
CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

Infectious diseases that are caused by a variety of waterborne organisms such as bacteria, viruses, protozoa and intestinal parasites remain a source of global morbidity and mortality. Many of these organisms are able to thrive and proliferate in contaminated natural sources as well as in man-made systems such as waterlines designed to provide water to the public for consumption, in building equipment, and in healthcare facilities (Avelar-González, Harel and Guerrero-barrera, 2015). More specifically, the microbial contamination of dental unit waterlines is a significant challenge in oral healthcare facilities. Water is an indispensable commodity in oral healthcare procedures as it is used for the functioning and cooling of dental chair unit handpieces, to cool tooth surfaces, to clear the oral healthcare work field, and to rinse oral cavities. The quality of the water supplied to a dental chair unit and the water exiting handpieces play a critical role in patient care and personnel health safety because both patients and oral healthcare personnel are regularly exposed to water and aerosols generated from the handpieces.

5.2 Study Summary

The results of studies that explored compliance with infection control associated with microbial water quality and the presence of biofilm-related organisms have been widely reported and were scrutinised. This study argues that the South African government currently has no explicit requirements for the quality of water that is supplied to and used in dental chair units (DCUs), and the literature review that was conducted for the current study affirmed this oversight. For instance, there is no infection prevention/control policy that regulates or guides oral healthcare providers to protect their patients and oral healthcare personnel in terms of the water safety of the DCUs they use. The National Health Policy also does not contain any regulation related to oral health aspects to curb the transmission of infectious diseases and to adhere to infection control issues related to the water used for oral healthcare (Hartshorne, 2010). Furthermore, only a very few studies have been conducted on the microbial quality of dental chair unit water in South Africa.

The current study was conducted in six oral healthcare facilities in the Mangaung region of the Free State Province of the Republic of South Africa. Both public and private oral healthcare facilities participated in the study. Three oral healthcare facilities using open system DCUs and three oral healthcare facilities using closed system DCUs were identified and recruited. Water is sourced directly from the municipal mains in some oral healthcare facilities and the DCUs used in these are classified in this study as open system DCUs. In a closed water system, reservoir bottles are filled with distilled water and attached to the DCU or fitted at a designated location within the oral healthcare facility. The dental chair units used in these facilities are thus classified in this study as either open system DCUs or closed system DCUs. It must be noted that the water used for distillation in the latter facilities are sourced from municipal water.

To analyse the water quality, water samples were collected at each facility from the municipal water supply (taps) and the fast handpieces used in both the open system and the closed system facilities. Ten samples were obtained from each site classified as an open system DCU and eleven samples were obtained from closed system DCUs. Additional water samples for analysis were collected from closed system DCUs' distiller and reservoir bottles.

Surface swab samples were taken for analysis of microbial load and to ascertain the potential presence of biofilm-associated organisms on the inner surfaces of the DUWLs at the various sampling sites. These swab samples were taken of the inner surfaces of taps and fast handpieces of open and closed DCUs, as well as the inner surfaces of distiller and reservoir bottles of closed system DCUs. The actual waterlines of the DCUs were thus not accessed due to cost implications.

5.3 Key Findings

This study highlights the environmental risk of contaminated source water that is supplied to dental chair units, as well as the risk of contaminated water that exits distal outlets of fast handpieces attached to open and closed system DCUs. It also highlights the disparity that exists for heterotrophic bacteria and total coliforms as stipulated by

SANS 241:1 (SANS, 2015) and the actual conditions at oral healthcare facilities, where these standards are not met in certain instances. The study also exposes a lack of legislation and guidelines to steer water quality assurance in oral healthcare facilities.

There was a statistically significant difference ($p = 0.040$) in the HPCs of tap water between open and closed system DCUs. The mean microbial count of the tap water used in closed system DCUs (1.71×10^6 CFU ml⁻¹) was higher than that of the tap water used in open system DCUs (1.50×10^6 CFU ml⁻¹). There was no statistically significant difference between the water exiting distal outlets of fast handpieces in open and closed system DCUs ($p = 0.099$). The HPCs of fast handpieces in both open and closed system DCUs were high and exceeded the SANS 241:1 (SANS, 2015) limitation, suggesting that further contamination occurred in the DCUs of both systems.

Water that is distributed by the municipality to the public is also used in open system DCUs and in closed system DCUs to fill distiller bottles, and it is naturally assumed that this water has been tested prior to public distribution and that it consequently meets the SANS 241:1 (SANS, 2015) standard for microbial water quality. The microbial load of distilled water that is used to fill reservoir bottles for closed system DCUs should be the same as, if not lower than, that of municipal water. However, the findings showed this was not the case, as there was statistical significance between the tap water supplied to open system DCUs and the tap water supplied to distiller bottles. Similarly, the one order magnitude of difference regarding tap water supplied to open system DCUs and closed system DCUs, and the water exiting the distal outlets of fast handpieces, was statistically significant for heterotrophic bacteria.

An increase in HPCs was evident (Figure 4.1) when the three closed system DCUs were compared, and it was found that the one order of magnitude difference between distiller and reservoir bottles was not statistically significant ($p = 0.21$). The HPCs of closed system reservoir bottles supplying fast handpieces with water also showed no statistical difference ($p = 0.476$). The minimum range in HPCs of reservoir bottles was 1.20×10^4 CFU ml⁻¹ and the maximum was 2.05×10^5 CFU ml⁻¹, while the counts for handpieces displayed a similar range as the minimum was 1.24×10^4 CFU ml⁻¹ and the maximum was of 2.11×10^5 CFU ml⁻¹ (Appendix A and Figure 4.2). These data support the suggestion that the distillers were not functioning optimally, suggesting

that the initial microbial load may have been too high for the distillation process to eliminate all microbes in the supply water, or the reservoir bottles may also have been contaminated, thus indicating potential biofilm presence.

The high HPCs of the supply water suggest optimal conditions, the opportunity for biofilm growth, and the proliferation of opportunistic pathogens. These were indicated by the microbial load detected on the internal surfaces of taps, distiller bottles, reservoir bottles, and handpieces. The most common causes of dental unit waterline contamination are the formation of biofilm and the subsequent sloughing off of biofilm from the surfaces of the lumens in DUWL tubing and, if not decontaminated, these phenomena potentially place immunocompromised individuals at risk.

The two DCU systems were compared for biofilm associated with HPC, and it was found that the HPC of the inner surfaces of taps in the closed system was lower than that of taps in the open system, and thus the one order magnitude of difference between the two systems was statistically significant ($p = 0.013$). When the HPCs of the inner surfaces of fast handpieces for the two DCU systems were compared, the mean HPC was not statistically significant ($p = 0.270$). This indicates that the distillation processes used to decontaminate the closed system DCUs were flawed and resulted in increased biofilm formation at the distal outlets of the inner surfaces of the fast handpieces.

Total coliforms were determined as an indicator organism of contamination and the possible detection of the presence of potentially pathogenic bacteria in the DCUs at the various sampling sites. Based on the measurement of the microbial load of total coliform bacteria, the findings indicated that the water used in the open system DCUs, as well as the water exiting the handpieces of the open system DCUs, did not comply with the SANS 241:1(SANS, 2015) standard for drinking water. This standard determines that water for consumption should not contain more than 10 colony forming units of coliforms per 100 ml of water. Conversely, the source water as well as the water exiting the handpieces of the closed system DCUs was compliant with the SANS 241:1(SANS, 2015) standard.

The one order magnitude of difference between the tap water used in open system DCUs and that used in closed system DCUs was statistically significant ($p = 0.043$). This means that, when comparing the two DCU systems, the tap water supplied to open system DCUs had higher total coliform counts than the water supplied to closed system DCUs. However, there was no statistically significant difference between the tap water samples of the two DCU systems ($p = 0.669$).

The total coliform mean counts of open and closed system DCUs were compared for the inner surfaces of taps that supplied source water to the DCUs. The inner tap water samples were statistically significant ($p = 0.032$) and the inner surfaces of the taps of open system DCUs contained total coliforms, while the taps used for the closed system DCUs did not have any total coliforms present on their inner surfaces. Biofilm was present on the inner surfaces of the taps used for the open system DCUs. The inner surfaces of the fast handpieces of both the open and closed system DCUs did not contain any total coliforms.

Opportunistic pathogens like *Pseudomonas aeruginosa* and *Legionella spp.* are prevalent in dental unit water and waterlines. However, there are no legislated parameters relating to the presence of these organisms and microbial water quality, which is an oversight as their presence can encourage the growth of other microorganisms whose presence contravenes the limitations for microbial water quality. The presence of opportunistic pathogens in dental unit water poses a high risk to patients and oral healthcare providers and their personnel who may be immunocompromised (Coleman et al., 2009). These organisms are not only intrinsically resistant to high temperatures and biocides, but the biofilms they inhabit also enhance their resistance (Moradali et al., 2017). *P. aeruginosa* was present in the source water and handpieces of open system DCUs, while no *P. aeruginosa* was detected in the water of closed system DCUs. When comparing the two DCU systems, statistical significance ($p = 0.019$) was found between the tap water of the open and closed systems. However, there was no statistical significance ($p = 0.128$) in the water exiting the fast handpieces of the two DCU systems.

The inner tap surfaces of open system DCUs also contained *P. aeruginosa*, which is indicative that the waterlines supplying source water was contaminated and could

have contained biofilm. The contamination of source water and tap surfaces presents an introductory pathway for *P. aeruginosa* into the waterlines of open system DCUs and the consequent colonisation and formation of biofilm within dental unit waterlines. This may also be the reason for the presence of *P. aeruginosa* in the water exiting handpieces. *P. aeruginosa* was also present in handpieces and the distiller and reservoir bottles of closed system DCUs.

The comparison of the inner surfaces of the taps supplying source water to open and closed system DCUs indicated a statistical significance ($p = 0.015$), where the inner surfaces of open system taps contained *P. aeruginosa* and the inner surfaces of closed system DCUs contained no *P. aeruginosa*. This finding is an indication of the presence of biofilm on the inner tap surfaces of open system DCUs. The inner surfaces of fast handpieces showed no statistical significance ($p = 0.173$), although the fast handpieces of closed system DCUs showed the presence of *P. aeruginosa*. This is an indication that biofilm could have been present in the DUWLs of the closed system DCUs.

Legionella spp. is another potentially pathogenic organism of concern. Only the water exiting handpieces were examined for *Legionella spp.*, and it was detected in only one open system DCU. The DCU was suspended for use of oral procedures for a period of 14 days. The waterlines of this unit, DCU O2.1, were flushed daily with 50 ml of 0.2% chlorhexidine mouthwash. Following the 14 days of suspension, the water at the distal outlet of the fast handpiece was sampled and analysed for the presence of *Legionella spp.* Upon examination, it was revealed that no *Legionella spp.* was present in the water of the fast handpiece of DCU O2.1, and it was concluded that the daily flushing with 0.2% chlorhexidine mouthwash had eliminated the *Legionella spp.* from the water.

Contamination of dental unit waterlines seems inevitable, as contamination may be caused by the water supply, retraction of biological fluids from handpieces during oral healthcare procedures, or continuous biofilm detachment or fragmentation in the narrow waterline tubes (Costa et al., 2015). The interior of small-diameter tubing in dental unit waterlines (DUWLs) creates an attractive environment for the growth of biofilm and bacteria. Oral healthcare providers in this study indicated various methods

for disinfection and flushing. However, the results indicating microbial load in tap water and on inner surfaces of all sampling sites undoubtedly proved that the protocols and interventions used were not effective in eliminating all microorganisms from the water and surfaces of DCUs, thus causing assumed DUWL contamination.

Various approaches to prevention and control of the microbial contamination of dental unit water are evolving. Currently, available methods and emerging technologies, along with regulatory and advisories on water quality in oral healthcare facilities, are considered and tested. Clean water systems, fully autoclavable systems, and a variety of devices designed to provide physical barriers to the influx and accumulation of microbial contaminants can all be used to assure the satisfactory quality of coolant and irrigant water (Depaola et al., 2002). It is therefore important that oral healthcare providers commit to the maintenance of DCUs if they are to be deployed successfully and safely. There should be strict adherence to the recommendations of the American Dental Association and the American National Standards Institute in the manufacturing and production of DCUs. Moreover, legislative enforced compliance with regulatory protocols for dental unit water will advance the cause of dental unit water quality control. Strict adherence to the SANS 241:1(SANS, 2015) limit for drinking water quality should also be ensured by all oral/dental healthcare providers when they execute oral healthcare procedures.

5.4 Limitations of the Study

- The current study was only exploratory and did not physically access waterlines to determine the actual rates of contamination and biofilm on their internal surfaces.
- Intervention measures for deep cleaning or the replacement of certain waterlines, bottles, and handpieces were not explored.
- It is acknowledged that only a few selected microbes were investigated and that a more extensive selection could have enhanced the findings of the study.
- The microbial diversity in DCU waterlines may be much more complex and requires investigation on genomic level, which this study did not perform..

- The study involved only six oral healthcare facilities in one region in the Free State Province. Future studies should expand similar investigations across regions and communities to enhance and expand knowledge on the topic under investigation.

5.5 General Recommendations

The onus is on oral healthcare providers to ensure a safe environment for their patients and oral healthcare personnel. Existing recommendations are presented by the Organisation for Safety, Asepsis and Prevention that can help accomplish this. The suggested recommendations that oral healthcare providers should adhere to are as follows:

- Keep abreast of the current recommendations for the use of water for oral healthcare procedures and the control of microbial biofilm contamination in dental unit waterlines.
- Review and adhere to instructions for use of the DCU by the device manufacturer to control contamination of the waterlines and ancillary equipment and to maintain the quality of oral healthcare procedural water.
- Obtain and review information regarding the safety, effectiveness, and compatibility of the dental equipment used when selecting germicidal products and devices for controlling biofilm colonisation in dental unit waterlines.
- Flush waterlines for twenty to thirty seconds at the beginning and end of each day and between patients to remove patient material that has (potentially or actually) been retracted during treatment.
- Develop and implement standard operating procedures for maintaining, monitoring, and documenting oral healthcare procedural water quality that is consistent with the recommendations by the CDC in terms of devices, germicides and monitoring methods used in the oral healthcare facility as part of an overall infection control plan.
- Train and educate all oral healthcare personnel on the importance of managing dental unit water quality and provide training in compliance with standard operating procedures to ensure a safe, infection free environment for patients, oral healthcare providers, and oral healthcare personnel.

- Apart from flushing waterlines, a recommendation by the CDC is that DCU water and air should also be discharged for a minimum of twenty to thirty seconds after each patient from any device connected to the dental water system that enters the patient's mouth. It does not recommend flushing between patients as a sole means to improve procedural water quality to perform oral healthcare procedures (Mills, Porteous and Zawada et al., 2018).

5.6 Recommendations for Future Research

- No regulations are in place to regulate the water quality of water used in DCUs in South Africa or for disinfection protocols for DUWLs. Oral healthcare providers can no longer rely on the assumption that source water is within the microbial water limitations of the SANS 241:1 (SANS, 2015) requirement. This study demonstrated the need for at least independent and consistent monitoring of water quality used in both open and closed system DCUs, and future studies should explore this as a matter of urgency.
- Future research should also fill the gaps left by this study by exploring the actual contamination of waterlines through accessing them. To do so, a partnership could be established with the DCU manufacturer for more a cost-effective investigation.
- South African municipal supply water quality is unlikely to improve in the near future, and cost-effective treatment options for DUWL supply water should be investigated.
- Future studies could explore training opportunities for oral healthcare facility personnel to support the recommendations offered above.

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ANNEXURES

Annexure A

Letter of consent and questionnaire provided to oral healthcare providers at the various oral healthcare facilities





To: The Head / Manager / Owner of the Oral Health Care Facility

To Whom It May Concern

RE: THE EXECUTION OF A RESEARCH PROJECT AT YOUR PRACTICE / DEPARTMENT

Your facility has been identified as a possible participant in a study to sample and analyse the quality of water used in dental unit waterlines in Mangaung.

This project will form part of my research for a Master's degree, "Bacterial water quality of dental unit waterline systems in Mangaung, Free State".

Purpose of this research study:
The purpose of this study is to analyse the bacterial water quality as well as biofilm associated organisms in dental practices in dental unit waterlines in both open and closed water systems of dental chair units, depending on the system used at your facility. From the results of this analysis recommendations will be made in terms of the water quality (Barbeau et al. 1996).

Aim:
The aim of this study is to determine the bacterial water quality and presence of biofilm associated organisms within open and closed water systems of dental unit waterlines. The aim will be met by following the following objectives:

Objectives

- Collect and analyse water samples to determine the bacterial water quality used at various points in the dental unit system for both open and closed dental unit systems of dental practices.
- Collect and analyse samples of the biofilm within the dental unit system and waterlines.

Compare the bacterial water quality of the open water systems, with the bacterial water quality of the water in the closed water system. Barbeau, J. et al., 1996. Multiparametric Analysis of Waterline Contamination in Dental Units. , 62(11), pp.3954–3959.

Momba, M.N.B., *INHIBITION OF BIOFILM REGROWTH IN Inhibition Of Biofilm Regrowth In Potable Water Systems,*

- (Momba n.d.)
- Compare the biofilm associated organisms of the open water system, with the biofilm associated organisms in the closed water system.
- Provide suggestions and remedies to manage poor water quality and reduce biofilm formation in dental units.

• Available sources of information:

I would sincerely appreciate your participation and making your facility available. Should you agree, you are kindly requested to respond by email [akasiikh@ufs.ac.za]. Please feel free to contact me should you require additional information by calling me at +273 324 9288 or +2751 401 2468 or enquiring by email. Finally, I assure you of my continued commitment to promote safe oral health care and add to the improvement of quality oral health care services provided. Thanking you in anticipation and I look forward to your reply.

Yours sincerely

Ms CB Keder

Contact Details for communication

Name of the Facility:					
Dentist in charge:					
Physical Address:					
Postal Address:					
Tel no:	()		Cell no:		
Fix no:	()		Email		
Contact person:				Capacity	
Consent to conduct and participate in this project			Approved		Not Approved

Head of Oral Health in the facility

Should you be willing to participate in this project, please fill in the following questionnaire:

1	What type of water supply does your dental unit use?	Open (Municipal) water supply.		Closed (Reservoir Bottle) water supply.	
2	How many dental chairs are in use at your facility?				
3	Age of Dental Unit				
4	In the case of more than one dental chair: Are the chairs connected to the same water supply or independently?	Same water supply		Independently	
5	Do you have a distiller in your facility?	Yes		No	
6	If reservoir bottles are used what type of water is used in these bottles?	Municipal		Distilled	
7	Do you use a cleaning regime for dental chair waterlines	Yes		No	
8	If <u>yes</u> please list the chemical(s) used	1			
		2			
		3			
9	Please indicate the frequency of the cleaning regime in the dental chair unit (hours/days/months)				
10	Please list any areas of concerns or comments:				

Barbeau, J. et al., 1996. Multiparametric Analysis of Waterline Contamination in Dental Units. , 62(11), pp.3954–3959.

Momba, M.N.B., INHIBITION OF BIOFILM REGROWTH IN Inhibition Of Biofilm Regrowth In Potable Water Systems,

Annexure B

Summary Statistics of Microorganisms: Water quality of dental until waterlines

Organism (unit)	Statistic	Tap		Handpiece		Reservoir Bottle	Distiller Bottle
		Closed	Open	Closed	Open	Closed	Closed
N		11	10	11	10	11	11
HPC (CFU ml⁻¹)	Mean	1.71 x 10 ⁴	1.48 x 10 ⁴	6.93 x 10 ⁴	3.52 x 10 ⁴	5.92 x 10 ⁴	4.67 x 10 ⁴
	Std. Dev	1.38 x 10 ⁴	2.05 x 10 ⁴	6.09 x 10 ⁴	2.29 x 10 ⁴	5.80 x 10 ⁴	5.84 x 10 ⁴
	Minimum	5.6 x 10 ³	9 x 10 ²	1.24 x 10 ⁴	1.15 x 10 ³	1.20 x 10 ⁴	6.50 x 10 ²
	Maximum	5.45 x 10 ⁴	6.32 x 10 ⁴	2.11 x 10 ⁵	7.20 x 10 ⁴	2.05 x 10 ⁵	2.08 x 10 ⁴
	SANS Compliance	0%	10%	0%	0%	0%	72.72%
Total	Mean	9.09 x 10 ⁻¹	9 x 10 ⁰	8.63 x 10 ¹	6 x 10 ¹	6 x 10 ⁻¹	1.36 x 10 ⁻¹
Coliforms (CFU 100 ml⁻¹)	Std. Dev	1.26 x 10 ⁻⁰	1.21 x 10 ¹	2.09 x 10 ⁰	1.31 x 10 ²	1.29 x 10 ⁰	4.52 x 10 ⁻¹
	Minimum	0	0	0	0	0	0
	Maximum	4 x 10 ⁰	2.95 x 10 ¹	7 x 10 ⁰	4.19 x 10 ²	4 x 10 ⁰	1.5 x 10 ⁰
	SANS Compliance	100%	50%	81.82%	70%	72.73%	90.91%
<i>Pseudomonas aeruginosa</i> (CFU 100 ml⁻¹)	Mean	0	1.26 x 10 ¹	1.41 x 10 ⁰	3.35 x 10 ¹	3.80 x 10 ⁰	1.5 x 10 ⁻¹
	Std. Dev	0	1.43 x 10 ¹	3.30 x 10 ⁰	6.29 x 10 ¹	9.60 x 10 ⁰	4.52 x 10 ⁻¹
	Minimum	0	0	0	0	0	0
	Maximum	0	3.35 x 10 ¹	1.05 x 10 ¹	1.93 x 10 ²	3.10 x 10 ¹	1.50 x 10 ⁰
	Presence	0%	27.73%	27.73%	40%	9.09%	9.09%
<i>Legionella</i> spp. (CFU 100 ml⁻¹)	Mean			0	2.2 x 10 ⁰		
	Std. Dev			0	6.97 x 10 ¹		
	Minimum			0	0		
	Maximum			0	2.2 x 10 ¹		
	Presence			0%	10%		

Annexure C

Summary Statistics of Microorganisms: Biofilm associated organisms

		Tap		Handpiece		Reservoir Bottle	Distiller Bottle
Organism (unit)	Statistic	Closed	Open	Closed	Open	Closed	Closed
HPC	N	11	10	11	10	11	11
(per 1 ml)							
	Mean	4 x 10 ³	1.47 x 10 ⁴	3.96 x 10 ⁴	1.39 x 10 ⁴	8.74 x 10 ⁴	2.08 x 10 ⁴
	Std. Dev	2.76 x 10 ³	1.01 x 10 ⁴	1.02 x 10 ⁵	1.19 x 10 ⁴	8.94 x 10 ⁴	6.84 x 10 ⁴
	Minimum	3.50 x 10 ²	6.50 x 10 ²	3.00 x 10 ²	0	9.50 x 10 ²	0
	Median	4.90 x 10 ³	1.48 x 10 ⁴	1.45 x 10 ³	1.23 x 10 ⁴	9.16 x 10 ⁴	0
	Maximum	7.30 x 10 ³	3.15 x 10 ⁴	3.45 x 10 ⁵	3.23 x 10 ⁴	3.10 x 10 ⁵	2.27 x 10 ⁴
	SANS Compliance	27.27%	10%	45.45%	20%	9.09%	90.90%
Total	Mean	0	5.09 x 10 ³	0	0	6.23 x 10 ³	0
Coliforms	Std. Dev	0	8.22 x 10 ³	0	0	1.27 x 10 ⁴	0
(per 100 ml)	Minimum	0	0	0	0	0	0
	Median	0	0	0	0	0	0
	Maximum	0	18.35 x 10 ⁴	0	0	4.06 x 10 ⁴	0
	SANS Compliance	100%	70%	100%	100%	27.27%	100%
<i>Pseudomo</i>	Mean	0	3.64 x 10 ⁴	1.5 x 10 ⁰	0	5.91 x 10 ¹	0
<i>nas</i>	Std. Dev	0	4.74 x 10 ⁴	3.47 x 10 ⁰	0	1.95 x 10 ²	0
<i>aeruginosa</i>	Minimum	0	0	0	0	0	0
(per 100 ml)	Median	0	0	0	0	0	0
	Maximum	0	1.06 x 10 ⁵	1.1 x 10 ¹	0	6.50 x 10 ²	0
	Presence	0%	40%	18.18%	0%	9.09 %	0%