

## MICROBIAL CONTAMINATION IN DENTAL UNIT WATERLINES: COMPARISON BETWEEN ER:YAG LASER AND TURBINE LINES

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**Abstract:** The investigation was carried out by evaluating the microbiological characteristics of the water before and after treatment with Er:YAG laser and turbine. The study was carried out in 2 dental surgeries. In both cases the laser and dental units were served by two independent circuits, fed by the same potable tap water. Samples were taken from the water supplying and the water leaving the turbine and laser before and after treatment on the same patient. Total heterotrophic plate count was measured at 36°C and at 22°C, and the presence of *Staphylococcus* species and non-fermenting Gram negative bacteria was investigated. Bacterial contamination was found within the circuit, especially in the laser device. *Pseudomonas aeruginosa* was detected in only 1 sample of supply water, in 11.1% and in 19.4% of the samples from the turbine and the laser respectively. No evidence of *Staphylococcus aureus* was found. The contamination of supply water was low, whereas that of the water leaving the handpieces of the 2 devices was high, especially in the laser. Attention should be paid to the control of the water leaving laser devices, given the increasingly wide use of such instruments in dental treatment exposed to risk of infection.

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### INTRODUCTION

The water leaving the waterlines of dental units is frequently contaminated by pathogenic and opportunist microorganisms [18, 22, 23, 25, 26, 28, 31]. These microorganisms have two main sources: from the patients by suck-back or from the incoming potable tap water or deionized water [19]; the presence of biofilm attached to the inner surfaces of the tubes enhances the survival and reproduction of the bacteria [29]. The biofilm is made up of a polymeric matrix containing bacteria, fungi and

protozoa, and it is particularly difficult to remove [16, 27]. Mechanical flushing at the beginning of each day brings about only a temporary removal of bacteria suspended in the water [3, 11]. The intermittent or continuous application of disinfectant products generally allows the microbial flora to be controlled, but not definitively eliminated [20, 24, 32]. It is now universally accepted, therefore, that dental unit water represents a potential vehicle of infection. The risk is not only for the staff, but more especially for medically compromised and immunocompromised patients who are undergoing dental

treatment and can become infected by swallowing or inhaling contaminated aerosol from the spray produced by the classic high speed rotating systems used for dental burrs, air/water pistols, turbines, etc. or through solutions of continuity of the oral mucosa [10].

In recent years, the scientific dentistry community has shown increasing interest in the use of laser instruments (Light Amplification by Stimulated Emission of Radiation) for the treatment of certain pathologies involving the oral mucosa and hard material (filling removal, enamel whitening, maintenance of root aseptis etc.) [6, 7, 9, 12, 13, 21]. In the endodontic field irradiation with Nd:YAG laser (1.5 watt; 15 Hz) has proved effective in blocking the growth of staphylococci and streptococci in root canals, producing results actually superior to those obtained with sodium hypochlorite irrigation [4]. The Er:YAG laser is normally used to remove dental caries. During laser treatment the temperatures reached are high, making it necessary to cool the devices with a stream of water that, if contaminated, can represent a risk for staff and patients alike.

The present study aimed to assess the level of contamination in the water leaving laser devices and turbines. The investigation was carried out by evaluating the microbiological characteristics of the water before and after treatment with both types of mechanism in order to highlight any variations that might occur after the use of the devices and in relation to the phenomenon of backflow.

## MATERIALS AND METHODS

The study was carried out on two units situated in two different private dental offices in the Emilia-Romagna region of Italy. None of the units had been treated to remove biofilm or reduce planktonic bacterial contamination either before or during the study.

In both surgeries the laser and dental unit were served by two independent circuits, but were fed by the same potable tap water. The model of laser used in the study has no internal water reservoir.

The type of laser used was the Er:YAG laser (wavelength = 2940 nm with articulated arm) produced by Fotona (Lubiana), the conditions of application were: energy = 250 mJ, frequency = 10-15 Hz. During the collection of water samples the protocol for the treatment of caries was applied.

After flushing with 300 ml of water, water samples (100 ml) were taken on Mondays at the start of the working week. Samples were taken from:

- the water supplying the two circuits;
- the water leaving the turbine before and after treatment on the first patient of the day;
- the water leaving the laser before and after treatment on the same patient of the day.

The samples, with the addition of 0.1 ml of a filter sterilized 10% solution of sodium thiosulphate to

neutralize any residual chlorine, were kept at a temperature of 4°C and were analyzed within 12 h.

Over a period of approximately 18 months, 38 sampling sessions were made producing a total of 190 samples.

For each sample the following bacteriological parameters were measured: total heterotrophic plate count at 36°C and at 22°C, *Staphylococcus* species and non-fermenting Gram negative bacteria, in particular *Pseudomonas aeruginosa*.

Heterotrophic plate counts were made by the pour plate method (Plate count agar - Oxoid) (APHA) [2].

For the count of the Pseudomonadaceae, sample portions were filtered and the membranes (Millipore 0.45 µm) were incubated in Pseudomonas agar base with CFC supplement (Oxoid) at 30°C for 24-48 h. All the different types of colonies underwent biochemical identification test using API 20 NE System (BioMérieux).

The presence of staphylococci was investigated by filtering suitable quantities of samples and incubating the filter membranes (Millipore 0.45 µm) in Staph 110 medium (Oxoid) at 36°C for 40-48 h. For the speciation API Staph System (BioMérieux) was used.

**Statistical analysis.** For statistical analysis the values of the heterotrophic plate counts were converted into  $\text{Log}_{10}x$  and those of the *Pseudomonas* species into  $\text{Log}_{10}(x+1)$ . The t test (paired) was used to compare the bacteriological contamination of the two water lines, and the simple correlation test to compare the contamination level of the supply water with that of the water leaving the two respective devices, and the degree of contamination before and after using the two devices. Significance of difference was assumed at  $p < 0.05$ .

## RESULTS

The mean values of the heterotrophic plate counts at 36°C and at 22°C for the water supplying the circuits and leaving the turbine and laser are shown in Table 1. Bacterial contamination was found within the circuit,

**Table 1.** Microbiological characteristics of the water at the various sampling points.

	No. samples	36°C Heterotrophic plate count		22°C Heterotrophic plate count	
		mean log cfu/ml	S.D. log cfu/ml	mean log cfu/ml	S.D. log cfu/ml
Supply water	38	1.54	0.92	1.58	1.16
Water leaving laser B*	38	3.91	1.09	3.92	0.90
Water leaving laser A°	38	3.90	0.97	4.04	0.89
Water leaving turbine B*	38	3.64	0.82	3.61	0.93
Water leaving turbine A°	38	3.53	0.65	3.41	0.58

B\* = before treating patient; A° = after treating patient

**Table 2.** Contamination by *Pseudomonas aeruginosa* at the various sampling points.

	No. samples	% positive samples	mean log cfu/100 ml	S.D. log cfu/100 ml	range log cfu/100 ml
Supply water	36	2.78	0.01	0.08	0-0.48
Water leaving laser B*	36	19.4	0.71	1.54	0-5.36
Water leaving laser A°	36	19.4	0.65	1.49	0-4.92
Water leaving turbine B*	36	11.1	0.15	0.48	0-2.13
Water leaving turbine A°	36	19.4	0.33	0.71	0-2.07

B\*=before treating patient; A°=after treating patient

especially in the laser device (36°C heterotrophic plate count ranging from 1.54 to 3.91 Log cfu/ml; 22°C heterotrophic plate count from 1.58 to 3.92 Log cfu/ml). However, no correlation was seen between the level of contamination of the water leaving the two devices and that of the water supply. Paired t test revealed a statistically significant association only between the total plate count at 36°C of the water leaving the laser and turbine before their use (t test =2.2; p<0.05).

No important variations in the total count were found after the use of the unit for treatment. The only statistical significance was between the total plate counts at 22°C in the water leaving the handpiece of the turbine before and after use (r=2.03; p<0.05).

*P. aeruginosa* was detected in only one sample of supply water at very low levels, while it was isolated in 11.1% of the samples taken from the turbine and in 19.4% of the samples from the laser, where the highest levels of bacteria were found (Tab. 2). After use for dental treatment an increase can be seen in the frequency of *P. aeruginosa* in the turbine samples (19.4%) (Tab. 2).

The other species of non-fermenting Gram negative bacteria isolated were: *Comamonas acidovorans* (203-70,000 cfu/100 ml) and *Xantomonas maltophilia* (328-350 cfu/100 ml) in respectively 2.6% and 4.2% of the water samples from the laser device; *Flavobacterium indologenes* (7-2,350 cfu/100 ml) was detected in 6.3% of samples and at all sampling points.

**Table 3.** Contamination by *Staphylococcus* spp. at the various sampling points.

	No. samples	<i>Staphylococcus</i>							
		<i>hominis</i> 1		<i>haemolyticus</i> 1		<i>warneri</i>		<i>epidermidis</i>	
		% positive samples	range (cfu/100 ml)	% positive samples	range (cfu/100 ml)	% positive samples	range (cfu/100 ml)	% positive samples	range (cfu/100 ml)
Supply water	36	0.0	0	0.0	0	0.0	0	0.0	0
Water leaving laser B*	36	77.8	0-1	0.0	0	25.0	0-3	0.0	0
Water leaving laser A°	36	25.0	0-1	0.0	0	0.0	0	30.6	0-1
Water leaving turbine B*	36	27.8	0-1	25.0	0-1	0.0	0	0.0	0
Water leaving turbine A°	36	25.0	0-1	0.0	0	0.0	0	0.0	0

B\*=before treating patient; A°=after treating patient

No evidence of *S. aureus* was found. Very low levels were found of the staphylococci normally present in the oral cavity, with a higher frequency in the water of the laser (Tab. 3).

## DISCUSSION

The results show that the microbiological contamination of the supply water was fairly low, whereas that of the water leaving the handpieces of the two devices was quite high, especially in the laser.

In fact, 66.6% of the supply water samples show lower heterotrophic plate count values at 22°C than those recommended by the EU directive 98/83 (<100 cfu/ml), whereas none of the samples taken from the distal outlets achieve the limits. Moreover, 66.6% of the supply water samples, but only 13.5% of those from the turbine, show counts ≤200 cfu/ml (target of the American Dental Association - ADA) [1]. Not only do these data confirm the reports of other authors [22, 31], but they also highlight a greater level of contamination in the laser devices, where recommended standards were never achieved. Several explanations could be offered: the less frequent use of this device and the lower quantity of water used during treatment enhance stagnation and the formation of biofilm. In addition, the waterline circuit linked to the laser device tends to be longer and narrower than that of the turbine (230 mm vs 110 mm and 2.0 mm vs 2.2 mm), with an increased surface-to-volume ratio which facilitates the bacterial colonisation of the inner walls [3]. Finally, since the movement of the water inside a narrow tube occurs mainly in the central part, leaving a layer of liquid virtually still near the walls, the optimal conditions are created for the attachment of water microflora and the consequent development of biofilm [3, 5].

It can be seen, therefore, that the contamination may be strongly influenced by the characteristics of the circuit and by the modality of use.

It should also be noted that on account of the intermittent use and small diameter of the tubes, the mechanical flushing [11] at the beginning of the day is not enough to keep the contamination under control, especially if applied after weekends, as in our case. On the other hand, the use of a disinfectant solution has

proved to be effective if applied at the end of every working day and after periods when the equipment has remained unused or the water in the circuits has stagnated [8, 17, 20, 32]

The predominant non-fermenting Gram negative bacteria detected belonged mainly to the Pseudomonadaceae, typical microorganisms of water environments that find no difficulty in reproducing and forming colonies due their low nutritional needs, but are notoriously recognised as opportunist pathogens. In particular *P. aeruginosa*, found only once (3 cfu/100 ml) in the supply water, was often isolated from the water leaving the turbine and, more especially, the laser handpiece, underlining the ability of this microorganism to colonise the inner surfaces of circuits and to reach potentially dangerous levels. Various cases of infection from *P. aeruginosa* have, in fact, been reported in the literature in immunocompromised patients undergoing dental treatment [15].

The frequency of isolation of *P. aeruginosa* was similar to that reported by Barbeau [3] but higher than that of a recent study carried out at a European level [30].

As far as the unusual rise in the *P. aeruginosa* seen in the water of the turbine after use, this could be explained by the fact that the greater flow of water occurring in the turbine during treatment may lead to the detachment of microcolonies from the biofilm.

Finally, the very low concentrations of certain species of staphylococci normally present in the oral cavity and sometimes responsible for hospital infections [14], confirms their poor ability to reproduce inside water circuits and underlines the low possibility of contamination due to backflow, shown also by the unremarkable rise in the total count at 36°C after the use of the devices for treatment.

## CONCLUSION

The results of this study confirm the need for the systematic application of measures to contain the contamination of dental chair water circuits in order to reduce them to acceptable levels and thus minimise the risk of infection for both staff and patients.

Particular attention should be given to the microbiological control of the water leaving laser devices, given the increasingly wide use of such instruments in dental treatment exposed to risk of infection.

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