to provide an accurate description of environmental microbial contamination in a local reality, and, secondly, to evaluate the results obtained on the basis of the benchmark proposed in the previous study.

Materials and methods

The study was carried out during the spring 2012. The environment analyzed was used for conservative and surgical practices. In order to evaluate weekly and daily modifications, microbial contamination of water, air and surfaces was assessed in the same room during the five working days of the week, before (T_0) and at the end of the daily activity (T_2), immediately after the last patient. In addition, air was sampled also during (T_1) clinical activity. Sampling was repeated for 4 non-consecutive weeks.

Evaluation of microbial water contamination

Water from the tap and from dental unit water systems (DUWS) was sampled to assess the Total Viable Count (TVC) at 36°C and 22°C, and the presence of *Legionella* spp. and *P. aeruginosa*. For DUWS, the TVC was measured on every handpiece (1 ml of water from cup filler, air-water syringe, turbine, microengine, ablator), while *Legionella* spp. and *P. aeruginosa* were measured from a single sample made up collecting water from all the five handpieces (200 mL from each handpiece for *Legionella* spp. and 50 mL from each handpiece for *P. aeruginosa*). All samples were collected in sterile bottles and immediately transported in a cool box (4-8°C) to the laboratory.
The Italian legislation n. 31 of February 2, 2001, based on the European Council Directive 98/83/EC on the quality of water intended for human consumption (1998), was used as the reference for TVC and *P. aeruginosa* detection [27, 36]. Legionella identification was performed according to the Italian guidelines for the prevention and control of legionellosis [37].
Water samples were processed as previously reported [34]. For TVC, Plate Count Agar plates were incubated at 22°C for 72 h and at 36°C for 48 h. Median values were compared with the threshold values established by both the European Council Directive 98/83/EC (20 cfu/mL at 36°C and 100 cfu/mL at 22°C) and CDC Guidelines (500 cfu/mL) [27, 28].
As for *P. aeruginosa* detection, Cetrimide agar incubated at 37°C for 48 h and API 20 NE Galleries (Bio Mèrieux Srl, Charbonnières-les-Bains, France) for biochemical confirmation were used. Absence of bacteria in 250 mL of sampled water was considered as threshold value [27].
To determine the presence of *Legionella* spp., we used glycine–vancomycin–polymyxin–cyclohexamide (GVPC) agar medium. Suspect colonies were subcultured on charcoal-yeast-extract (CYE) agar and buffered-charcoal-yeast-extract (BCYE) agar. Serological identification was performed by latex test, and by a monoclonal antibodies trial (DenkaSeiken Co. LTD, Tokyo, Japan). Regarding the presence of *Legionella* spp., a threshold value of 1,000 cfu/L was used [37] (Tab. I).

Evaluation of microbial air contamination

Microbial air contamination was evaluated through both active and passive sampling [38]. Active sampling was performed using the Surface Air System (SAS) sampler (International PBI, Milan, Italy), with a flow rate of 180 liters per minute (L/min) and a suction volume of 500 L.

Tab. I. References adopted for contamination threshold values.

	Water				Air		Surfaces	
Reference	TVC 22°C cfu/mL	TVC 36°C cfu/mL	*Pseudomonas aeruginosa* (cfu/250 mL)	*Legionella* spp. (cfu/L)	Active Sampling (cfu/m³)	Passive Sampling (cfu/plate 9cm∅/h)	Countertop	Dental unit switches
European Council Directive	100	20	0					
CDC Guidelines	500	500						
Italian Guidelines for the prevention and control of legionellosis				1,000				
Pasquarella et al., Sci Tot Environ 2012					Target 166 Alert 278	Target 27.5 Alert 43.7	0.64	0.63

The number of colony forming units was adjusted using the conversion table provided by the manufacturer and was expressed in colony forming units per cubic meter (cfu/m^3).
Passive sampling was performed to determine the number of cfu grown on a Petri dish of a 9 cm diameter placed for 1 hour on a surface (Index of Microbial Air contamination, IMA) [29].
Both samplers were placed in the monitored room about 1 m above the floor and about 1 m away from dental unit, walls and any other obstacle.
Total Viable Count (TVC) was performed using Tryptone Soya Agar plates incubated at 36°C for 48 h.
Presence of *Legionella* spp. was evaluated only through active sampling, using RODAC plates containing glycine–vancomycin–polymyxin–cyclohexamide (GVPC) agar.
Target and alert values proposed by Pasquarella et al. have been used as benchmark to evaluate the contamination level of the air (Tab. I) [35].

Evaluation of surface microbial contamination

Microbial contamination of the countertop serving the monitored dental unit and that of dental unit switches surfaces were evaluated. A RODAC plate, 55 mm in diameter, was pressed on the surface to be tested, and then incubated at 36°C for 48 h. The results were expressed as cfu/cm^2.
Threshold values reported by Pasquarella et al. have been used to evaluate the contamination level of the surfaces (Tab. I) [35].

Statistical analysis

Descriptive statistical analysis was performed to provide median, minimum-maximum values range, and mean and standard deviation.
Parametric or nonparametric tests were applied on the basis of data distribution.
The Wilcoxon signed-rank test was used to analyze differences in microbial water contamination median values between T_0 and T_2, and those in microbial surface contamination median values between T_0 and T_2. Differences in microbial air contamination mean values between T_0 and T_1, and between T_1 and T_2 were evaluated by Student's t-test for paired samples. Differences among the microbial contamination recorded on different days of the week were evaluated by Friedman two-way Analysis of Variance by Ranks; possible differences among the results from Monday and Friday (the first and the last day of the weekly activity respectively) were also analyzed by using the Wilcoxon signed-rank test. Possible interference between the presence of *P. aeruginosa* and *Legionella* spp. was tested by one-tailed Fisher's exact test. A p value below 0.05 was considered statistically significant.

Results

Median values and ranges, together with mean and standard deviation for water contamination results are reported in Tables II and III. The number and percentage of tap and DUWS water samples above the threshold values were also reported.
As for tap water TVC, both median values at 36°C and at 22°C decreased from T_0 to T_2, but the reduction was statistically significant only for mesophilic counts ($p = 0.005$). This is confirmed by the decreasing number of samples which exceeded the limits established by the European Council Directive, registered only for those kept at 36°C (from 50% at T_0 to 30% at T_2). Both mesophilic and psycrophilic contamination of water samples from DUWS showed a significant decrease ($p = 0.00$) from the start (T_0) to the end (T_2) of clinical practice. In total, *P. aeruginosa* was detected in 5 (12.5%) tap water samples and in 20 (50%) samples from DUWS, while *Legionella* spp. exceeded the limit value in 8 (20%) tap water samples and in 6 (15%) samples collected from DUWLs (data not shown). No statistically significant differences were found in *P. aeruginosa* contamination of both tap and DUWLs water ($p = 0.27$ and $p = 0.16$ respectively) collected before and at the end of the dental practice. The same results were registered for *Legionella* spp. contamination ($p = 0.71$ and $p = 0.10$ respectively). With one only exception, contamination by *P. aeruginosa* or *Legionella* spp. registered before the start of the activities at one sampling point was confirmed at the same

Tab. II. Microbial contamination values of water samples from tap water before (T_0) and after (T_2) clinical practice.

	T_0				T_2			
	TVC 22°C cfu/mL	TVC 36°C cfu/mL	*Legionella* cfu/L	*Pseudomonas* cfu/250mL	TVC 22°C cfu/mL	TVC 36°C cfu/mL	*Legionella* cfu/L	*Pseudomonas* cfu/250mL
Median	16.50	32.50	0	0	6	3.50	0	0
Range	0-9,000	0-39,000	0-3,700	0-8	0-300,000	0-300,000	0-5,300	0-6
Mean (SD)	1,140 (2,536.8)	2,937.5 (8,818.5)	420 (961.68)	0.6 (1.8)	16,118.1 (66,861.6)	15,108.75 (67,056.8)	540 (1,327.6)	0.45 (1.4)
N. (%) of samples above threshold value*°	5 (25)	10 (50)	4 (20)	3 (15)	5 (25)	6 (30)	4 (20)	2 (10)

* Council Directive 98/83/EC 3 November 1998 on the quality of water intended for human consumption.
° Guidelines for the prevention and control of Legionellosis, 2000.

Tab. III. Microbial contamination values of water samples from dental unit water system before (T_0) and after (T_2) clinical practice.

	T_0				T_2			
	TVC 22°C cfu/mL	TVC 36°C cfu/mL	*Legionella* cfu/L	*Pseudomonas* cfu/250mL	TVC 22°C cfu/mL	TVC 36°C cfu/mL	*Legionella* cfu/L	*Pseudomonas* cfu/250mL
Median	61,000	23,500	0	1	9,000	5,000	0	0.5
Range	0-360,000	0-300,000	0-2,200	0-102	0-300,000	0-300,000	0-3,100	0-110
Mean (SD)	117,863.8 (1.2)	99,487.4 (121,762.3)	270 (675.9)	19 (34.6)	68,456.1 (1.1)	53,967.3 (98,868.4)	420 (1,032.4)	9.3 (24.3)
N. (%) of samples above threshold value*□°	88 (88) 87 (87)	93 (93) 83 (83)	3 (15)	10 (50)	78 (78) 72 (72)	86 (86) 68 (68)	3 (15)	10 (50)

* Council Directive 98/83/EC 3 November 1998 on the quality of water intended for human consumption.

□ CDC Guidelines for Environmental Infection Control in Health-Care Facilities, 2003.

° Guidelines for the prevention and control of Legionellosis, 2000.

Tab. IV. Prevalence of *Legionella* spp. and *Pseudomonas aeruginosa* in samples from dental unit water systems.

	Pseudomonas aeruginosa		
Legionella spp.	Negative n. (%)	Positive n. (%)	Total n. (%)
Negative	20 (50)	14 (35)	34 (85)
Positive	0 (0)	6 (15)	6 (15)
Total n. (%)	20 (50)	20 (50)	40

One-tailed Fisher's exact test $p = 0.01$

point at the end of the work. Table IV shows the relation between *Legionella* spp. and *P. aeruginosa* found in the DUWS. *Legionella* spp. was found more frequently when *P. aeruginosa* was present ($p = 0.01$). Six samples (15%) showed both *Legionella* spp. and *P. aeruginosa* presence.

Regarding air samples, Table V shows as median values of microbial contamination decreased only at the end of the clinical practice (T_2), without notable variation between T_0 and T_1. The reduction was statistically significant only for passive sampling (33.5 to 15 cfu/plate, p=0.001) and not for active sampling (78 to 55.5 cfu/m^3, p = 0.736). This is also confirmed by the number of samples above limit values adopted; only in a few cases active sampling gave samples out of the benchmarks. *Legionella* spp. was never detected.

Microbial surface contamination showed an increase between T_0 and T_2 both for countertops and dental unit switches, but this variation was not statistically significant (p = 0.611 and p = 0.084 respectively) (Tab. VI). Samples from dental unit switches exceeded threshold values more often at the end than at the start of the activities.

Neither water samples, nor air and surfaces samples, showed statistically significant differences in microbial contamination levels among the different days of the week. The comparison among results obtained on Mondays and Fridays did not give any significant difference, so for water, as for air and surfaces (data not shown).

Discussion

This study has been realized on the basis of the previous experiences of the SItI Working Group "Hygiene in Dentistry". In particular, monitoring procedures and limit values are the same adopted in the last multicenter studies carried out in six dental clinics of Italy [34, 35]. The results of this study support the importance of analyzing the environmental microbial contamination in dental settings, in order to control infective risks for patients and staff.

As previously reported [30, 34, 35], contamination of water from taps as for DUWS, which is probably consequent to night stagnation, showed a decrease during clinical activity. Contamination by *P. aeruginosa* was registered in a high number of samples (50%) from DUWS, while the contamination of tap water was always lower. In contrast to what registered in previous experiences, the presence of *Legionella* spp. was not favored by the lack of *P. aeruginosa*. On the contrary, they were both present in the 15% of the samples, and this probably demonstrates the presence of risk factors which could favor the development of critical situations.

The air contamination did not show increases during activity, while a decrease was registered at the end of the day, both for active and passive sampling. This could be explained with the presence of other factors independent by clinical activity, which can influence the quality of the air. Probably the status of air conditioning systems, which were often turned off at the end of the daily activity, could have an important role in determining these results.

Tab. V. Microbial air contamination values assessed by active sampling (cfu/m³) and passive sampling (IMA) before (T_0), during (T_1) and after (T_2) clinical practice.

	Active sampling (cfu/m³)			Passive sampling (cfu/plate 9cm∅/h)		
	T_0	T_1	T_2	T_0	T_1	T_2
Median	75	78	55.5	36	33.5	15
Range	14-190	9-163	17-246	10-101	9-141	7-45
Mean (SD)	86.6 (50.6)	82.3 (48.3)	86.5 (64.8)	38.3 (21)	39.6 (28.2)	20 (11.5)
N. (%) of samples above target value*	2 (10)	0	3 (15)	13 (65)	13 (65)	5 (25)
N. (%) of samples above alert value*	0	0	0	7 (35)	6 (30)	1 (5)

* Benchmark proposed for dental clinics by Pasquarella et al. [35].

Tab. VI. Microbial contamination values on countertop and switches before (T_0) and after (T_2) clinical practice.

	Countertop (cfu/cm²)		Dental unit switches (cfu/cm²)	
	T_0	T_2	T_0	T_2
Median	3,000	4,200	4,000	7,600
Range	0.08-2.44	0.04-2.16	0.04-2.64	0.12-10.40
Mean (SD)	0.47 (0.52)	0.57 (0.48)	0.61 (0.67)	1.5 (2.3)
N. (%) of samples above threshold value*	5 (25)	4 (20)	4 (20)	11 (55)

* Benchmark proposed for dental clinics by Pasquarella et al. [35].

IMA values resulted more often above both target and alert values proposed by Pasquarella et al. [35]. Since passive sampling has the role to evidence risks deriving from particles fallout, while active sampling has to reveal those deriving from aerosols diffusion [39], it seems that in the analyzed dental clinic settled particles could represent the higher critical element.

This was also demonstrated by surfaces biocontamination, which increases at the end of the activity. This surely represents the effect of working activities, but probably it could also derive from the fallout of airborne particles. Here, as in other experiences of our Working Group, Rodac plates represented the best choice among the surface sampling systems, both for accuracy and user-friendliness [34, 35, 40].

Surprisingly, no differences between the contamination levels at the start (on Monday) and at the end (on Friday) of weekly clinical activity have been registered. Evidently, facility closing along the weekend did not increase the effects of water stagnation and microbial particles fallout insomuch as the levels of microbial contamination show a significant modification. Regarding air contamination, the switching off of malfunctioning air conditioners, as above suggested, could have contributed to this result.

Conclusions

In conclusion, data reported highlight the need to improve disinfection procedures and air treatment systems in the considered environment, so during as at the end of the activities. Furthermore, our study underlines that microbial monitoring could represent an important element to detect alert values which indicate the presence of risk factors and require the adoption of control measures.

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■ Correspondence: Giorgio Liguori, Chair of Hygiene and Epidemiology, Department of Studies of Institutions and Territorial Systems, University of Naples "Parthenope", via Medina 40, 80133 Naples, Italy - Tel./Fax + 39 081 5474790 - E-mail: giorgio.liguori@uniparthenope.it