MICROBIAL CONTAMINATION OF DENTAL UNIT WATERLINES IN DENTAL PRACTICES IN HESSE, GERMANY: A CROSS-SECTIONAL STUDY

Mardjan Arvand* and Alfons Hack

Hesse State Health Office, Centre for Health Protection, Dillenburg, Germany

Received: December 14, 2012; Accepted: January 2, 2013

The quality of water from dental units is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit. This study analyzed the microbial quality of water obtained for periodical monitoring from 56 dental units in different dental practices in Hesse. Contamination by *Legionella* spp., *Pseudomonas aeruginosa*, and increased total colony counts were detected in 27.8%, 3.5%, and 17% of samples. *Legionella pneumophila* serogroup 1 accounted for 28% of *Legionella* isolates. The *Legionella* concentration was >100 cfu/100 ml in 84% of contaminated samples. Samples collected from an instrument channel were more frequently contaminated by *Legionella* than those from cup filler (41.7% vs. 18.6%, $p = 0.02$).

After release of these results, decontamination measures were performed in units that had revealed unsatisfactory results. The outcome of the intervention was followed-up by microbiological analysis. At follow-up, 65.2% and 72.7% of waterlines that had previously been contaminated by *Legionella* or had shown increased total colony counts were free of contamination.

Our results show a high rate of contamination of water from dental units in dental practices in Hesse. They highlight the risk of exposure for patients and personnel and the need for effective strategies to reduce microbial contamination.

Keywords: water, contamination, dental unit, *Legionella*, *Pseudomonas*, prevention

Introduction

The general problem of microbial contamination of dental unit waterlines (DUW) is well known [1–3]. However, the dimension of this problem in our own environment under the current conditions is less well studied. Aerosols generated during dental procedures are an important source for the transmission of *Legionella* and other bacterial pathogens in the dental practice [3, 4]. *Legionella pneumophila* is the major agent of Legionnaire's disease (LD) and Pontiac fever. The estimated incidence rate of LD in the European Union (EU) is 100 per million [5]. Approximately two third of reported cases of LD in the EU are community-acquired [5, 6]. Recent studies have shown that *Legionella* is one of the most common agents of community-acquired pneumonia in Germany [7].

It has been suggested that *Legionella* contaminated DUW may contribute to respiratory illness among dentists, dental nurses, and patients. Increased levels of *Legionella*

antibodies suggest that dental staff may experience sub-clinical infection or mild Pontiac fever upon continuous exposure to contaminated aerosols [8, 9]. However, the significance of the microbial contamination of DUW has been discussed controversially in the past, mainly because systematic studies on transmission of *Legionella* via DUW were lacking. In a recent study, Ricci et al. reported on transmission of *L. pneumophila* from a dental unit to a patient, who developed a fulminant LD and died from septic shock [10]. The role of the dental unit as source of infection was proved by molecular fingerprinting techniques.

In Germany, the microbial quality of water from dental units is regulated by the German Drinking Water Ordinance (GDWO) and the recommendations of the German Commission for Hospital Hygiene and Infection Prevention at the Robert Koch Institute. They generally recommend a *Legionella* concentration of <100 cfu/100 ml and a total colony count of <100 cfu/ml for all dental units. When

*Corresponding author: Mardjan Arvand, M.D., Hessisches Landesprüfungs- und Untersuchungsamt im Gesundheitswesen, Zentrum für Gesundheitsschutz, Wolframstrasse 33, D-35683 Dillenburg, Germany; Phone: +98 2771 320631; Fax: +98 2771 36667; E-mail: mardjan.arvand@hlpug.hessen.de

immune-compromised patients are treated, no detectable amounts of *Pseudomonas aeruginosa*, Cryptosporidia, and Legionella are required [11, 12]. In order to maintain the high microbial quality, it is recommended that only water of drinking water quality or sterile water should be fed into dental units [12]. Guidelines on preventive measures to reduce the contamination burden in DUW have been issued by governments and national dental associations [11, 13, 14]. They recommend a periodical (yearly) monitoring of water from DUW. In the laboratories of the Hesse State Health Office, we routinely perform microbiological analysis of water from DUW as a diagnostic service. The aim of this study was to evaluate the microbial quality of water from dental units that were analyzed in the context of periodical analysis in our laboratory between 2009 and 2011. In addition, we evaluated the success rate of decontamination measures performed in units which had revealed unsatisfactory results upon periodical analysis.

Material and methods

The study was carried out between January 2009 and December 2011 and included water samples from DUW, which were analyzed in the context of periodical monitoring in our laboratories. A total of 90 samples were collected from 56 dental units in 22 dental practices in different districts of Hesse. We generally recommend the analysis of at least two samples per dental unit, one from a high-speed rotor or a syringe, and one from the cup filler. However, some dentists insisted on investigation of only one DUW per unit. In these cases, one sample per unit was analyzed. Together, each 2 samples were analyzed from 34 dental units and only one sample from 22 units.

Samples were generally collected during routine working hours on a week-day by a specialized team. About 250 ml water was collected from a DUW and subjected to analysis for Legionella, *P. aeruginosa* and total aerobic colony count in our laboratory. Legionella detection was carried out according to the recommendations of the German Federal Environment Agency [15]. In detail, 2 aliquots of 0.5 ml water were inoculated directly to GVPC agar, and 100 ml sample was filtered through a cellulose-nitrate membrane (pore size 0.45 µm). The filter was overlaid with 20 ml 0.2 M HCl-KCl [pH 2.2] for 4 min. The buffer was discarded, and the filter was rinsed with 10 ml sterile water and placed on a third GVPC agar plate. The cultures were incubated at 37 °C for 10 days. Differentiation of the Legionella isolates was performed with a latex agglutination test (Oxoid), which discriminates between *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 2–14, and non-pneumophila *Legionella* species [16].

Detection of *P. aeruginosa* was performed according to the recommendations of the GDWO and DIN EN ISO 16266 [12]. Briefly, 100 ml sample was filtered through a cellulose-nitrate membrane, which was subsequently placed on Cetrimid agar and incubated at 37 °C for up to 2 days. Total colony counts were determined in 1 ml

sample at 37 °C by using the plate pour method according to the GDWO [12].

Decontamination procedures were independently organized and tailored by the owner of the practice or institution in accordance with the manufacturer's recommendations and national guidelines. Follow-up investigation was carried out in 23 dental units that had revealed contamination upon periodical monitoring.

Statistical analysis was carried out with the chi-square test or Fisher's exact test, when appropriate. A *p* value of <0.05 was considered significant.

Results

We analyzed the microbial quality of water from DUW in a random sample of dental practices in Hesse, Germany. Of 90 samples analyzed, 25 (27.8%) were contaminated with Legionella (Table 1). Three samples (3.5%) were contaminated with *P. aeruginosa*, and 15 (17%) displayed increased total colony counts (>100 cfu/ml), as defined by the GDWO (Table 1). Two different contaminating agents, e.g. Legionella and *P. aeruginosa*, were detected in 8% of samples. Taken together, 32 (35.6%) of 90 samples did not comply with the German drinking water standards.

The encountered Legionella isolates were further differentiated by serological methods. *L. pneumophila* serogroup 1 was detected in 7 (28%) of 25 contaminated samples. One sample (4%) contained additionally *L. pneumophila* serogroup 2–14 and 18 (72%) samples contained non-pneumophila Legionella species.

Twenty-one (84%) of 25 samples which were contaminated by Legionella organisms displayed a concentration of ≥100 cfu/100 ml (Table 2). A contamination with less than 100 cfu/100 ml was detected in 4 (16%) samples (Table 2). This concentration is below the threshold defined by the GDWO for drinking water, but exceeds the concentration recommended by the Robert Koch Institute for dental units used for the treatment of immune-compromised patients [11, 12].

We next analyzed whether there is a relationship between the type of DUW used for sample collection and the rate of contamination. The DUW were assigned to two categories: (i) Instrument Channel, which included a high-speed drill, a micro-motor, or a syringe, and (ii) cup filler. Fifteen (41.7%) of 36 samples collected from an instrument channel and 10 (18.6%) of 54 samples collected from a cup filler were contaminated by Legionella. The difference between the channels was statistically significant (*p* = 0.016). The contamination rates for the other parameters did not differ significantly (Table 3).

Decontamination measures were introduced after the release of the results of periodical monitoring in the units with contamination problems. Microbiological follow-up was performed in 23 of these units. Of 23 DUW that had previously been contaminated with Legionella, 15 (65.2%) were rendered free of Legionella after intervention (Table 4). Similarly, of 11 DUW that had previously

Table 1. Microbial contamination of water from dental units in dental practices in Hesse, Germany, 2009–2011

Microorganism	Contaminated samples (<i>n</i>)	Contaminated samples (%)	Total number of samples analyzed
Legionella contamination*	25	27.8	90
Pseudomonas contamination*	3	3.5	86
Increased total colony count [§]	15	17.0	88

*≥1 cfu/100 ml

§≥100 cfu/ml

Table 2. Legionella concentration in DUW analyzed in the context of periodical microbiological monitoring

Legionella concentration cfu/100 ml	Contaminated samples (<i>n</i>)	Contaminated samples %
1–99	4	16.0
100–999	13	52.0
1000–9999	7	28.0
>10,000	1	4.0
Total	25	100

Table 3. Frequency of microbial contamination in relation to the type of channel used for sampling. DUW were assigned to two categories, (i) Instrument channel, which included high-speed drill, micromotor and 3-in-1 syringe, and (ii) cup filler

	Instrument channel <i>n</i> (%)	Cup filler <i>n</i> (%)	<i>p</i> value
Legionella contamination*	15 (41.7)	10 (18.6)	0.016
Pseudomonas contamination*	1 (2.8)	2 (3.7)	1
Increased total colony count [§]	9 (25.0)	6 (11.1)	0.099
Total	36	54	90

*≥1 cfu/100 ml

§≥100 cfu/ml

Table 4. Results of follow-up analysis after accomplishment of decontamination measures in dental units that had revealed microbial contamination during periodical analysis. A total of 34 paired samples that collected prior to and after the first intervention were compared

	DUW with contamination during periodical monitoring	DUW without contamination upon follow-up	Success rate of intervention (%)
Legionella contamination*	23	15	65.2
Pseudomonas contamination*	2	1	50.0
Increased total colony count [§]	11	8	72.7

*≥1 cfu/100 ml

§≥100 cfu/ml

revealed increased total colony counts, 8 (72.7%) displayed normal colony counts after intervention (*Table 4*). Additional measures and repeated intervention were necessary to achieve acceptable results in all other cases.

Discussion

In this study, Legionella was the most prevalent pathogen which was detected in approximately 28% of samples from

DUW. The virulent *L. pneumophila* serogroup 1 accounted for 28% of the Legionella isolates. Our results are very similar to the results of a German study from 1986 [17]. They are also in accordance with recent studies from Italy, Switzerland, and South Africa that revealed Legionella contamination rates of 33%, 24%, and 33% in samples from DUW in dental practices [1, 3, 18]. Our results suggest that despite the enormous technical progress of the recent decades, the risk for transmission of Legionella and other microorganisms during dental surgery is still unacceptably high in Germany.

According to the European Centre for Disease Prevention and Control, more than two third of reported cases of LD in the EU were community-acquired [5, 6]. An environmental follow-up was performed in less than 10% of latter cases [5]. It can be hypothesized that some of these cases might have been related to a dental treatment, but remained undetected because of lacking environmental follow-up. Further investigation and intensified surveillance are necessary to evaluate this hypothesis. We recommend including questions about recent dental treatment in the questionnaire for environmental follow-up of LD.

In this study, we found significantly different contamination rates between samples collected from an instrument channel and those collected from the cup filler. The contamination level may correlate with different parameters including the internal diameter of the tubing, the internal surface-area-to-volume ratio, water pressure, flow rate, and stagnation periods. We therefore recommend analysing at least two channels – an instrument channel and the cup filler – in the context of periodical monitoring.

This study evaluated the success rate of individual decontamination measures, which were performed upon release of the results of periodical analysis in units with contamination. They included intensified flushing, intensified circulation, and closer adherence to the manufacturer's recommendation regarding routine cleaning and disinfection measures. To our knowledge, this is the first study to evaluate the success rate of an intervention in such a diverse sample of dental practices. We found a success rate of up to 73% after the first intervention, indicating that good adherence to established protocols can effectively reduce the microbial contamination of DUW.

In summary, our results show a high rate of microbial contamination, especially with Legionella, in water from DUW in dental practices in Hesse. Further investigation is needed to evaluate the risk of microbial transmission by contaminated DUW. Implementation of periodical monitoring of the water quality of dental units is an important measure of infection prevention in the dental practice.

Acknowledgements

We thank the team of the Infection Control Laboratory, Bärbel Hain, Kerstin Losacker, and Oliver Roth, for excellent technical assistance.

References

1. Castiglia P et al.: Italian multicenter study on infection hazards during dental practice: control of environmental microbial contamination in public dental surgeries. *BMC Public Health* 8, 187 (2008)
2. Pankhurst CL et al.: Microbial contamination of dental unit waterlines: the scientific argument. *Int Dent J* 48, 359–368 (1998)
3. Singh T, Coogan MM: Isolation of pathogenic Legionella species and legionella-laden amoebae in dental unit waterlines. *J Hosp Infect* 61, 257–262 (2005)
4. Atlas RM et al.: Legionella contamination of dental-unit waters. *Appl Environ Microbiol* 61, 1208–1213 (1995)
5. European Centre for Disease Prevention and Control (ECDC), surveillance report: Legionnaires' disease in Europe 2009 (2009).
6. Joseph CA, Ricketts KD: Legionnaires disease in Europe 2007–2008. *Euro Surveill* 15, 19, 493 (2010)
7. von Baum H et al.: Community-acquired Legionella pneumonia: new insights from the German competence network for community acquired pneumonia. *Clin Infect Dis* 46, 1356–1364 (2008)
8. Fotos PG, Westfall HN, Snyder IS, Miller RW, Mutchler BM: Prevalence of Legionella-specific IgG and IgM antibody in a dental clinic population. *J Dent Res* 64, 1382–1385 (1985)
9. Luck PC et al.: Legionellae in dental units – a hygienic risk? *Dtsch Zahn Mund Kieferheilkd Zentralbl* 80, 341–346 (1992)
10. Ricci ML et al.: Pneumonia associated with a dental unit waterline. *Lancet* 379, 684 (2012)
11. Robert-Koch-Institut: Infektionsprävention in der Zahnheilkunde-Anforderungen an die Hygiene. *Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz* 49, 1–15 (2006)
12. Trinkwasserverordnung 2011. Verordnung über die Qualität von Wasser für den menschlichen Gebrauch in der Fassung vom 28.11.2011 (BGBl. I S. 2370)
13. Health-Protection-Surveillance-Centre. National Guidelines for the Control of Legionellosis in Ireland (2009)
14. Centre for Disease Prevention and Control. Guidelines for Infection Control in Dental Health-Care Settings – 2003. *MMWR* 52, 1–68 (2003)
15. Umweltbundesamt. Nachweis von Legionellen in Trinkwasser und Badebeckenwasser. Empfehlung des Umweltbundesamtes nach Anhörung der Trink- und Badewasserkommission des Umweltbundesamtes (Federal Environment Agency, Detection of Legionella in drinking water and bathing water). *Bundesgesundheitsbl* 43, 911–915 (2000)
16. Arvand M et al.: Contamination of the cold water distribution system of health care facilities by *Legionella pneumophila*: do we know the true dimension? *Euro Surveill* 16 (16)
17. Reinthaler F, Mascher F: Demonstration of *Legionella pneumophila* in dental units. *Zentralbl Bakteriol Mikrobiol Hyg B* 183, 86–88 (1986)
18. Barben J et al.: Water quality in dental chair units. A random sample in the canton of St. Gallen. *Schweiz Monatsschr Zahnmed* 119, 976–985 (2009)