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Primary Colonization of DUWL by *P. aeruginosa* and its Eradication by Ozone

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INTRODUCTION

Pseudomonas aeruginosa (P.a.) is a gram negative bacteria that poses a considerable risk on immuno-compromised and in particular cystic fibrosis patients. It is one of the bacteria that inhabits water sources. Along with Mycobacteria and Legionella it forms the most significant environmental bacteria in terms of health hazard to patients treated in the dental office.

Ozone is a potent microbicide and is used for disinfection of water in swimming pools and drinking water.[1] In dentistry, it was shown to be effective in the treatment of Primary Root Caries and Primary Occlusal Pit and Fissure Caries. [2-4]

AIM

To test Ozone (O_3) efficacy in eradicating primary colonisation of DUWL by *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Two newly installed dental units (KaVo, Germany) were treated and monitored weekly for DUWL contamination. The first unit was treated with continuous hydrogen peroxide (H_2O_2) (Oxygenal, KaVo, Germany) delivery system according to the manufacturer regulations. The second unit, a control, was treated with flushing with water for two minutes every morning before treating the first patient and for 30 seconds between patients according to BDA recommendations.

After appearance of P.a. in the control unit, it was treated with O_3 (HealOzone, CurOzone USA and KaVo, Germany – fig. 1). The concentration of O_3 produced was 2100 ppm and the flow rate was 615ml/ min. The O_3 machine was connected to the unit's water bottle using a quick-release coupling and O_3 was bubbled into the water for 5 minutes followed by flushing the high-speed handpiece waterline with ozonated water

MATERIALS AND METHODS (cont'd)

for 10 minutes. After a week water was sampled and after another week, O_3 treatment was repeated. 20-ml water samples were collected before and after each O_3 treatment and cultured on nutrient agar medium and incubated for 3 days at 25°C. Total viable count (TVC) was done after that.

Fig. 1: Ozone machine connected to the water bottle of the dental unit via a quick-release coupling

RESULTS AND DISCUSSION

First Unit:

Oxygenal treatment continuously produced water with TVC of less than 100 CFU/mL in the range of 0 – 80 CFU/mL. This conforms with the European regulations concerning drinking water which require that the bacterial load should be less than 100 CFU/mL. Hydrogen peroxide may be the most investigated biocide for the control of DUWL contamination and has been shown to be effective when delivered continuously. However, it has not been shown yet to be able to remove the already existing biofilms in old dental units. Also long term follow up is required to draw confirmative conclusions.

Second Unit:

Bacterial TVC of water from the control unit was 2.3 x 10^4 and 3.4 x 10^4 CFU/mL after 1 and 2 weeks of installation respectively. The primary coloniser was identified using API 20 NE kit as pure *Pseudomonas aeruginosa* (fig. 2). After the first O_3 treatment the TVC was reduced to 60 CFU/mL. After one week TVC rose to 3.9 x 10^4 CFU/mL with few Pseudomonas colonies.

RESULTS AND DISCUSSION (cont'd)

After two weeks, TVC became 2.8 x 10^3 CFU/mL with no detected P.a. and became 0 CFU/mL after the treatment. Repeated sampling of the unit for 9 weeks showed no re-growth of P.a.

Our previous work have shown that Ozone is effective in the treatment of DUWL contamination and biofilm removal This experiment widens the range of Ozone effect to include both the classical bacteria present in DUWL and the more significant P.a.

It would be required to undertake experiments to test the sensitivity of similar significant bacteria as Mycobacteria and Legionella.

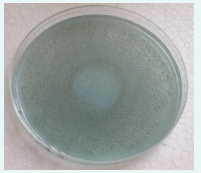

Fig. 2: Green P.a. colonies as appeared on the Nutrient Agar medium.

CONCLUSIONS

- *Pseudomonas aeruginosa* can be the primary coloniser of DUWL
- *Pseudomonas aeruginosa* contamination can be eradicated with ozone.
- Flushing DUWL with water could not maintain a satisfactory microbiological standard of water.
- Oxygenal can maintain DUWL count of less than 100 CFU/mL.

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Pseudomonas aeruginosa (P.A.) may colonise Dental Unit Waterlines (DUWL) and be a risk for immunocompromised adults and cystic fibrosis children. **Objectives:** To test Ozone (O_3) efficacy in eradicating primary colonisation of DUWL by P.A. **Methods:** Two new dental units (KaVo, Germany) were monitored weekly for DUWL contamination. The first unit was treated with continuous H_2O_2 (Oxygenal) delivery. The second unit, a control, was flushed with water according to BDA recommendations. After appearance of P.A. in the control unit, it was treated with O_3 (HealOzone, CurOzone USA; KaVo, Germany). The O_3 machine was connected to the unit's water bottle and O_3 was bubbled into the water for 5 minutes followed by flushing the waterline with ozonated water for 10 minutes. After a week water was sampled and after another week, O_3 treatment was repeated. 20-ml water samples were collected before and after each O_3 treatment and cultured on nutrient agar and incubated for 3 days at 25°C. **Results:** Oxygenal treatment continuously produced water with TVC of less than 100 CFU/mL. TVC of water from the control unit was 2.3 x 10^4 and 3.4 x 10^4 CFU/mL after 1 and 2 weeks of installation. The primary coloniser was identified (API 20 NE kit) as pure P.A. After the first O_3 treatment the TVC was reduced to 60 CFU/mL and rose to 3.9 x 10^4 CFU/mL after a week with few Pseudomonas colonies. After two weeks, TVC was 2.8 x 10^3CFU/mL CFU/mL with no detected P.A. and became 0 CFU/mL after the treatment. Repeated sampling of the unit for 9 weeks showed no re-growth of P.A. **Conclusion:** Oxygenal can maintain DUWL count of <100 CFU/mL while flushing with water could not. P.A. can be the primary coloniser of DUWL and can be eradicated with O_3. *Supported by CurOzone, USA & KaVo, Germany.*