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# Water Quality in Dental Chair Units

## A Random Sample in the Canton of St. Gallen

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**Summary** This study aimed to identify the microbial contamination of water from dental chair units (DCUs) using the prevalence of *Pseudomonas aeruginosa, Legionella species* and heterotrophic bacteria as a marker of pollution in water in the area of St. Gallen, Switzerland. Water (250 ml) from 76 DCUs was collected twice (early on a morning before using all the instruments and after using the DCUs for at least two hours) either from the high-speed handpiece tube, the 3 in 1 syringe or the micromotor for water quality testing. An increased bacterial count (>300 CFU/ml) was found in 46 (61%) samples taken before use of the DCU, but only in 29 (38%) samples taken two hours after use. *Pseudomonas aeruginosa* was found in both water samples in 6/76 (8%) of the DCUs. Legionella were found in both samples in 15 (20%) of the DCUs tested. *Legionella anisa* was identified in seven samples and *Legionella pneumophila* was found in eight. DCUs which were less than five years old were contaminated less often than older units (25% und 77%, $p<0.001$). This difference remained significant ($0=0.0004$) when adjusted for manufacturer and sampling location in a multivariable logistic regression. A large proportion of the DCUs tested did not comply with the Swiss drinking water standards nor with the recommendations of the American *Centers for Disease Control and Prevention (CDC).*

## Introduction

The transmission of pathogens to patients during dental treatment has been a topic of discussion for years, which is why dental unit water has been investigated again and again (Exner et al. 1981, Panagakos et al. 2001). Because irreversible damage may be caused to teeth when using high-speed handpieces without sufficient cooling, water spray, both to clear the working area and for cooling, is an indispensable component of the modern dental unit. Aerosol from these instruments has not only increased the danger of infection for patient and dentist, but also continually increased the germ count in the dental surgery (Dombrowsky et al. 1980). In particular, bacterial biofilm formation in the dental chair units (DCU) presents a big problem and poses a risk of infection not only for dental staff but also for immunocompromised patients or persons suffering from other severe diseases (Pankhurst et al. 1995, Williams et al. 1996, Barbeau et al. 1998, Walker et al. 2000). Patients with cystic fibrosis (CF), an inherited disease with thickened mucous in the lung, are particularly prone to infection with *Pseudomonas aeruginosa,* which can be found in stagnant water (Govan et al. 1996, Saiman et al. 2003).

Several previous investigations found that the water which reached the DCU failed to meet drinking water standards. In Switzerland there are few studies on this topic, perhaps because the water quality here is believed to be good. One study from Bern showed that only 10% of DCUs complied with all criteria required for drinking water and that the tolerated margin of 100 microbes per ml was frequently exceeded, however, three minutes rinsing was sufficient to guarantee the water quality (Tonetti-Eberle et al. 2001). In recent years various efforts have been made and numerous methods of disinfection have been investigated with a view to improving the water quality hindering the formation of biofilm in DCUs (Wirthlin et al. 2003, McDowell et al. 2004). For this purpose, the American *Centers for Disease Control and Prevention (CDC)* have recently published new guidelines for infection control in dental healthcare settings (Kohn et al. 2003).

The aim of the present study was to determine whether the DCU water quality in the St. Gallen region of Switzerland met

the Swiss drinking water standards or whether dental treatment might pose a risk for patients with diseases such as CF.

## Materials and Methods

Water samples were taken from a total of 80 DCUs in 40 randomly chosen dental practices in the St. Gallen area. The samples were taken either from the high-speed handpiece tube, the 3 in 1 syringe or the micromotor. The dentist decided where the sample was taken from, but the water was sampled twice at the same location first on a Monday morning, before the DCU had been used, and also after use for at least two hours. The water was collected by the dentist, or by another member of staff, in a sterile 250 ml glass containing sodium thiosulfate to inactivate oxidising disinfectants. All samples were brought to the *Department of Health and Consumer Protection* on the same day and were processed within 24 hours. The DCU type and age were recorded on a questionnaire.

The standard method for water quality determination in Switzerland was used to determine the number of bacteria (aerobic mesophilic bacteria) as follows: one ml of water was transferred into a Petri dish with 25 ml sterile liquid methods Agar (Difco, 279740) which, once it had set, was incubated at 30 °C for 72 hours. Then bacteria present were counted as colony forming units (CFU) but not specified further. According to official Swiss hygiene guidelines for the drinking water supply, values below 300 CFU/ml are regarded as normal, but pathogens should not be present (Eidgenössisches Departement des Innern 2005).

*Pseudomonas aeruginosa* was isolated and identified from the water samples according to the method officially recommended in Switzerland. 100 ml of water was filtered through a sterile membrane filter (EZ-Pak Membran Filter 0.45 µm, Ø 47 mm, Millipore EZHAWG474). The membrane filter was then incubated on Cetrimide Agar *(Pseudomonas* CFC Agar, Oxoid CM559) with 10 ml glycerol/l and nalidixic acid (Oxoid SR102E) at 37° for 48 hours. Colonies which clearly showed pyocyanine production (blue-green colonies fluorescing at 360 nm) were presumed positive for *Pseudomonas aeruginosa*. Questionable colonies were inoculated onto Cetrimide Agar (see above), incubated at 42 °C for 48 hours, and then tested for a positive oxidase reaction with Merck test strips (Merck 1.13300).

Legionella were identified by culture in water using BCYE Agar according to the recommendations in the German "Gesundheitsblatt" (Umweltbundesamt 2000). Values below the cut-off point (10 CFU/L) were regarded as normal. Colonies of the positive cultures were examined by means of latex slide Agglutination (Oxoid, DR0800) for the occurrence of *Legionella pneumophila* or *Legionella species*. Additionally, *Legionella species* were identified by sequencing the mip gene (Ratcliff et al. 2003).

*Statistical analyses:* The data were analysed using STATA (StataCorp. 2007. Stata Statistical Software: Release 10.0. College Station, TX: StataCorp LP). As the microbial count data were not normally distributed, we used medians rather than means to describe average microbial counts. Our main outcome (dependent variable) was the proportion of water samples with a microbial count above the cut-off (300 CFU/ml for total microbial count, 10 CFU/l for Legionella und < 1 CFU/ml for *Pseudomonas aeruginosa*). These values were given as percentages with a 95% confidence interval (95% CI). In order to determine whether the proportion of positive samples was associated with the age and type of DCU as well as the sampling site, the data were first cross-tabulated and then further investigated using univariate and multivariate logistic regression analyses. Statistical significance was tested using a Chi2 test for the cross-tabulation and Likelihood ratio tests for the logistic regressions.

## Results

Two 250 ml water samples were taken correctly from 76 of the 80 DCUs tested and could be used for investigation. In two cases, too little water was collected in the bottle (< 200 ml). In another case, the second sample was collected too early, and in another, the sample was stored for longer than 24 hours. Of the 76 water samples, 20 were taken from the high-speed handpiece tube, 38 from the 3 in 1 spray and 16 from the micromotor; in two cases the sampling location was not recorded. Most (46%, 35/76) of the DCUs investigated were made by Sirona (C1, C1+, C2, C2+, M1, Sirodont), 25% (24/76) by KaVo (ESTETICA, ORTHOcenter), the rest were from various manufacturers. 24 (33%) DCUs were less than five years old, 20 (28%) were five to ten years old, and 32 (42%) were over ten years old.

Forty-six (61%, 95%CI 49%–72%) of the DCUs investigated had a total microbial count over the limit of 300 CFU/ml in the first sample (mean: 5,088 CFU/ml; median: 3,750 CFU/ml; max. 26,000 CFU/ml). In 29 DCUs (38.2%; 95%CI 27%–49%) the total microbial count was still too high after two hours use (mean: 2,338 CFU/ml, median: 1,100 CFU/ml; max. 19,000 CFU/ml). Only 30 of the DCUs (39.5%; 95%CI 28%–51%) met the Swiss drinking water requirements (see Table I).

In seven cases (9.2%; 95%CI 2.6%–15.9%) *Pseudomonas aeruginosa* was found in the first water sample, and in six cases (7.9%; 95%CI 1.7–14.1) it was shown in both samples. Of these six cases, four were very heavily contaminated (> 100 CFU/100 ml). All sampling locations were involved: *Pseudomonas aeru-*

Tab. I Proportion of water samples positive for bacteria (above the respective cut-off) for total bacterial count (colony forming units, CFU), Legionella and *Pseudomonas aeruginosa*

| | **Both samples *negative*** | **1st Sample *positive* 2nd Sample *negative*** | **Both samples *positive*** |
|---|---|---|---|
| Total bacterial count (negative < 300 CFU/ml) | 30 (39.5%) | 17 (22.4%) | 29 (38.2%) |
| Legionella (negative < 10 CFU/L) | 60 (79.0%) | 3 (4.0%) | 15 (19.7%) |
| *Legionella pneumophila* (negative < 10 CFU/L) | 64 (84.2%) | 3 (4.0%) | 9 (11.8%) |
| *Pseudomonas aeruginosa* (negative < 1 CFU/100 ml) | 69 (90.8%) | 1 (1.3%) | 6 (7.9%) |

*ginosa* was found four times in water from the 3 in 1 syringe, twice in water from the high-speed handpiece tubing and once in water from the micromotor.

Legionella were detected in 18 DCUs (23.7%, 95%CI 13.9%–33.5%). In 15 cases (19.7%, 95%CI 10.6%–28.9%) they were found in both samples. The median of the 15 positive first samples was 1,200 CFU/L, ranging from 200 to 50,000 CFU/L. This did not decrease after using the unit for two hours: in the second samples the median was even higher (3,200 CFU/L), ranging from 35 to 25,000 CFU/L. *Legionella species* were shown in the first but not in the second samples for three units. *Legionella pneumophila* was detected in zwelve DCUs (15.8%, 95%CI 7.4%–24.2%), in nine of these (7.9%, 95%CI 1.7%–14.1%) in both samples. *Legionella anisa* was identified seven times in both water samples. *Legionella species* were also found in all three sampling locations (13 times in the 3 in 1 syringe, four times in the micromotor and once in the high-speed handpiece tubing.

The 300 CFU/ml cut-off point was exceeded significantly less often in both first and second samples if the DCU was new (less than five years old) compared to older units (Table II). Furthermore, Sirona and KaVo units tended to be less contaminated compared to the other units, however no differences were found as a function of sampling location.

In a multivariate logistic regression in which all three possible factors were considered simultaneously, age of the unit (odds ratio [OR] 5.7 and 15.1 for 5–10- and >10-year old units compared to <5-year old units, p=0.0004) and manufacturer (OR 0.16 for Sirona and 0.13 for KaVo compared to other units, p=0.0243) remained significantly associated with the microbial count (Table III).

## Discussion

This study of water quality in dental chair units (DCU) in the region of St. Gallen showed that 60% of the units tested had a total bacterial count over that allowed by the Swiss guidelines for drinking water. *Legionella species* were found in almost 20% of the units and *Pseudomonas aeruginosa* was also found in some. Older units were affected more frequently than those under five years of age.

**Tab. II** Proportion of water samples with a bacterial count above the cut-off (300 CFU/ml) in both samples, by sampling location, age and type of dental chair unit

| | | Sample 1 | | Sample 2 | |
|---|---|---|---|---|---|
| | N | n | % | n | % |
| **Age of DCU (years)** | | | | | |
| <5 | 24 | 6 | 25% | 4 | 17% |
| 5–10 | 20 | 14 | 70% | 9 | 45% |
| >10 | 32 | 26 | 81% | 16 | 55% |
| p | | | <0.001 | | 0.030 |
| **Type of DCU** | | | | | |
| Sirona | 35 | 20 | 57% | 13 | 37% |
| KaVo | 17 | 8 | 47% | 4 | 24% |
| Other | 23 | 18 | 78% | 12 | 52% |
| p | | | 0.076 | | 0.145 |
| **Sampling location** | | | | | |
| High-speed handpiece tubing | 20 | 13 | 65% | 8 | 40% |
| Micromotor | 16 | 12 | 75% | 9 | 56% |
| 3 in 1 syringe | 38 | 21 | 55% | 12 | 32% |
| Not specified | 2 | 0 | 0% | 0 | 0% |
| p | | | 0.166 | | 0.243 |

p: from Chi2 test

**Tab. III** Association of characteristics of DCU with an increased total bacterial count in the first sample (univariable and multivariable logistic regression, with bacterial count over the cut-off of 300 CFU/ml as a dependent variable, N=74)

| | Univariate | | | Multivariate* | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p | OR | 95% CI | p |
| **Age (years)** | | | | | | |
| <5 | 1.0 | | 0.0002 | 1.0 | | 0.0004 |
| 5–10 | 6.22 | 1.63–23.76 | | 5.68 | 1.20–26.86 | |
| >10 | 11.56 | 3.18–42.05 | | 15.11 | 3.37–67.84 | |
| **Manufacturer** | | | | | | |
| Other | 1.0 | | 0.0782 | 1.0 | | 0.0243 |
| Sirona | 0.43 | 0.13–1.44 | | 0.16 | 0.03–0.76 | |
| KaVo | 0.22 | 0.06–0.86 | | 0.13 | 0.02–0.86 | |
| **Sampling location** | | | | | | |
| High-speed handpiece tubing | 1.0 | | 0.3655 | 1.0 | | 0.3285 |
| Micromotor | 1.62 | 0.38–6.94 | | 1.99 | 0.34–11.58 | |
| 3 in 1 syringe | 0.67 | 0.22–2.04 | | 0.60 | 0.14–2.65 | |

OR: odds ratio 95% CI: 95% confidence for OR p: from Likelihood ratio test * adjusted for all variables in this table

The upper limit for heterotrophic bacteria in drinking water was set at 500 CFU/ml by the *American Environment Protection Agency (EPA)* and the *American Public Health Association (APHA)* (Eaton et al. 1999), while in Switzerland 300 CFU/ml is considered acceptable as an upper limit for bacteria in the drinking water supply (Eidgenössisches Departement des Innern 2005). The *American Dental Association* (ADA 1996) and the *Centers for Disease Control and Prevention (CDC)* (Kohn et al. 2003) set an upper limit of 200 CFU/ml in dental unit water in their recommendations. This requirement was fulfilled by only 40% of the units tested in this study.

Since the aim of the study was to record the level of contamination of DCUs, no attempt was made to further differentiate individual bacteria. *Pseudomonas aeruginosa* and aerobic mesophilic bacteria were used as markers for contaminated water and this is an internationally accepted method to determine water quality.

Water is an integral component of a modern DCU: for example high-speed micromotors require sufficient water-cooling, whereby a fine aerosol is also created. Although suction systems reduce aerosol formation, saliva and microorganisms from the mouth still reach the system (Panagakos et al. 2001). Therefore, many handpieces are equipped with special backflow flaps to reduce backflow of contaminated water (Epstein et al. 2002). The humidity of the suction system and connecting tubes provide optimal conditions for the growth of microorganisms which can develop into resistant biofilms and attach themselves to the insides of the tubes (Wirthlin et al. 2003). These biofilms protect bacteria from being washed away by the water stream and also aid in resisting many disinfectant measures (Donlan 2002). There are several methods to disinfect a DCU; frequently a microfilter is placed at the end of a water tube, or periodical shock treatment with aggressive chemicals is used (McDowell et al. 2004). Numerous methods including alcohol-, chlorine-, iodine- and peroxide-based agents have been tested for DCU disinfection (Mills et al. 1986, Tuttlebee et al. 2002, Wirthlin et al. 2003, Szymanska 2006). But often the biofilm is not destroyed and can release bacteria into the water system again after the disinfection has taken place (Epstein et al. 2002, Walker et al. 2000). In our study we did not specifically ask which method of disinfection had been used, but all participants assured us that they disinfected according to the DCU manufacturer's instructions. We found that the bacterial colonisation significantly increased with increasing age of the DCU. This could be due to an accumulated biofilm in older units, or also to difference in construction. Moreover, it appeared, in particular in the multivariable analyses, that the DCU type (manufacturer) influenced the result. However, this would have to be tested in larger studies. A DCU may be contaminated not only through the suction system by microorganisms from patients' mouths, but also by pathogens present in the public water supply. In an earlier study we were able to show that drinking water quality in the St. Gallen area is generally very good and that *Pseudomonas aeruginosa* could only be identified in rare cases in stagnant water (Barben et al. 2005). This does not explain the high number of contaminated DCUs found in the present study. Nevertheless, it is certain that even a small number of bacteria may cause a hygiene problem if they gain access to a DCU as the narrow tubes and periodically stagnant water provide optimal conditions for bacterial growth. It is more likely that DCU is contaminated by oral flora from patients, since *Pseudomonas aeruginosa* can be shown in 4% of healthy people (Botzenhart et al. 1987).

In the early 80's a German study showed that 74% of DCUs were contaminated with *Pseudomonas aeruginosa,* but these results were barely heeded (Exner et al. 1981). In our study *Pseudomonas aeruginosa* was found in only 9% of the DCUs tested, possibly as a result of improved disinfection procedures in the last 20 years. A contamination with *Pseudomonas aeruginosa* poses a risk particularly for immunocompromised patients and those with special medical problems such as cystic fibrosis (CF). The actual risk of transmission to patients has never been thoroughly investigated. One paper reports the development of a localised *Pseudomonas aeruginosa* infection in two immunocompromised patients (Martin 1987). As our study merely reported the prevalence of pathogens in DCUs we cannot say what the real risk of infection for patients is. Over the duration of the study we had three CF-children with a newly acquired *Pseudomonas aeruginosa* infection, but none of the three had visited a dentist in the previous six months.

Dental units are often contaminated with Legionella, and biofilm formation can aid survival of dangerous pathogens such as *Legionella pneumophila* (Fotos et al. 1985, Walker et al. 1995, Szymanska 2004). The literature reports a prevalence of between 10 and 50% for *Legionella pneumophila* in DCUs (Reinthaler et al. 1988, Oppenheim et al. 1987, Lück et al. 1992). In our study the Legionella prevalence was 21% (16/76), and Legionella were found more frequently in the 3 in 1 syringe. The high degree of DCU contamination with Legionella is worrying because the aerosol created during dental treatment is sufficient to infect both patients and dental staff (Fotos et al. 1985). One study reported that 34% of dental staff showed a positive Legionella antibody titre in blood, whereas in the control group only 5% had a positive titre; dentists had the highest titre at 50%, followed by assistants (38%) and technicians (20%) (Reinthaler et al. 1988).

For optimal DCU water quality, the CDC recommends running water in all instruments, which (may) come in contact with the mouth, for two minutes in the morning before beginning treatment and for 20–30 seconds between patients for normal dental treatment. After a weekend, longer rinsing is required (Kohn et al. 2003, Williams et al. 1996). In our study this measure was obviously not enough to ensure sufficient water quality. Therefore more extensive measures than those recommended by the CDC are necessary to reduce the bacterial count in water. For example, these might be the use of a separate water reservoir with sterile water independent of the drinking water supply, a periodical or even continuous disinfection of the DCU with bacteriocidal agents like, for example, microfilters which would prevent the transmission of pathogens to patients. Moreover, on account of our study occasional testing of DCUs for pathogens followed by suitable measures would also make sense.

In summary, our study has shown that the water quality of 60% of the DCUs tested in the St. Gallen region failed to reach the Swiss drinking water standard and the recommendations of the American Centers for Disease Control and Prevention (CDC). Furthermore, several DCUs contained *Pseudomonas aeruginosa* and *Legionella pneumophila,* which poses a risk of infection for both patients and dental staff. However, no conclusion may be made about the actual risk of infection based on the results of this study.

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## Résumé

L'objectif de l'étude était d'identifier la contamination microbienne de l'eau provenant des fauteuils dentaires en utilisant la prévalence de *Pseudomonas aeruginosa, Legionella species* et de bactéries hétérotrophiques comme marqueurs de pollution de l'eau dans la région de St-Gall, Suisse. L'eau (250 ml), soit du tuyau de turbine, de la seringue multifonctionnelle ou du micromoteur, de 76 fauteuils dentaires a été prélevée deux fois (tôt le matin avant la mise en service de tous les instruments et après une utilisation minimum de deux heures du fauteuil dentaire). Avant la mise en service, le nombre total de germes tolérés a augmenté (>300 CFU/ml) dans les échantillons de 46 fauteuils. Après utilisation du fauteuil, cette augmentation n'est observée que dans 29 (38%) fauteuils. *Pseudomonas aeruginosa* ont été mises en évidence dans 6 (8%) fauteuils dans les 2 échantillons. Des légionelles ont été détectées dans les 2 échantillons de 15 (20%) fauteuils: *Legionella anisa* dans 7 échantillons, *Legionella pneumophila* dans 8 échantillons. Les fauteuils dentaires de moins de 5 ans étaient moins souvent contaminés que les plus anciens (25% vs. 77%; $p<0,001$). Cette différence persiste ($p=0,0004$) malgré l'ajustement dans le modèle multivarié par le fabriquant et le lieu de collection. En conclusion, la majeur partie des fauteuils dentaires examinés ne correspond ni aux normes d'hygiène suisses pour l'eau potable, ni aux recommandations des «Center for Disease Control and Prevention (CDC)" américain.


*Mots clés:* eau potable, qualité de l'eau, hygiène des cabinets, *Pseudomonas aeruginosa, Legionella pneumophila*

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