

Department of Clinical Dentistry

Faculty of Health Sciences

Quality of Dental Unit Water at University Dental Clinic, Private and Public Clinics in Tromsø

Investigation of bacteria levels in water samples in dental chair water lines

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Abstract

Exposure to water with high bacterial counts violates the basic principles of clinical infection control in dental clinics. Therefore, bacterial counts in water that comes from the dental chair unit should always be evaluated. As guideline water that comes from hand pieces/three-way syringes should have less than 200 colony forming units per one mL of water.

Aims: The aim of this study is to investigate levels of bacteria in dental unit water at University Dental Clinic (UDC), public and private clinics in Tromsø, and assess the hygiene procedures for dental unit water at the different clinics.

Materials and methods: Water samples from dental chair at UDC, public and private clinics were obtained for microbiological analysis. Water samples were cultivated in agar plates and in prefabricated sample kits to investigate the growth of bacteria. Digital droplet PCR (ddPCR) was used to evaluate the bacterial load in these samples.

Results: Differences in the amount of bacteria in water samples between UDC, private and public clinics were detected. UDC had lower bacterial load in the dental unit water than the private and the public clinics. There was a significant difference in level of *E.coli* counts, Heterotrophic plates counts (HPC) and the ddPCR counts between the UDC and the other two groups. There was also a significant difference between the private and the public clinics, in level of bacteria determined by the ddPCR. The public clinics had the highest level of bacteria in the water from the dental chair units.

Conclusion: It seems that water coming from the dental chairs at UDC have the lowest bacterial load than the water obtained from the dental chairs at public and private clinics. The reason of high bacterial loads in the private and public clinics could be suboptimal infection control measures in these places. In addition, high bacterial load could be attributed to old pipe system and lack of stringent water filtration system within the clinics. Even though the study has shown high levels of bacterial load we cannot conclude that dental unit water is considered to be a risk for immunocompromised patients. This is because of, no attempt has been done to identify, what kind of bacteria that are present in the water and if these bacteria are pathogenic or not.

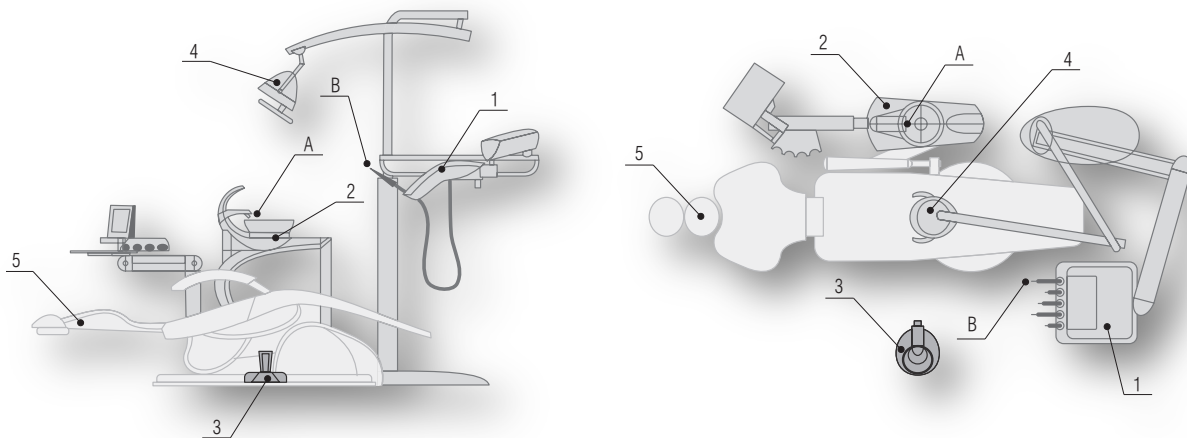
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1. Dental unit water

1.1 Construction of a dental chair unit

Dental chair unit (DCU) is an essential item of equipment necessary for the practice of clinical dentistry. It provides a variety of different demands like air supply, electrical power and water. A basic dental chair unit includes the chair, a light source, a tool panel, a spittoon and water sucks. Water is used for the purpose to cool instruments like conventional dental hand pieces, high-speed turbines, ultrasonic scalers and the three-way syringe. Next to the chair is a spittoon that also supplies a valve that fills a disposable cup with water for the patient to rinse (Figure 1). The water conduit in the DCU is composed of approximately 5 meters of narrow bore plastic tubes having an internal diameter of 2-6 mm and contains approximately 35 mL of water (1). This network of tubes is called dental unit waterlines (DUWLs) (2).



1- tool panel, 2 – spittoon, 3 – chair foot control, 4 – dental light, 5 – dental chair. B – sampling point.

Figure 1: Construction of dental unit and sampling points. Adopted from: Michalkiewicz M., Ginter-Kramarczyk D., Kruszelnicka I.K.; Is water in dental units microbiologically safe? *Medycyna Pracy* 2015; 66(6):763-770

1.2 Quality and guidelines of dental unit water

Water supplied for dental chair units in Tromsø is municipal water that is provided from Tromsø municipality. The water quality is of drinking water standard that means it is free of pathogens and dangerous levels of chemicals/minerals. In Norway there are guidelines for basic microbiological and chemical requirements for general drinking water. Regulations that govern the quality of drinking water stated that the water must be free from physiological, chemical and biological components that is a potential risk for human health (3). Recommendation from the European Union (EU) concerning drinking water is set to no more than 100 colonies forming units of mesophilic, aerobic and heterotrophic bacteria per millilitre (>100 CFU/mL) (4). There are no specific requirements concerning the quality of water that is supplied to dental units in European countries (5, 6). However, reference is made in some countries to the drinking water standards (4). Norway does not have specific recommendations regulating dental unit water quality.

In the USA, The American Dental Association (ADA) and The Centers for Disease Control and Prevention (CDC) stated that use of poor water during dental treatment is inconsistent with patient expectations of safety and standards of modern dentistry. ADA recommends that coolant water used in non-surgical dental procedures meets regulatory standards for drinking water, which is less than or equal to 200 CFU/mL (6-8). They also recommend that infection control measures should be included in dental practices to maintain bacteria counts within a safe range.

1.3 Microbial contamination of dental unit water

A DCU is categorized as a medical device under the EU Medical Devices Directive. In health care environments, medical devices have been shown to be an important cause of cross-contamination and cross-infection (2).

At dental clinics the DCU is used in treatment of many patients each day and microbial contamination of the water supply can be a potential source of cross-contamination. Colonization of microorganisms within the waterlines may not be a concern to healthy individuals. However, it might place elderly or immunocompromised patients at unnecessary risk.

Within the narrow bore tubes of the DCU water flows freely at the centre, leaving a thin layer of undisturbed water around the walls (9). This thin immobile layer of fluid is called the hydrodynamic

boundary layer, and act as the interface of the lumen wall and the moving water within the DUWL (10). That allows formation of a conditioning pellicle that let bacteria in water adhere through weak, reversible van der Waals forces and afterwards attach themselves more permanently by other cell attachment and adhesion mechanisms (Figure 2). Early colonizers in the DCU supply water provide more diverse adhesion sites for other microorganisms, called secondary colonizers, giving rise to microcolonies (11). This interaction with solid surfaces, like the tubes of DCU, will eventually give rise to biofilm formation (1).

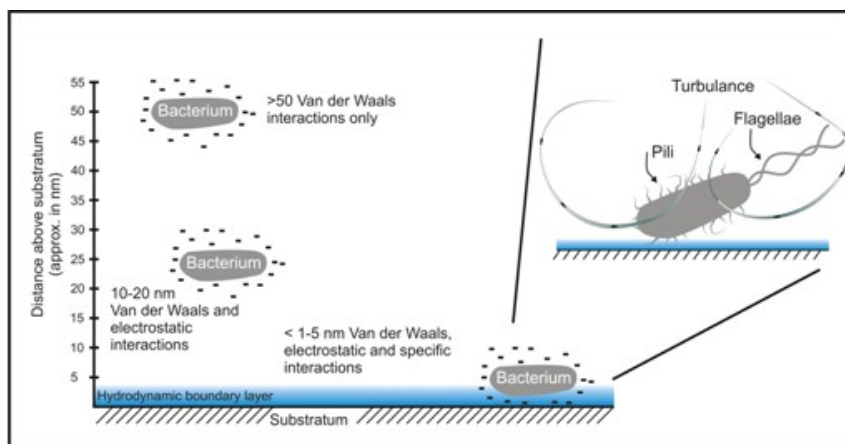


Figure 2: Hydrodynamic boundary layer and Van der Waals forces. Adopted from:

http://www.hypertextbookshop.com/biofilmbook/working_version/contents/chapters/chapter002/section002/blue/page001.html. Date: 27.03.2017

The tubes of DCU are composed of polyurethane and polyvinylchloride (PVC). This is a favourable substrate for biofilm formation because of the hardeners and additives that can be used as nutrient for bacteria (12). In addition water stagnation during non-working hours/days when the equipment is not being used encourage proliferation of bacterial growth and biofilm formation (1).

When water flows through DUWLs microorganisms detach from the biofilm and consequently contaminates the output water flowing through the water system. That may cause development of a biofilm in another part of the installation, and it may also flow directly into the patients' mouth during dental treatment procedure. In addition, dental-health care workers may be exposed to aerosols generated by dental units. Detachment of microorganisms from the biofilm is substituted by new attachments of bacteria during water stagnation (1, 13).

Microorganisms from the human oral cavity may initiate the development of biofilm in DUWLs as oral fluids (e.g. saliva and blood) can be retracted into DUWLs during the treatment procedure (2). Therefore, the dental instruments should contain integrated anti-retraction valves that prevent backflow of fluids from the oral cavity into the DUWLs (13). Previous studies have detected oral bacteria in DUWLs and provided convincing evidence for failure of previously installed anti-retraction valves (2).

The first report describing the presence of microorganisms in DUWLs was published in 1963, and it was recognized that water contained large number of organisms in the range of 10^4 to 10^6 CFU/mL (14). In 2002 the first report concerning DUWLs was published in Norway (15), followed by the another Norwegian report from 2005 (1). A Norwegian review article from 2002 concluded that studies have shown far higher bacteria counts in DUWLs than that of drinking water (15).

A wide variety of bacteria, protozoa and fungi have been recovered from DUWLs. The majority of contaminants are gram-negative aerobic species, which are non-pathogenic heterotrophic bacteria (16). Although opportunistic respiratory pathogens such as *Legionella pneumophila*, *Pseudomonas aeruginosa* and non-tuberculosis *Mycobacterium* have been detected (2, 9, 17). *Pseudomonas* and related species are the predominant bacteria found in DUWLs, and *Pseudomonas aeruginosa* is a common colonizer. *P. aeruginosa*, can survive and grow in low nutrient environments such as distilled water, and furthermore, in diluted disinfectants such as chlorhexidine and it express resistance to a wide range of antibiotics (7). In healthy individuals the infective dose of this bacteria is $> 1,5 \times 10^6$ CFU/mL (18). Fortunately, in DUWLs such high numbers of bacteria is not found, but because of antibiotic resistance and immunocompromised patients, like cystic fibrosis patients, become more sensitive to this opportunistic pathogen (19).

1.4. Biofilm in DUWLs

Biofilm is an assemblage of microorganisms that are irreversible attached to a surface and embedded in a extracellular matrix that consists of water and macromolecules derived from microbes (20). The matrix provides architectural structure and mechanical stability to the attached bacteria. It contains internal channels and void spaces in which nutrients can circulate, and therefore biofilm communities are able to develop thickness and complexity. Biofilm cells have phenotypic properties

that are distinct from their unattached counterparts. For example, within the biofilm genes are exchanged by natural transformation or conjugation (21). Additionally to work as a three-dimensional network, biofilm also has a protective role to its members, by protecting them against the action of antimicrobial agents. It is suggested that the matrix works like a physical barrier, preventing entry of those agents into the microbial community (22, 23).

Biofilms grow on living or inert surfaces, and can attach to a variety of materials such as human tissue (teeth, mucosa, heart valves and contact lenses), industrial water pipes and in health-care settings such as DUWLs. Surfaces that are exposed to an aqueous medium are coated with molecules that form a conditional film. In DUWLs this layer may be composed of organic or inorganic molecules that reflects the chemistry of the water (4).

1.5 Biofilm control in DUWLs

DUWLs must be regularly maintained in order to deliver water of an optimal microbiologic quality. It has been shown that the level of microorganisms in untreated dental unit waterlines can reach more than 500 CFU/mL, which exceeds the drinking water standard (4). CDC recommended in 2016 that dental unit waterlines should be flushed in the beginning of the clinic day to reduce the microbial load. Flushing with fresh water does reduce the density of microbes, but this practice has been shown in different studies that it does not remove the established biofilm in the waterlines (2, 8, 24). Therefore, one or more commercial devices and procedures to disrupt the biofilm and improve the quality of water should be employed.

To improve the water quality microbial filters placed before the water flows into the DCU is effective as it filters the municipal water that is not sterile. The filters have to be changed regularly as they become clogged after some time. However, filters have no effect whatsoever on existing biofilm in the DUWLs (2).

The most efficient method for removing biofilm from DUWLs is regular disinfection with chemical, biocide or cleaning agent, which will result in good quality water (4, 13, 25-28). Common products that are used for disinfection and removal of biofilm in DUWLs are based on a range of compounds, including sodium hypochlorite, chlorine dioxide, chlorhexidine, peroxides and citric acids (29-35).

University Dental Clinic (UDC) in Tromsø has a manual for disinfection routines. Before every treatment and between patients the air/water syringe, turbine, hand piece and scaler are flushed through. This procedure reduces the number of bacteria in the water system and prevents water that has been retracted during treatment to become a source of infection.

Once a week the syringe, high speed turbine, hand piece and scaler will be set on a rinse of disinfection cycle which will run through DUWLs and clean them. At UDC the disinfection agent Oxygenal is regularly used. Oxygenal contains hydrogen peroxide which will reduce the microbial load in the waterlines (36). Studies have shown this procedure to be highly effective in reducing biofilm, which has shown to reach 99,2% reduction of the biofilm in DUWLs after one single treatment (26). Hydrogen peroxide-based disinfectant has shown to achieve no more than 200 CFU/mL of mesophilic heterotrophic bacteria in unfiltered output water (34).

Additionally, before the waterline comes into dental units at the UDC, it goes through several filters (Figure 3). The first filter is just after municipal water enters into the UDC and then the water flows through additional fine filter system. Before the municipal water goes into the DUWLs in all units, it flows again through a second filter and then a last filtration inside the unit.

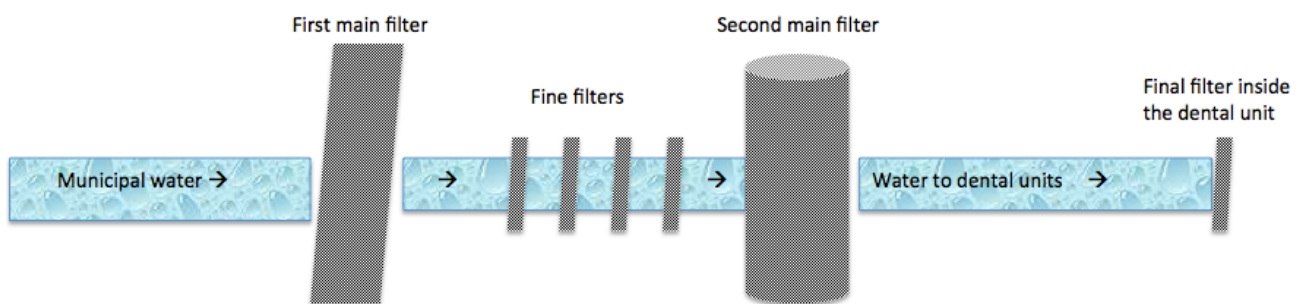


Figure 3: The filtration system at University Dental Clinic

All filters in the UDC are changed once a year. In case of clogging they are changed even more frequently. The technical manager is responsible for these tasks at UDC. Filters will have no effect on the development of biofilm in the waterlines, but will remove microorganisms as the water is flushed out of the water system.

1.6 Infection control of DUWLs

To prevent waterborne contamination of DUWLs, a proper infection control precautions should be performed. This includes using water that meets recommendations for potable water (less than 200 CFU/mL). Between patients, water and air should be discharged for a minimum of 20–30 seconds from any dental device connected to the DUWLs that enter the patient's mouth (for example handpieces and three-way syringe). The DUWLs manufacture should be consulted to determine suitable methods and equipment to obtain the recommended water quality, and methods for monitoring the water to ensure quality is maintained. Different dental chair manufactures may recommend specific products to be used, and before any chemical agent is introduced to the DCU the manufacture should be consulted. In addition, the dental practitioner should consult the dental unit manufacturer on the need for periodic maintenance of anti-retraction mechanisms (4, 24).

2. Digital droplet polymerase chain reaction (ddPCR)

In our project we have used digital droplet PCR (ddPCR) to quantify bacteria in water samples. The 16S rRNA gene was used as a genetic marker for bacterial presence. The 16S rRNA gene is a very short section of prokaryotic DNA, with about 1542 nucleotide bases, found in all bacteria. Although these genes will have different nucleotides scattered in the gene sequence, the gene has conservative regions in the beginning and the end of the gene sequence between organisms (37).

In ddPCR the samples will be divided into a very large number of small droplets in a water-oil emulsion system. The amplification/PCR reaction takes place in each droplet. Thermal cycling is performed to endpoint. Wells with target gene 16S rRNA present, will become brightly fluorescent. The droplet reader will analyse and count both positive (target gene present) and negative reactions (target gene not present). The total number of positive reactions is then used to calculate the number of original target genes in the original sample.

3. The aim of our study

The aims of this study were to investigate the presence and levels of bacteria in dental unit water at the UDC, public and private clinics in Tromsø, and assess the hygiene procedures for maintaining proper quality of water in the dental units at the three different dental clinics. Since there are no recommendations in Norway concerning how many colonies per millilitre in DUWLs are acceptable,

we also investigated the current levels of bacteria in the DUWLs. It was also our interest to assess knowledge about hygiene procedures and routines among dentists in Tromsø. In other words, our main research question was “Is the dental unit water at UDC, private and public dental clinics in Tromsø, microbiological safe?”

4. Materials and methods

4.1 Research methods

Before we started on our laboratory project we did pilot tests to decide what kind of cultivation plates most suitable for our project. We used two different cultivation mediums. The first one is anaerobe blood agar and the second plate is aerobic blood agar.

The anaerobe agar plate contains horse blood and peptones. This is a medium for fastidious bacteria, such as *Neisseria gonorrhoea*. Vitamin K and sodium succinate may be added to give essential growth factors for some anaerobes (38).

The aerobic agar plate, also known as Mueller Hinton, contains beef extract and acid hydrolysate of casein that supplies amino acids, nitrogenous substances, vitamins and minerals necessary for growth. It also contains small levels of thymine and thymidine. Sheep blood may be added to improve the growth of fastidious bacteria, such as *Campylobacter species* (39).

We also used three different prefabricated samplers test kits. One kit that provides growth for *E-coli*, one for heterotrophic bacteria and one for yeast. We transferred 18 ml of each water sample to each filter at the samplers test kits. These were incubated at 37°C for 72 hours.

4.2 Water samples

In order to compare the water quality between the three clinics we collected data from growth of anaerobic bacteria, aerobic bacteria, *E.Coli*, *HPC* and bacterial counts obtained from ddPCR results. The water samples were collected from the air/water syringe from five different dental clinics in Tromsø; the UDC (n=15), two public dental clinics (n=12) and two private dental clinics (n=12). All water samples were collected in the morning at 08:00 am in 50 mL sterile Falcon tubes. Instructions were made to the clinics that the air/water syringe should not be flushed through before the water was sampled.

The water samples were then transferred to the laboratory at the Department of Clinical Dentistry (IKO) for further analysis. All were immediately plated into two different blood agar plates (500 µL on each plate) and placed in the incubator at 37°C for 48-72 hours. Both the aerobic agar plates and the anaerobic agar plates were grown aerobically.

4.3 Digital Droplet PCR (ddPCR)

The ddPCR was used to find the quantity of bacteria in water samples. The ddPCR mastermix consists of 10µL supermix (ddPCR supermix for probes (no dUTP)), 1µL primer/probe for 16S rRNA gene (FAM) and 9µL water sample (DNA). Then the master mix was transferred into microtiter plate wells after droplets generated to start the ddPCR reaction. In brief, each sample will be divided into approximately 20 000 small droplets. The DNA amplification takes place in each droplet by using PCR machine (C1000 Touch™ Bio-Rad). When amplification was completed, each well were analysed individually by the droplet reader (Droplet Reader QX200™ Bio-Rad). After the reading is completed, the data will directly be transferred into QuantaSoft™ software, where the quantity of the target DNA is calculated statistically.

4.4 Statistical analysis

The results obtained from the cultivation plates, Samplers test kits and the ddPCR were analysed by SPSS Statistical software (version 22.0) for any significant difference between the three clinics. Since the data was not normally distributed we applied the nonparametric Mann-Whitney U Test, with colony count as the dependent variable, and the clinic as the independent variable.

4.5 Questionnaire

A questionnaire was delivered to each participating clinic to collect information regarding infection control procedures and routines of the DUWLs, any history of water system problems encountered in the clinics, and other issues that might influence or explain presence of bacteria in DUWLs. In addition, information about filtration systems and cleaning routines at UDC was obtained from the custodian.

5 Results

5.1 Results of the microbiological tests

Table 2, 3 and 4 shows the results of the microbiological tests from the 39 water samples obtained from the different clinics. The laboratory results obtained from a total of 15 dental chairs from UDC are presented in Table 2. The laboratory results obtained from a total of 12 dental chairs from two private dental clinics are presented in Table 3. The laboratory results obtained from a total of 12 dental chairs from two different public dental clinics are presented in Table 4. Yeast was not found in any of the 39 water samples. *E.coli* were found in 15 of the 39 water samples. *HPC* were found in 13 out of 39 water samples. A total of 29 samples showed growth in anaerobe plates whereas a total of 28 water samples showed bacterial growth in aerobic plates. At agar plates and samplers kits, bacterial counts ranged from 0 to over 400 CFU per mL.

In UDC we found growth of anaerobic colonies in 73.3% of the water samples. Range from 2 to 14 colonies. In both private and public clinics we found growth in 75% of the plates with a range from 2 to more than 400 colonies. Private clinics had only one water sample with more than 400 colonies, and public had two water samples over 400 colonies. Mean value of the anaerobic colonies in UDC was 4.67 CFU/mL and median at 4.0. The privates mean value at 62.50 CFU/mL and median at 2.0. The public had a mean value at 71.58 CFU/mL, median 4.0. There was an equal number in percent of clean and filthy water samples from the three clinics, but difference in grade of filthiness in between the groups. Anyhow, the median tells us that there are not as big difference between the three groups since there are only a few water samples that contain considerably more anaerobic bacteria increasing the mean value. There was no significant difference between the clinics.

There were growth of aerobic bacteria in 86.7% of samples in UDC, 66.6% in private and 58.3% in public. Anyhow, the UDC had more water samples with aerobic colonies than private and public, the mean value was lowest with 4.27 and median 2.0 CFU/mL, with a range 0-14 colonies. Mean value of aerobic colonies in private was 49.5 CFU/mL with a median at 7.0, range from 0 to over 200 colonies. For the public, mean value at 47.5CFU/mL and median 3.0 CFU/mL, range from 0 to over 400 colonies. There was no significant difference between the clinics.

No growth of *E.coli* at UDC. In private clinics 66.6% of the water samples contained *E.coli*, with a

mean value at 23.5 CFU/mL and median 3.0 CFU/mL, range from 0 to over 200 colonies. In public clinics, 58.3% of the water samples contained *E.coli*, mean value 11.58 CFU/mL, and median 1.0 CFU/mL, range from 0 to 74 colonies. There was a significant difference between UDC and private clinics, UDC and public clinics, but no significant difference between public and private clinics.

No growth of *HPC* was found in water samples from UDC. Private clinics showed *HPC* in 58.3% of the water samples, and public clinics in 50% of the water samples. Private clinics had a mean value at 72.17 CFU/mL and median 5.0 CFU/mL, range from 0 to over 200. Public clinics had a mean value at 16.33 CFU/mL and a median 0.5 CFU/mL, range from 0 to 140 colonies per ml. From the public clinics, 50% of the water samples had no *HPC* and only four of the water samples contained more than 1 colony per mL. There was a significant difference between UDC and private clinics, UDC and public clinics, but no significant difference between public and private clinics.

Results from the ddPCR showed a big difference between the groups. ddPCR tells us the exact number of copies of the gene *16S rRNA* that is present in all bacteria. Mean value of copies in the water samples from UDC was 360.33, with a range from 0 to 1925. Mean value in private clinics was 32 148.67 with a range from 183 to 68 148. Mean value in public clinics was 109 280.50, with a range from 26 852 to 612 592. The results of the amount of copies from ddPCR we can use as an estimate of amount of bacteria per millilitre. With this estimate we can say that the water sample containing the highest amount of bacteria, was one from the public clinics and had 612 592 bacteria/mL. There was a significant difference between all the groups.

Table 1: Statistics showing mean value, median and range of bacterial counts for University Dental Clinic, private and public clinics

Place of sample		Anaerobic count	Aerobic counts	E.coli counts	HPC counts	Yeast counts	ddPCR
UDC	N Valid	15	15	15	15	15	15
	Mean	4,7	4,3	0	0	0	360,3
	Median	4	2	0	0	0	111
	Mode	0	2	0	0	0	48
	Range	14	14	0	0	0	1906
	Minimum	0	0	0	0	0	19
	Maximum	14	14	0	0	0	1925
Private	N Valid	12	12	12	12	12	12
	Mean	62,5	49,5	23,5	72,2	0	32148,7
	Median	2	7	3,0	5	0	32870,5
	Mode	2	0	0	0	0	183
	Range	400	200	200	200	0	67965
	Minimum	0	0	0	0	0	183
	Maximum	400	200	200	200	0	68148
Public	N Valid	12	12	12	12	12	12
	Mean	71,6	47,5	11,6	16,3	0	109280,5
	Median	4	3	1	0,5	0	62592
	Mode	0	0	0	0	0	26852
	Range	400	400	74	140	0	585740
	Minimum	0	0	0	0	0	26852
	Maximum	400	400	74	140	0	612592

Table 2: Microbiological findings of the 15 samples from the University Dental Clinic

UDC Unit number	Plates (CFU/mL)		E-Coli	Sample Kits		ddPCR 16S rRNA/mL
	Anaerobe	MH		HPC	Yeast	
1	-	12	-	-	-	22
2	8	2	-	-	-	19
3	2	4	-	-	-	156
4	2	-	-	-	-	111
5	6	2	-	-	-	537
6	6	2	-	-	-	611
7	4	8	-	-	-	259
8	14	8	-	-	-	56
9	-	2	-	-	-	133
10	-	4	-	-	-	81
11	-	2	-	-	-	85
12	4	-	-	-	-	48
13	10	2	-	-	-	1314
14	8	14	-	-	-	48
15	6	2	-	-	-	1925

Table 3: Microbiological findings of the 12 samples from the Private Dental Clinics.

First Private Clinic	Plates (CFU/mL)		E-Coli	Sample Kits		ddPCR 16S rRNA/mL
	Anaerobe	MH		HPC	Yeast	
1	-	8	-	106	-	35 593
2	12	158	46	-	-	183
3	320	180	-	-	-	56 222
4	-	-	11	-	-	740
5	>400	-	>200	>200	-	2407
6	2	6	3	-	-	30 148
Second Private Clinic						
1	2	-	1	2	-	68 148
2	-	-	-	-	-	344
3	2	24	-	8	-	51 592
4	2	4	4	>200	-	24 111
5	4	200	3	>200	-	57 037
6	6	14	14	150	-	59 259

Table 4: Microbiological findings of the 12 samples from the Public Dental Clinics

First Public Clinic	Plates (CFU/mL)			Sample Kits		ddPCR
	Anaerobe	MH	E-Coli	HPC	Yeast	16S rRNA/mL
1	12	-	16	1	-	67 777
2	3	116	42	10	-	57 407
3	>400	20	74	36	-	131 111
4	18	-	-	-	-	100 000
5	4	2	-	-	-	39 370
6	>400	>400	1	-	-	26 852
Second Public Clinic						
1	-	-	2	8	-	43 555
2	-	4	-	-	-	41 407
3	16	-	3	1	-	41 296
4	-	24	-	140	-	612 592
5	4	4	1	-	-	75 185
6	2	-	-	-	-	74 814

Table 5: Statistical differences in bacterial counts between University Dental Clinic, Private and Public Dental Clinics

	Private Clinics			Public Clinics		
	High E-coli counts	High HPC counts	High 16S rRNA counts	High E-coli counts	High HPC counts	High 16S rRNA counts
UDC	$p<0.0001^*$	$p<0,001^*$	$p<0,0001^*$	$p<0,001^*$	$p<0,003^*$	$p<0,0001^*$
Private clinics						$P<0,015^*$

*Statistically significant

We categorized the samples into low, medium or high estimated on the number of copies that were found using ddPCR. In UDC all of the 15 samples had beneath 2000 copies and categorized as low. In the private dental clinics three of the samples were categorized as low, one sample as medium and eight samples as high. In the public clinics, all the 12 samples were categorized as high.

Table 6: The samples categorised as low, medium and high for the number of 16S rRNA that were found using ddPCR.

Clinic	Low (1-2000)	Medium (2000-5000)	High (>5000)
Private	3	1	8
Public	0	0	12
UDC	15	0	0

5.2 Results of the questionnaire

We were interested to find out about the hygiene routines performed by the private and public clinics. All the private and public clinics informed that the dental assistant has the responsibility to implement the hygiene procedures. Flushing through the hand pieces is carried out daily in all clinics. Disinfectant is also used in a daily basis. The public clinics used the disinfectant Metasys, one of the private used Orotol, and the other private used Oxygenal same as UDC. The differences in the effectiveness of these disinfectants are not known.

There is a great variation in when the units were installed at the clinics. The private clinics had units that ranged from 0-10 years old, while the public clinics have units that range from 5 to more than 10 years old.

Only one private clinic knows how many filters the water runs through, and answered three to four, all clinics are aware that the filters are getting changed annually, in conjunction with service or by instruction from the unit. Only one of the clinics has experienced problems with the water quality the last year, where the problem was discoloration of the water caused by digging and construction work in the area.

Questions to obtain information regarding sterilization procedure and which type of autoclave the clinics use revealed that, the public clinics and one of the private clinics use Class B autoclave. The last private clinic did not know what class of autoclave they have. Neither of the private clinics knew the temperature and time required for sterilization in autoclave. One of the public clinic answered that the autoclave is used at 121°C in 15 minutes, and the second public clinic answered 134°C for 5 minutes. The public clinics validated their autoclaving monthly, and private answered monthly or yearly. Questions to obtain information regarding the different systems for validating of autoclavation process revealed that both private clinics and one public clinic have system in place for validation. However, in the next question where they were asked which type of validating system they used, all three clinics answered “Don’t know”.

We were also interested to know if the public and private clinics have special procedures for handling immunocompromised and MRSA affected patients in their daily practice. Both private and public clinics have reported the same hygiene procedures in handling immunocompromised patients and MRSA patients compared to other normal patients. All clinics do not also treat these patients at a special time of the day. All of the clinics answered that they do not ask patients if they have been treated medically and/or dentally abroad in the last 6 months.

6. Discussion and conclusion

The questionnaire pointed out that all dental clinics included in this study do have hygiene routines and protocols to maintain appropriate water quality that is safe for all patients.

All clinics reported flushing water through the air/water syringe and hand pieces every morning before handling of any patients. In addition, all the clinics reported the use of disinfection through the tubing system once a day. Unfortunately, our study has shown that the water supplied in some of the obtained samples is no even of a drinking water quality as it exceeds 200 CFU/mL. Even small amount of bacteria in the water may eventually lead to development of biofilm on the inner surface of the tubing system. Biofilm is considered a reservoir of different colonies of bacteria and will eventually affect the quality of the water if left undisturbed.

In our analysis of the DUWLs it is revealed that not all of the dental clinics have water in their DUWLs that meets the recommendations of drinking water. In some of the tested samples, there

were high concentrations of heterotrophic and anaerobe bacteria. Some of the samples contain more than 200 colonies of heterotrophic bacteria and more than 400 colonies of anaerobe bacteria. Even the presence of *E.coli* was found in some of the samples. The DUWLs should not constitute any health hazard to patients, because the water provided to the system, is municipal water which theoretically should meet the requirements of drinking water quality. However, biofilm formation in the tubing system could be a source of this contamination.

In our study we, did not report the presence of specific bacteria in the cultured samples because of cost and time limitation. However, detection of high amounts of heterotrophic bacteria counts suggests that there are ideal conditions for bacteria growth and biofilm formation, and opportunistic pathogens could be among these bacteria. The identification of specific bacteria in a biofilm has been done in other studies and there are numerous examples of bacteria found in dental unit water. Pathogens such as *Legionella pneumophila* and *Pseudomonas aeruginosa* were among those bacteria detected, and these could infect immunocompromised patients (40-42).

The results obtained from bacteria culturing on plates and sample kits are generally lower than that obtained from ddPCR. The ddPCR showed great numbers of copies of the gene 16s rRNA, especially at the private and public clinics. We know that on cultivation plates and sample kits only detect live bacteria that can grow on those plates, but in ddPCR we detect both dead and alive bacteria. It is also a possibility that some of the bacteria could not grow on the media used, because of lacks of optimal growth conditions for specific bacteria. On the other hand, those bacteria detected in in ddPCR can be of different types and may be they would have grown under other growth conditions not provided with the plates used herein. Furthermore, the gene used in ddPCR, i.e. 16S rRNA, as a genetic marker for bacteria could be present in each bacterium from 1 to 10 copies. Since we do not know exactly what bacteria is present in the DUWLs, and, hence, how many copies of the 16S rRNA gene, for simplification we assumed that each bacterium in our water samples have approximately 6 gene copies and we calculate the number of bacterial counts obtained by the ddPCR accordingly.

Our main question in the current study was “is the water from DUWLs safe, especially for immunocompromised patients?” The private and public clinics do not have any special routines relative to these patients. Their hygiene procedures are assumed to be of such good quality that they

can treat any patients at any time of the day. However, our result suggests otherwise. Even if DUWLs are flushed through and treated with disinfectant every morning, the obtained results from these clinics suggest more procedure should be implemented to reduce bacterial counts in DUWLs.

There are strict guidelines to water quality in Norway. However, national guidelines are still non-existing for DUWLs in Norway. The difference between UDC and public/private clinics regarding amount of bacteria detected in the current study is remarkable. There are also big differences within the same clinic. One could assume that the same clinic, which is provided with the same municipal water, should have the same microbiological results. Surprisingly, there were differences in microbiological results from the same clinic that have several DCUs. This could be explained by differences in hygiene procedures for different DCUs. Other explanations could be age differences between the DCU, or DCU that are not being used in a regular bases which in some cases allow for stagnation of water and, hence, encouragement of bacterial growth in the DUWLs in these DCUs. Other causes for lower bacterial counts in UDC, are the water pipes that are more modern since it is a newer building. UDC also has a technical manager who controls the water lines as a part of his daily routine and therefore maintenance and follow up is of good quality.

Our study has shown that there were high levels of bacteria in many of the collected water samples. There were differences between the samples collected in UDC and the other clinics. Water collected at UDC showed low levels of bacteria while most of the samples collected in the private and public clinics in Tromsø showed remarkably high levels of bacteria. Analysis of the cultivation plates showed that UDC has low levels of anaerobe and aerobe bacteria with a range between 0-14 CFU/mL. Analyses of private clinics showed a higher rang of anaerobe (0->400 CFU/mL) and aerobe (0->200 CFU/mL) bacteria, while analyses of water for the public clinics showed overall the highest levels of both anaerobe (0->400 CFU/mL) and aerobe (0->400 CFU/mL) bacteria.

The difference regarding *E.coli*, *HPC* and ddPCR between UDC and private clinics is found to be statistically significant. In addition, the difference between UDC and public clinics regarding *E.coli*, *HPC* and ddPCR is also found to be statistically significant. Regarding the results of ddPCR, the difference between private and public clinics is found to be statistically significant.

We can conclude that water supplied to DCUs at UDC is much cleaner than water supplied to DCUs at public and private clinics. However, all clinics have equal hygiene procedures like flushing of water before and between patients and use of disinfection once a day. Therefore, the source of water contamination could be because of hygiene procedures that are not followed carefully. In addition old pipe system and lack of water filtration within the building where the clinics are situated could also contribute to the high bacteria levels.

Even though the study has shown high levels of bacteria we cannot conclude with confidence or role out that dental unit water is considered to be a risk for immunocompromised patients. This is because of what kind of bacteria that are present in the water is still an open question. However, guidelines for drinking water is set to less than 200 CFU/mL and our study has shown higher levels than what is recommended for drinking water.

The incidence of infections due to contaminated water unit is probably low and has so far not represented a serious problem for immunocompromised patients treated in dental clinics. However, it is of concern that dental health professionals make great efforts to sterilize the outer surface of the DCU and instruments, but little care is still the case when it comes to DUWLs. Failure to deal with this issue is inconsistent with good infection control practices and hygiene in dental clinics.

7. Questionnaire

Spørreskjema

Vi er tre 5. års tannlegestudenter ved UiT og vi skriver en masteroppgave om vannkvaliteten i uniter og infeksjonskontroll i klinikken. Vi skal undersøke mengde bakterier i vannet tatt fra treveissprøyten på Universitetstannklinikken, offentlige tannklinikker og private tannklinikker i Tromsø. Vi har i den forbindelse også et spørreskjema der vi ønsker å undersøke hygienerutiner og håndtering av immunsupprimerte pasienter ved klinikken. Spørreskjemaet skal besvares av klinikkssjef/daglig leder. Analysene av vannprøvene og spørreskjema anonymiseres. Skriv inn et kryss(X) bak svaret ditt der hvor det kreves.

Samtykke til deltakelse i prosjektet

Sted og dato:

Signatur:

1. *Hvor mange jobber på klinikken?*
Antall tannleger:
Antall tannhelsesekretærer:
Antall tannpleiere:
2. *Hvem er daglig leder?*
- Kjønn:
- Alder:
3. *Hvem har hovedansvaret for gjennomføring av hygieneprosedyrene på klinikken?*
A. Tannhelsesekretær
B. Tannlege
C. Tannpleier
4. *Når ble unitene installert?*
A. 0-5 år siden
B. 5-10 år siden
C. Mer enn 10 år siden
5. *Er det rutiner på gjennomspyling av håndstykkene og treveissprøyten?*
A. Daglig
B. Annenhver dag
C. En gang i uka
6. *Desinfiseres/renses håndstykkene med egnet desinfiseringsmiddel?*
A. ja

B. nei

7. Hvis **ja** på spørsmål nr. 6;
 - A. daglig
 - B. ukentlig
 - C. månedlig
8. Hvilket desinfiseringsmiddel brukes for desinfisering av unitene?
 - A. Oxygenal
 - B. Bioblue
 - C. Dentasept
 - D. Andre:
9. Hvor mange filtre går vannet gjennom fra det kommer inn i bygget til det kommer ut i uniten?
 - A. 1-2
 - B. 3-4
 - C. 5 eller mer
 - D. vet ikke
10. Hvor ofte byttes filteret/filtrene?
 - A. månedlig
 - B. 2 ganger i året
 - C. årlig
 - D. aldri
11. Har dere hatt noen problemer med vanntilførselen-/kvaliteten siste året?
 - A. Ja
 - B. Nei
12. Hvis **ja** på spørsmål 8;
 - A. kokevarsel fra kommunen
 - B. utskifting av vannrørdeleler
 - C. annet
13. Hvordan håndteres immunsupprimerte pasienter på klinikken(for eksempel organtransplanterte)?
 - A. ingen forskjell i forhold til andre pasienter
 - B. ekstra hygienetiltak
 - C. ekstra tildekking/påkledning behandler
14. Når på dagen behandler dere immunsupprimerte pasienter?
 - A. begynnelsen av arbeidsdagen
 - B. rundt lunsjtider
 - C. slutten av arbeidsdagen
 - D. ingen fast tidspunkt

15. Hvilken type autoklav brukes ved sterilisering av utstyr?
- A. Type A
 - B. Type B
 - C. Type C
 - D. Ingen av delene/vet ikke
16. Temperatur og tid på autoklaveringen?
- A. 121 °C i 15 minutter
 - B. 134 °C i 5 minutter
 - C. Andre
17. Hvor ofte valideres autoklaveringen?
- A. Hver dag
 - B. Ukentlig
 - C. Årlig
18. Det finnes ulike valideringssystemer for autoklavering, kjenner du til dette?
- A. ja
 - B. nei
19. Hvis **ja** på spørsmål; hvilket valideringssystem for autoklavering bruker dere?
- A. Klasse I
 - B. Klasse II
 - C. Klasse III
 - D. Klasse VI
 - E. Vet ikke
20. Vet du hva MRSA er for noe?
- A. Mange resistente stafylokokkus type A
 - B. Multiresistente stafylokokkur auricularis
 - C. Meticilinresistente stafylokokkus aureus
 - D. Vet ikke
21. I helseskjemaet, blir det spurt om pasienten har hatt medisinsk behandling/tannbehandling i land utenfor Norden de siste to år?
- A. ja
 - B. nei
22. Dersom pasienten er MRSA-positiv, har dere særlige tiltak for behandling av disse pasientene?
- A. ja
 - B. nei

Takk for dine svar!

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