Testing for aerobic heterotrophic bacteria allows no prediction of contamination with potentially pathogenic bacteria in the output water of dental chair units

Der Nachweis aerober heterotropher Bakterien ist kein Prädiktor für eine Kontamination von Dentaleinheiten mit potentiellen Pathogenen

Abstract

Background: Currently, to our knowledge, quality of output water of dental chair units is not covered by specific regulations in the European Union, and national recommendations are heterogeneous. In Germany, water used in dental chair units must follow drinking water quality. In the United States of America, testing for aerobic heterotrophic bacteria is recommended. The present study was performed to evaluate whether the counts of aerobic heterotrophic bacteria correlate with the presence of potentially pathogenic bacteria such as Legionella spp. or Pseudomonas aeruginosa.

Methods: 71 samples were collected from 26 dental chair units with integrated disinfection device and 31 samples from 15 outlets of the water distribution pipework within the department were examined. Samples were tested for aerobic heterotrophic bacteria at 35°C and 22°C using different culture media and for Legionella spp. and for Pseudomonas aeruginosa. Additionally, strains of Legionella pneumophila serogroup 1 were typed with monoclonal antibodies and representative samples of Legionella pneumophila serogroup 1 were typed by sequence based typing.

Results: Our results showed a correlation between different agars for aerobic heterotrophic bacteria but no correlation for the count of aerobic heterotrophic bacteria and the presence of Legionella spp. or Pseudomonas aeruginosa.

Conclusion: Testing for aerobic heterotrophic bacteria in output water or water distribution pipework within the departments alone is without any value for predicting whether the water is contaminated with potentially pathogenic bacteria like Legionella spp. or Pseudomonas aeruginosa.

Keywords: dental chair unit, water, disinfection, Legionella spp., Pseudomonas aeruginosa, aerobic heterotrophic bacteria

Zusammenfassung

Hintergrund: Entsprechend unserer Kenntnis sind die Anforderungen an austretendes Wasser in Wasser führenden Elementen von Dentaleinheiten in keiner Europäischen Richtlinie geregelt. Nationale Empfehlungen sind heterogen. In Deutschland muss in Detaleinheiten eingespeistes Wasser der Trinkwasserverordnung entsprechen. In den Vereinigten Staaten wird die Überprüfung auf aerober heterotrophe Bakterien jedoch empfohlen. Das Ziel der vorliegenden Studie war es zu überprüfen, ob die Zahl aerob heterotropher Bakterien mit der Anwesenheit potentiell pathogener Erreger wie Legionella spp. oder Pseudomonas aeruginosa korreliert.

Methoden: 71 Proben von 26 Dentaleinheiten mit integrierter Desinfektionsanlage und 31 Proben von 15 Wasserauslässen des Wasserversor-
gungsnetzes einer Zahnbehandlungsabteilung wurden untersucht. Sämtliche Proben wurden auf Vorhandensein von aeroben heterotrophen Bakterien bei 35 °C und 22 °C sowie auf das Vorkommen von *Legionella* spp. und *Pseudomonas aeruginosa* analysiert. Zusätzlich wurden alle *Legionella pneumophila* Serogruppe 1 Isolate mittels monoklonalen Antikörpern typisiert und ein Teil dieser mittels Sequenzierung molekularbiologisch näher bestimmt. **Resultate:** Die Untersuchung ergab für aerobe heterotrophe Bakterien eine Korrelation zwischen den unterschiedlich verwendeten Nährmedien, jedoch keine Korrelation zwischen der Anzahl aerober heterotropher Bakterien und der Anwesenheit von *Legionella* spp. oder *Pseudomonas aeruginosa*.

**Schlussfolgerung:** Die Untersuchung von aeroben heterotrophen Bakterien im Auslasswasser oder Wasser der wasserführenden Systems allein ist demzufolge kein Prädiktor für eine Kontamination mit potentiell pathogenen Erregern wie *Legionella* spp. oder *Pseudomonas aeruginosa*.

**Schlüsselwörter:** Dentaleinheit, Wasser, Desinfektion, *Legionella* spp., *Pseudomonas aeruginosa*, aerobe heterotrophe Bakterien

**Introduction**

Dental chair units (DCUs) are equipped with different water lines for supplying instruments like handpieces, turbines or ultrasonic scalers with cooling water, for supplying water/air syringes or for providing the patient with water for rinsing the mouth before/after treatment. These waterlines have been shown to host biofilms resulting in a high density of microorganisms in output water [1], [2], [3]. The clinical relevance of this finding has been discussed controversially. A review article including all relevant Medline publications for the time from 1996 to February 2007 revealed that infections caused by water from DCUs represent rare events [4]. However, contamination of water with bacteria should be avoided for medico-legal reasons. Growth of biofilms in the waterlines was shown to depend on the quality of the supply water [5].

Studies have shown that adding an antimicrobial compound, especially hydrogen peroxide and silver-ion-containing disinfectants, will result in a significant reduction of aerobic heterotrophic bacteria in the output water of a DCU [6]. Regrowth of these microorganisms can be demonstrated already one week after discontinuing the disinfectant [6].

The guidelines of the Centers for Disease Control and Prevention (CDC) and the American Dental Association (ADA) established limits of 500 colony-forming units (cfu)/ml and 200 cfu/ml, respectively [7], [8]. Tests for *Legionella* spp. or *Pseudomonas aeruginosa* are only recommended in certain situations [8]. The advise to test only aerobic heterotrophic bacteria is based on the assumption, that *Legionella* spp. or *Pseudomonas aeruginosa* are part of the biofilm. Disinfection of the water lines of the DCU should cover the entire biofilm. Therefore according to the guideline of the CDC “... no rationale is seen for routine testing for such specific organisms” [8]. Currently, to our knowledge, there is no European standard regarding the quality of output water, and national recommendations are heterogeneous. A symposium held at Trinity College, Dublin, Ireland, in September 2006 reached the consensus that output water quality should comply with the ADA standard (<200 cfu/ml). However, it was noted that this count should not include human pathogens [9]. In Germany, it is recommended that only water of drinking water quality may be used in dental chair units [10].

The present study was intended to evaluate whether the count with aerobic heterotrophic bacteria correlates with the count of clinically relevant pathogens such as *Legionella* spp. or *Pseudomonas aeruginosa*, and whether such testing for aerobic heterotrophic bacteria allowed for any prediction of bacterial load with *Legionella* spp. or *Pseudomonas aeruginosa*. Additionally, the *Legionella pneumophila* serogroup 1 strains identified were typed with monoclonal antibodies and isolates belonging to the same subtype were additionally examined with the sequence based typing.

**Material and methods**

Seventy-one samples from 26 DCUs and 36 samples from 15 outlets of the water distribution pipework within the departments (three independent water lines) of the Bernhard Gottlieb University Clinic of Dentistry were included in this evaluation. The DCUs were exclusively supplied with drinking water from the Vienna water supply system. All DCUs are equipped with an integrated disinfection device. The following DCU types are being used in the various departments: Sirona C3 (Sirona Dental Systems, Bensheim, Germany), Kavo 1062C and 1065T, 1066R (Kavo Dental GmbH, Biberach/Riß, Germany). The setup of the integrated disinfection system shall be described for the type Sirona C3 as representative example. The system consists of a tank being filled with a disinfectant containing hydrogen peroxide and silver ions – Dentosept P (1.41% H₂O₂ and 25 ppm silver ions) /
typing of *L. pneumophila* was performed using the consensus sequence-based scheme (SBT) described by Gaia et al. [16] and the EWGLI (European Working Group for Legionella Infections) SBT database (version 3.0) [17]. Isolates of non-pneumophila *Legionella* spp. were identified using a sequence-based classification scheme targeting the mip gene [18]. The data were evaluated in SPSS – Version 16.0 – (SPSS Inc., Chicago, USA). Apart from frequency calculations (minimum, maximum, median and interquartile range), correlation according to Pearson were tested. For all statistical analyses, p-values <0.05 were considered as significant.

### Results

#### Output water of DCUs

The 26 DCUs showed a median age of 4.8 years. The amount of colony forming units (cfu) of aerobic heterotrophic bacteria differed between the agar according to the ISO EN 6222 and the R2A agar for the output water of DCU. Detailed results are shown in Figure 1. With the agar described in EN 6222 a mean cfu of 20/ml was detected at 36°C and a mean cfu of 37/ml at 22°C. With the R2A agar at 35°C and 3 days incubation period a mean cfu of 220/ml and after 5 days of incubation a mean cfu of 590/ml was detected. With the R2A agar and incubation at 22°C for 5 or 7 days a mean cfu of heterotrophic bacteria of 535/ml or 800/ml, respectively, could be detected.

With the agar according to EN 6222 66.4% of the counts were within the limits specified in the ADA guidelines (<200 cfu/ml) at 36°C and 64.8% at 22°C. With R2A agar (35°C for 3 days or 5 days, 22°C for 5 or 7 days) only 49.3, 42.3, 38.6 and 37.7% of the samples were below the limit of 200 cfu/ml for aerobic heterotrophic bacteria.

A significant correlation (p<0.05) was seen between the two different culture media for aerobic heterotrophic bacteria regardless of the incubation temperature and incubation period. *Legionella* spp. could be found in 39 samples. Out of these 39 samples, in 15 samples either *Legionella pneumophila* or *Legionella anisa* could be differentiated. In 9 samples both *Legionella pneumophila* and *Legionella anisa* could be detected.

All of the 24 *Legionella pneumophila* isolates belonged to the *Legionella pneumophila* SG 1. Typing with the monoclonal antibodies was performed on 9 samples, showing that 8 isolates belonged to the MAb type Oxford/Olda and 1 isolates to the MAb type Bellingham All isolates of the MAb type Oxford/Olda belonged to the SBT 1 while the one isolate of MAb type Bellingham turned out as previously new SBT type now assigned as SBT 847. In 13 samples, the cfu of *Legionella pneumophila* SG 1 was less than 200 cfu/100ml (Figure 2). Only in two samples very high counts (11,000 and 14,000/100 ml)
Figure 1: Aerobic heterotrophic bacteria of 71 samples from the output water of dental chair units. The boxplot diagram shows the median, interquartile range and outlier. Abbreviations: R2A3572 = R2A agar at 35 °C for 72 h; R2A35120 = R2A agar at 35 °C for 120 h; R2A22120 = R2A agar at 22 °C for 120 h; R2A22168 = R2A agar at 22 °C for 168 h.

Figure 2: *L. pneumophila*, *L. anisa* and *P. aeruginosa* of 71 samples from the output water of dental chair units; the boxplot diagram shows the median, interquartile range and outlier.
could be seen. Both samples derived from the same DCU and were taken at an interval of one month (October and November 2009). This DCU was disinfected with chlorine solution by an external contractor after the second detection of high counts of *Legionella pneumophila* SG 1. *Pseudomonas aeruginosa* could not be demonstrated in one of the examined samples. No correlation could be found between the cfu of aerobic heterotrophic bacteria (regardless of the culture medium used) and the count of *Legionella pneumophila* and/or *Legionella anisa*.

In Figure 3, a scatter blot is shown, correlating the cfu of *Legionella* spp. with the cfu of aerobic heterotrophic bacteria yielded on the R2A agar at 35 °C after 3 days of incubation.

**Distribution pipework**

For the samples from the distribution pipework within the departments similar results as in the output water could be seen. Higher amounts of aerobic heterotrophic bacteria could be detected with the R2A agar as compared with the agar used in the EN 6222 (Figure 4). A significant correlation (p<0.05) between the two culture media for aerobic heterotrophic bacteria could be seen, but the correlation depended on the incubation temperature and incubation period. *Legionella* spp. could be found in 24 samples. Out of these 24 samples, in 9 samples *Legionella pneumophila* and in 10 samples *Legionella anisa* could be differentiated. In 5 samples both *Legionella pneumophila* and *L. anisa* could be detected. All 14 *Legionella pneumophila* strains belonged to the SG 1. Only in two of the samples a cfu higher than 200/100 ml was found (Figure 5). Typing of the 7 strains of *Legionella pneumophila* SG 1 with monoclonal antibodies showed that 6 belonged to the Oxford/Olda and 1 to the Bellingham type. Typing by means of sequence base typing showed that the Oxford/Olda belonged to SBT Type 1 and the one Bellingham to the new sequence type SBT 847. *Pseudomonas aeruginosa* was detected in only one case. No correlation could be seen between the load of *Legionella pneumophila* and/or *Legionella anisa* or *Pseudomonas aeruginosa* and the cfu of aerobic heterotrophic bacteria regardless of the culture medium used.

**Discussion**

The R2A agar showed higher counts of aerobic heterotrophic bacteria as compared to the agar used in the EN 6222. The R2A agar is seen as the “golden standard” for testing of output water of DCU [19]. However, there was a significant correlation between the counts obtained with the agar used in the EN 6222 and the R2A agar and also among the different incubation temperatures and incubation periods from the samples of the output water.
Figure 4: Aerobic heterotrophic bacteria of 36 samples from the water of distribution pipework. The boxplot diagram shows the median, interquartile range and outlier.

Abbreviations: R2A3572 = R2A agar at 35 °C for 72 h; R2A35120 = R2A agar at 35 °C for 120 h; R2A22120 = R2A agar at 22 °C for 120 h; R2A22168 = R2A agar at 22 °C for 168 h.

Figure 5: *L. pneumophila*, *L. anisa* and *P. aeruginosa* of 36 samples from the water of distribution pipework. The boxplot diagram shows the median, interquartile range and outlier.
Independent of the agar used, the incubation time, and the incubation period, no prediction can be made regarding the contamination with *Legionella* spp. or *Pseudomonas aeruginosa* based on the number of cfus of aerobic heterotrophic bacteria in the water evaluated. It was surprising that none of the samples of the output water of the DCUs showed contamination with *Pseudomonas aeruginosa*. This finding is in contrast to examinations of DCUs in other locations than the Bernhard Gottlieb University Clinic of Dentistry in Vienna, which frequently showed detection of *Pseudomonas aeruginosa*. The importance of this pathogen had already been pointed out by Martin in 1987 [20]. The reason for the failure to detect this pathogen in the output water may be due to the fact that the pathogen was only once identified in the supply water.

As already described in literature, the contamination with bacteria in DCU is the direct result of the contamination of the supply water [21]. Therefore, the water of the distribution pipework was also examined in this study. It could be shown that only *Legionella pneumophila* SG1 could be isolated and that these isolates derived from 2 strains as seen on the basis of sequence based typing. Depending on the method used only 49.3% (R2A agar 35 °C for 3 days) of the samples in our study were below the limits defined by the ADA. This shows that the integrated disinfection system of the DCU alone is not capable of coping with the growth of aerobic heterotrophic bacteria.

**Conclusion**

It can be concluded that exclusive evaluation for aerobic heterotrophic bacteria in DCU as contamination indicator does not allow for any conclusion as to the presence of contamination with *Legionella* spp. or *Pseudomonas aeruginosa*. Therefore, examination of distribution pipework or output water of DCUs for *Legionella* spp. and *Pseudomonas aeruginosa* is strongly recommended.

**Notes**

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgements**

We thank Radica Paunovic, Maria Voboril, Sonja Rehak and Petra Hasenberger for excellent technical assistance.

**References**

1. Pankhurst CL. Risk assessment of dental unit waterline contamination. Prim Dent Care. 2003;10(1):5-10. DOI: 10.1308/135576103322504030
2. Walker JT, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, Østergaard E, Ten Cate JM, Moorer WR, Schel AJ, Mavridou A, Kamma JJ, Mandilara G, Stösser L, Kneist S, Araujo R, Contreras N, Gorony-Cermes P, O'Quinlane D, Burke F, Forde A, O'Sullivan M, Marsh PD. Microbiological evaluation of dental unit water systems in general dental practice in Europe. Eur J Oral Sci. 2004;112(5):412-B. DOI: 10.1111/j.1600-0722.2004.00151.x
3. Singh T, Coogan MM. Isolation of pathogenic Legionella species and legionella-laden amoebae in dental unit waterlines. J Hosp Infect. 2005;61(3):257-62. DOI: 10.1016/j.jhin.2005.05.001
4. Pankhurst CL, Coulter WA. Do contaminated dental unit waterlines pose a risk of infection? J Dent. 2007;35(9):712-20. DOI: 10.1016/j.jdent.2007.06.002
5. Pankhurst CL, Johnson NW, Woods RG. Microbial contamination of dental unit waterlines: the scientific argument. Int Dent J. 1998;48(4):359-68. DOI: 10.1111/1785-595X.1998.tb00697.x
6. O'Donnell MJ, Shore AC, Coleman DC. A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: a one-year study. J Dent. 2006;34(3):648-61. DOI: 10.1016/j.jdent.2005.12.006
7. ADA statement on dental unit waterlines. J Am Dent Assoc. 1996;127(2):185-6. Available from: http://adajournal.com/content/127/2/185.full.pdf+
8. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM; Centers for Disease Control and Prevention (CDC). Guidelines for infection control in dental health-care settings - 2003. MMWR Recomm Rep. 2003;52(RR-17):1-61.
9. Coleman DC, O'Donnell MJ. Guest editorial. J Dent. 2007;35(7):699-700. DOI: 10.1016/j.jdent.2007.07.001
10. Trinkwasserordnung (TrinkwV). BGBl. 2001:959
11. ISO 6222:1999. Water quality–Enumeration of culturable microorganisms – Colony count by inoculation in a nutrient agar culture medium. May 15, 1999.
12. Eaton AD, Clesceri LS, Greenberg AE. Standard methods for the examination of water and wastewater. 19th ed. Washington D.C: APHA; 1995.
13. ÖNORM EN ISO 16266:2008. Water quality – Detection and enumeration of Pseudomonas aeruginosa – Method by membrane filtration. May 1, 2008.
14. ISO 11731-2:2004. Water quality – Detection and enumeration of Legionella spp. Part 2: Direct membrane filtration method for waters with low bacterial counts. May 01, 2004.
15. Helbig JH, Bernander S, Castellani Pastoris M, Etienne J, Gaia V, Lauwers S, Lindsay D, Lück PC, Marques T, Mentula S, Peeters MF, Pelaz C, Struelens M, Uldum SA, Wewalka G, Harrison TG. Pan-European study on culture-proven Legionnaires’ disease: distribution of Legionella pneumophila serogroups and monoclonal subgroups. Eur J Clin Microbiol Infect Dis. 2002;21(10):710-6. DOI: 10.1007/s10096-002-0820-3
16. Gaia V, Fry NK, Harrison TG, Peduzzi R. Sequence-based typing of Legionella pneumophila serogroup 1 offers the potential for true portability in legionellosis outbreak investigation. J Clin Microbiol. 2003;41(7):2932-9. DOI: 10.1128/JCM.41.7.2932-2939.2003
17. The European Working Group for Legionella Infections (EWGLI). Legionella pneumophila Sequence-Based Typing. Available from: http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php
18. Ratcliff RM, Lancer JA, Manning PA, Heuzenroeder MW. Sequence-based classification scheme for the genus Legionella targeting the mip gene. J Clin Microbiol. 1998;36(6):1560-7.

19. Karpay RI, Plamondon TJ, Mills SE, Dove SB. Validation of an in-office dental unit water monitoring technique. J Am Dent Assoc. 1998;129(2):207-11.

20. Martin MV. The significance of the bacterial contamination of dental unit water systems. Br Dent J. 1987;163(5):152-4. DOI: 10.1038/sj.bdj.4806220

21. Coleman DC, O'Donnell MJ, Shore AC, Russell RJ. Biofilm problems in dental unit water systems and its practical control. J Appl Microbiol. 2009;106(5):1424-37. DOI: 10.1111/j.1365-2672.2008.04100.x

**Corresponding author:**
Dr. Margit Bristela
Bernhard Gottlieb University Clinic of Dentistry, Medical University Vienna, Sensengasse 2a, 1090 Wien, Phone: +43 (0)1 40070/4901, Fax: +43 (0)1 40070/4909 margit.bristela@meduniwien.ac.at

**Please cite as**
Bristela M, Skolka A, Schmid-Schwap M, Pieslslinger E, Indra A, Wewalka G, Stauffer F. Testing for aerobic heterotrophic bacteria allows no prediction of contamination with potentially pathogenic bacteria in the output water of dental chair units. GMS Krankenhaushyg Interdiszips. 2012;7(1):Doc12. DOI: 10.3205/dgkh000196, URN: urn:nbn:de:0183-dgkh0001966

**This article is freely available from**
http://www.egms.de/en/journals/dgkh/2012-7/dgkh000196.shtml

**Published:** 2012-04-04

**Copyright**
©2012 Bristela et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc-nd/3.0/deed.en). You are free: to Share — to copy, distribute and transmit the work, provided the original author and source are credited.