

Water quality and biofilm formation in dental unit waterline systems in Mangaung, Free State

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DECLARATION OF INDEPENDENT WORK

I, Chantal Bronwyn Kader (student number _____), hereby declare that this research project submitted to the Central University of Technology, Free State, for the degree Master of Health Science in Environmental Health is my own independent work. This research project was conducted at the Central University of Technology, Free State under the supervision of Drs O de Smidt and J Oosthuysen.

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LIST OF ABBREVIATIONS

%	percentage
ADA	American Dental Association
CAFSaB	Centre for Applied Food Sustainability and Biotechnology
CDC	United States Centers for Disease Control and Prevention
CFU ml ⁻¹	colony forming units per millilitre
CFU	colony forming unit
cm ²	centimetre square
CUT	Central University of Technology
DCU	dental chair unit
DUWL/s	dental unit waterline/s
<i>E. coli</i>	<i>Escherichia coli</i>
FC	faecal coliforms
HIV	Human Immunodeficiency Virus
HPC/s	heterotrophic plate count/s
min	minutes
ml	millilitres
nm	nanometres
NTM	non-tuberculosis mycobacterium
°C	degree Celsius
OHCP/s	oral healthcare provider/s
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PCR	polymerase chain reaction
ppm	parts per million
PVC	polyurethane and polyvinylchloride
SANS 241:1 (SANS) 2015	South African National Standard 241:1 of 2015
sec	second/s
TC	total coliforms
UV	ultraviolet
WHO	World Health Organization
µM	micrometre

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ABSTRACT

Microbial contamination of dental unit water exposes personnel and patients to a plethora of potential infectious organisms. The objective of this study was to analyse bacterial water quality and determine the presence of biofilm-associated organisms in dental chair waterlines in 21 oral healthcare facilities in Mangaung, South Africa. Ten dental chair units (DCUs) with an open-water system (i.e., water sourced from municipal water) were analysed, while eleven DCUs with a closed-water system (i.e., utilising a reservoir bottle attached to the DCU) were also analysed. A set of questions was administered to survey, amongst others, the age and cleaning methods of the DCUs utilised at each facility. Standard water sampling procedures were followed in accordance with the South African National Standard 241:1(2015) (SANS, 2015) for acceptable drinking water quality. Heterotrophic plate counts and total coliforms were determined. Heterotrophic plate counts have been widely adopted as a standard technique for microbiological testing and safety management of drinking water. The heterotrophic plate count is a microbiological parameter that can quantify the formation of biofilm in water systems. The permissible number of heterotrophic bacteria in drinking water according to the SANS 241:1 should be less than 1 000 colony forming units per millilitre of water (SANS,2015). Total coliform bacteria were analysed because they are used as indicators to measure the degree of pollution and sanitary quality of water. The presence of these bacteria in drinking water does not necessarily mean that the drinking water will cause disease. However, it is an indication that the water supply may be vulnerable to contamination. Their presence therefore indicates that disease-causing organisms may be present in drinking water. The permissible number of total coliform bacteria in drinking water according to the SANS 241:1 should be less than 10 colony forming units per 100 millilitres of water. *Pseudomonas*

aeruginosa and *Legionella spp.* were detected at the end usage point of the dental water in the DCUs under study. *Pseudomonas aeruginosa* lives in the environment and can be spread to individuals in healthcare settings when they are exposed to water or soil that is contaminated with this bacteria. Resistant strains *P. aeruginosa* can also spread in healthcare settings from one person to another through contaminated hands, equipment, or surfaces. *Pseudomonas aeruginosa* causes infections in the blood, lungs (pneumonia), or other parts of the body post-surgery or when inhaled into the lungs. *P. aeruginosa* from biofilm that colonises DUWLs contaminate the water that is used in oral healthcare procedures. *Legionella* is a rod-shaped (bacillus) gram-negative bacteria that causes a form of pneumonia. It exists in natural water, such as lakes or rivers, but in small numbers do not cause a potential health risk. However, with favourable conditions and nutrients it can live and proliferate in domestic water systems. Legionnaires' disease is caused by the *Legionella pneumophila* bacteria. It is the most serious and potentially fatal of the legionellosis illnesses. Other less serious diseases caused by *Legionella spp.* are Pontiac fever and Lochgoilhead fever. *Legionella* can be transmitted to individuals if they inhale air droplets of contaminated water. In an oral healthcare facility this can be from the aerosols created from high-speed drills or an ultrasonic scaler. The presence of *P. aeruginosa* and *Legionella spp.* in dental chair unit water poses a serious risk for patients who are immunocompromised.

Total bacterial counts significantly exceeded the SANS 241:1 (SANS, 2015) standard of $<10 \times 10^3$ CFU ml⁻¹ of water for both the open and closed systems ($1.48-6.94 \times 10^4$ CFU ml⁻¹). Total coliform counts revealed that coliforms were present in the water source (tap water) that was supplied to the DCUs and reservoir bottles. Even though the fact that the total coliform counts were below the SANS 241:1 (SANS, 2015)

recommendation of <10 CFU 100 ml^{-1} , the presence of coliforms suggested the presence of faecal contamination that could have served as a precursor for biofilm formation. The level of total coliforms present in the distiller and reservoir bottles may have occurred as a result of improper bottle handling and inappropriate aseptic techniques. *P. aeruginosa* was not detected in the tap water of the closed system DCUs, but it was detected in fast handpieces, reservoir bottles, and distiller bottles. *Legionella spp.* (22 CFU ml^{-1}) were present in the output water from one fast handpiece of an open system DCU. The internal surfaces of all the sampling sites were swabbed to determine the presence of biofilm-associated organisms. It was found that the internal surfaces of taps, fast handpieces, distiller bottles, and reservoir bottles all exhibited mean counts that exceeded the SANS 241:1 (SANS, 2015) standard for heterotrophic bacteria. Total coliforms, that exceeded the SANS 241:1 (SANS, 2015) standard for total coliform bacteria, were identified in the fast handpieces of both the open and closed system DCUs and in the distiller bottles of the closed systems. Total coliform counts in reservoir bottles were lower but were not detected on the inner surfaces of taps in either the open or closed systems. *P. aeruginosa*, at a mean count of 3.64×10^4 CFU ml^{-1} , was detected on the inner tap surfaces of the open system DCUs. No *P. aeruginosa* was detected in the fast handpieces of either the open or the closed system DCUs, but it was present on the inner surfaces of reservoir bottles (5.9×10^1 CFU ml^{-1}) and the fast handpieces (1.5×10^0) of closed system DCUs.

CHAPTER 1: INTRODUCTION TO THE STUDY

1.1 Introduction

The oral healthcare environment is characterised by unique oral healthcare procedures, instrumentation, and patient care (Barker et al., 2013). However, oral healthcare is also associated with the risk of exposure to a range of environmental and human infectious agents that may affect both oral healthcare providers and patients. Infectious agents can be transmitted to patients and oral healthcare providers via blood, oral and oropharyngeal secretions, air, and water. Frequent exposure to blood and other biological fluids results in high occupational risk as diseases such as the human immunodeficiency virus (HIV), hepatitis B and C, meningococcal disease, mononucleosis, and herpes may be contracted (Manfredi, 2010; Baghla et al., 2015). Moreover, a further risk of infection and contamination for oral healthcare providers and patients that has assumed great importance in infection control in oral healthcare is the exposure of people to contaminated waterline water in dental units (Lizzadro et al., 2019).

1.2 Statement of the Problem

Water contamination in dental unit waterlines (DUWL) poses a potential threat to the effective control of infection, as these sources can easily become contaminated with both patient and water-derived impurities (Lizzadro et al., 2019). The reason for the facile spread of infections in the oral healthcare environment is generally twofold. First, the mere fact that oral healthcare procedures are performed within the highly dense microbial location of the oral cavity contributes to the increased risk that infectious agents can be transmitted to patients and oral healthcare providers alike (Nejatidanesh et al., 2013). Secondly, the use of high-speed instruments to perform oral procedures also requires the use of water for optimal functioning. This water is a pivotal cooling and cleansing agent in dental treatments (O'Donnell et al., 2011), but it may be contaminated with microbial agents that can pose a risk to patients and oral healthcare workers.

During the execution of oral healthcare procedures, water is supplied to the dental chair unit (DCU) to cool dental unit equipment and tooth surfaces and also to rinse oral tissue (Tilak Geetha and Raja, 2012; Szymańska and Sitkowska, 2013). Water used for clearing the dental field exposes oral healthcare providers (OHCPs), oral healthcare personnel, as well as patients to splatter and contaminated water aerosols. The splatter and aerosols are emitted from handpieces during oral healthcare procedures when rotating and ultrasonic instruments are utilised (Prasanth et al., 2010). Aerosol emissions can usually be observed as a cloud of material that is emitted from the handpieces held above the working field of the OHCP (Aurangjeb, Zaman and Badruddoza., 2013). Oral healthcare providers and oral healthcare personnel spend long hours in close proximity to patients and are continuously exposed to traumatised tissue, saliva, blood, as well as water and aerosols generated by DCU instruments (Harfst, 1991). In no other occupation is OHCPs placed at a higher risk of disease transmission because of this reason. It therefore follows that it is of critical importance that the application of standard precautionary strategies during contact with DCU water and aerosols in the oral healthcare environment complies with recommended infection prevention and control measures (Mansourian et al., 2011).

Water is supplied to the DCU either directly from a tap that is linked to the municipal water supply, or a reservoir attached to the DCU. This source water flows through the internal network of the DCU through flexible multichannel probes before it is channelled to handpieces and other instruments. The water that is supplied to the DCU should be of an acceptable microbiological quality and should ideally contain low levels of microorganisms (Lizon et al., 2016). However, due to the complex architecture of the DCU, water contamination occurs as a result of biofilm formation on the inner surface of the tubing of DUWLs. The water flow at the interface of the narrow diameters of the plastic DUWLs is almost negligible compared to that in the centre of the tubing because, over time, chemicals and organic substances from the source water are deposited onto the surfaces of the DUWL tubing (Olewi, 2017). Microorganisms attach to these deposits and flourish as multispecies biofilms that are able to contaminate DUWL water. Biofilms thus serve as a constant source of contamination of the water passing through the tubing of the DUWL, so that the quality of the water exiting handpieces will not be the same as the quality of the source water that is supplied to the DCU (Coleman, Donnell et al., 2009). Contaminated water exiting handpieces is

either aerosolised into the environment, settle on surfaces of the oral healthcare facility, or can settle directly in patients' mouths and wounds (Chua, Fathilah, and Himratul-Aznita et al., 2018).

Over the last five decades, studies have indicated that the water from DUWLs and DCU handpieces is usually heavily contaminated with microorganisms (O'Donnell et al., 2009; Lal et al., 2018; Alkhulaifi et al., 2019; Oosthuysen et al., 2020). The threat also exists that aerosols produced by DCU instruments connected to dental unit waterlines (DUWLs) during oral healthcare procedures may contain microorganisms that can be opportunistic pathogens that infect patients and OHCPs. This poses a public health concern as vulnerable patients, such as those who are medically and immunocompromised, are put at great risk of infection by contaminated water and aerosols (Ditommaso et al., 2016; Mishima et al., 2016). Moreover, as microbial proliferation inside DUWLs is inevitable, it presents a low but persistent risk of infection (Barker et al., 2013).

The safety of patients and OHCPs exposed to DUWL water is a critical issue globally that warrants attention and intervention by both oral healthcare governing bodies and equipment manufacturers. Internationally, the microbiological content of drinking water is regulated by legislation using coliform counts as an indicator of faecal and sewage contamination (Tyagi, Kulkarni and Krisna Prasad, 2010). The United States Centers for Disease Control (CDC) issued guidelines for the limitation of microbial counts in water to ensure effective oral healthcare procedures. The CDC recommends that water used for non-surgical oral healthcare procedures should always be maintained at the regulatory standards for drinking water; this means that heterotrophic bacterial levels should be less than 500 colony forming units per millilitre of water (CDC, 2016). The American Dental Association (ADA) recommends that heterotrophic bacterial levels in the water from DUWLs used for oral healthcare procedures should be 200 colony forming units per millilitre (Watanabe et al., 2016). These international guidelines are often domesticated in the legislation of specific countries. However, a grave concern regarding the quality of the water that passes through DUWLs is that there is no microbiological guideline or legislation in existence for the quantification of opportunistic Gram-negative bacterial species or respiratory pathogens that are found in low numbers in the main water supply. These organisms are numerically the most

important microorganisms in source water, the water flowing through the DUWL, and the water flowing out of DCU handpieces (Tyagi, Kulkarni and Krisna Prasad 2010). Water that flows from dental instruments is not intended for human consumption, but it should at least sensibly, reasonably, and responsibly approximate the standards of drinking water quality. This means that the quality of the water used during oral healthcare procedures should at least be within the stipulated regulations for human consumption, as this water enters oral cavities and the respiratory tract as aerosols (Coleman et al., 2010). A definitive reference for the acceptable limits for drinking water quality in South Africa is the South African National Standard 241:1 of 2015 (SANS, 2015).

Infection control and public health awareness within oral healthcare facilities should be closely monitored to ensure that the bacterial quality of the water that is used complies with all regulations and procedures (Lal et al., 2014). The source water used in oral healthcare facilities is often of local origin, and oral healthcare providers thus depend on the local municipality to ensure that this water is regularly tested for microbial water quality in municipal laboratories and that it is safe for use in oral healthcare procedures. Oral healthcare providers generally do not conduct regular water quality testing of the water they use when performing oral healthcare procedures, nor do they request laboratory results of the microbial or chemical quality of the water they source to ensure that it is safe for patients. Oral healthcare providers can therefore provide very little assurance to their patients that the quality of the water reaching the DCU is safe for dental procedures (Cicciù, 2020).

According to national and international guidelines and recommendations, the onus is on the oral healthcare provider to closely and constantly monitor the bacterial water quality of the water that is used to perform oral healthcare treatments (Ricci et al., 2012; Peralta et al., 2016; Oosthuysen et al., 2020; USEPA, 2021). This suggests that the OHCP should understand and adhere to dental waterline best practices and strategies to ensure that patients and personnel are not put at risk of infection or be exposed to contaminated water resulting from biofilm formation in the tubes of DCUs (Hartshorne, 2010).

1.3 Aim and Objectives of the Study

The aim of this study was to assess the bacterial water quality in DCU waterlines by determining the presence of biofilm-associated organisms in the waterlines of both open and closed systems in the DCUs.

To address this aim, the following objectives were formulated:

- Identify at least twenty oral healthcare facilities in the Mangaung region in the Free State Province - ten utilising open- and ten utilising closed-water system DCUs.
- Collect water samples at various points in the respective DCU systems and analyse these for bacterial quality.
- Collect and analyse swab samples of fast handpieces and distiller/reservoir bottles to determine the presence of biofilm-associated organisms on their internal surfaces and, potentially, in the waterlines of the surveyed dental chair units.
- Compare the bacterial quality of the water in the open system units with that of the water in the closed system units.
- Compare the prevalence of biofilm-associated organisms in the open system units with that of the biofilm-associated organisms in the closed system units.

1.4 Chapter Layout

This thesis is presented in five chapters:

Chapter 1: Introduction to the study provides a brief background. The study problem is discussed and the aim and objectives are presented. The chapter is concluded with a summary of each chapter in this thesis.

Chapter 2: Bacterial water quality of dental unit waterlines. This chapter provides a systematic review of recent literature on the bacterial water quality and biofilm-associated organisms in dental unit waterlines. The review explores literature pertaining to dental unit waterline contamination and the factors that contribute to water contamination and the formation of biofilm in dental unit waterlines. Recommended

water standards for oral healthcare procedures are presented and legislation pertaining to microbial water quality is discussed. The discourse further outlines the indicators of microbial water quality and the risks associated with the presence of opportunistic pathogens in drinking water and biofilm.

Chapter 3: Materials and methods details the study design and methodology used to achieve the aim and objectives. Sampling protocols are discussed for the collection and microbiological analysis of water and swab samples. It is explained that the determination of bacterial water quality in dental unit waterline systems was achieved by methods that detected specific indicator bacteria and biofilm associated organisms. It is also discussed how data were analysed using descriptive statistical analysis methods.

Chapter 4: Results and Discussion. The data presented in this chapter and the results are discussed with specific reference to the bacterial water quality of the tested dental unit waterlines and the biofilm-associated organisms that were traced in the dental unit waterlines that were tested. The results of the bacterial water quality and the swab assessments of the dental unit hard-pieces are presented and discussed.

Chapter 5: Conclusion. The main findings emanating from the study are presented and integrated with the existing body of knowledge. Recommendations are offered for safe water quality assurance during oral healthcare procedures that will benefit patients, oral healthcare practitioners, and other personnel by protecting them against exposure.

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CHAPTER 2: BACTERIAL WATER QUALITY OF DENTAL CHAIR UNITS: A SYSTEMATIC REVIEW OF THE LITERATURE FROM 2009 TO 2021

2.1 Introduction

Globally, infection control continues to be one of the most critical issues in the provision of healthcare services. Infection prevention and the control of potential cross-infection are important if a risk-free environment for patients and healthcare workers is to be established in healthcare facilities in general and in oral healthcare facilities in particular (Dagher et al., 2017). Cross-infection can easily occur while oral healthcare procedures are performed; this occurs through the transmission of infectious agents between patients and oral healthcare providers (Ibrahim et al., 2017). A career in oral healthcare is thus fraught with occupational risks as oral healthcare providers are exposed to a range of occupational hazards that are not necessarily diminished with years of practice (Singh et al., 2014). The risk of infection may occur directly through contact with blood, splatter, oral fluids, and other secretions, or indirectly through contact with contaminated instruments and environmental surfaces.

Moreover, direct transmission of infection to patients, OHCPs, and oral healthcare personnel may occur by exposure to contaminated dental chair unit (DCU) water through aspiration or contact with microorganisms present in aerosols or splatters of oral and respiratory fluids that are channelled through DUWLs (Cristina et al., 2009; Dallocio et al., 2014). Water is an indispensable commodity for the execution of oral healthcare procedures because it is used for the irrigation and cooling of both DCU ancillary equipment and tooth surfaces, while it is also used for oral rinsing. The acceptable microbial quality of DCU water is therefore of fundamental importance in the treatment of patients and for OHCPs who perform oral healthcare procedures (Zhang et al., 2018).

DUWL contamination is related to a diverse consortium of microorganisms that may include pathogenic and non-pathogenic bacteria, fungi, viruses, protozoa, and nematodes (Puttaiah *et al.*, 2012). These microorganisms colonise and replicate on the inner surfaces of DUWLs where they form biofilm. They also occur in water that is used for oral healthcare procedures and therefore, even if some may present with low

pathogenicity, they are of significant concern when oral healthcare procedures are performed on vulnerable patients such as immune- and medically compromised individuals (Lauritano et al., 2017). Microorganisms that slough off from biofilm formed on the inner surfaces of DUWLs are continuously released into the water used for oral healthcare procedures. Once these microorganisms have become resident within DUWLs, they can be ingested, aspirated, and even directly inoculated into surgical sites, which may most certainly result in the development of systemic infections or an oral abscess (Yadav, Priyanka and Archana 2015).

This systematic literature review that was conducted covered the theoretical aspects of microbial water quality of DUWLs and biofilm within these systems. The review explored existing findings and identified gaps for further exploration. The current discourse elaborates on how DCU unit water quality is compromised due to the presence of biofilm and biofilm-associated microorganisms, and elucidates the impact thereof on infection control practices in oral healthcare facilities. The review further highlights dental water quality guidelines as well as guidelines that are legislated for drinking water quality in South Africa.

2.2 Research materials and methods

A systematic review of recent literature on dental unit waterline quality in DCUs was undertaken. This review covered international studies published from January 2009 up to December 2022 and focused mainly on bacterial water quality in DCUs. International electronic databases were searched, including Medline (EBSCOhost), Academic Search Premier (EBSCOhost), Science Direct, SA ePublications, SACAT, ISAP, and the National Library of South Africa. In this process various relevant peer reviewed articles as well as theses and dissertations were scrutinised. The search terms included 'bacterial water quality', 'dental waterline contamination', 'water in dentistry', 'biofilm in dental unit waterlines', and 'dental chair units'. Responses to these search terms were then searched again in more depth. The search produced a total of 3 370 publications, of which 132 (Figure 2.1) were selected based on the fact that they contained qualitative and quantitative data, while those that contained mere information and those that had been published before the 2009 were excluded.

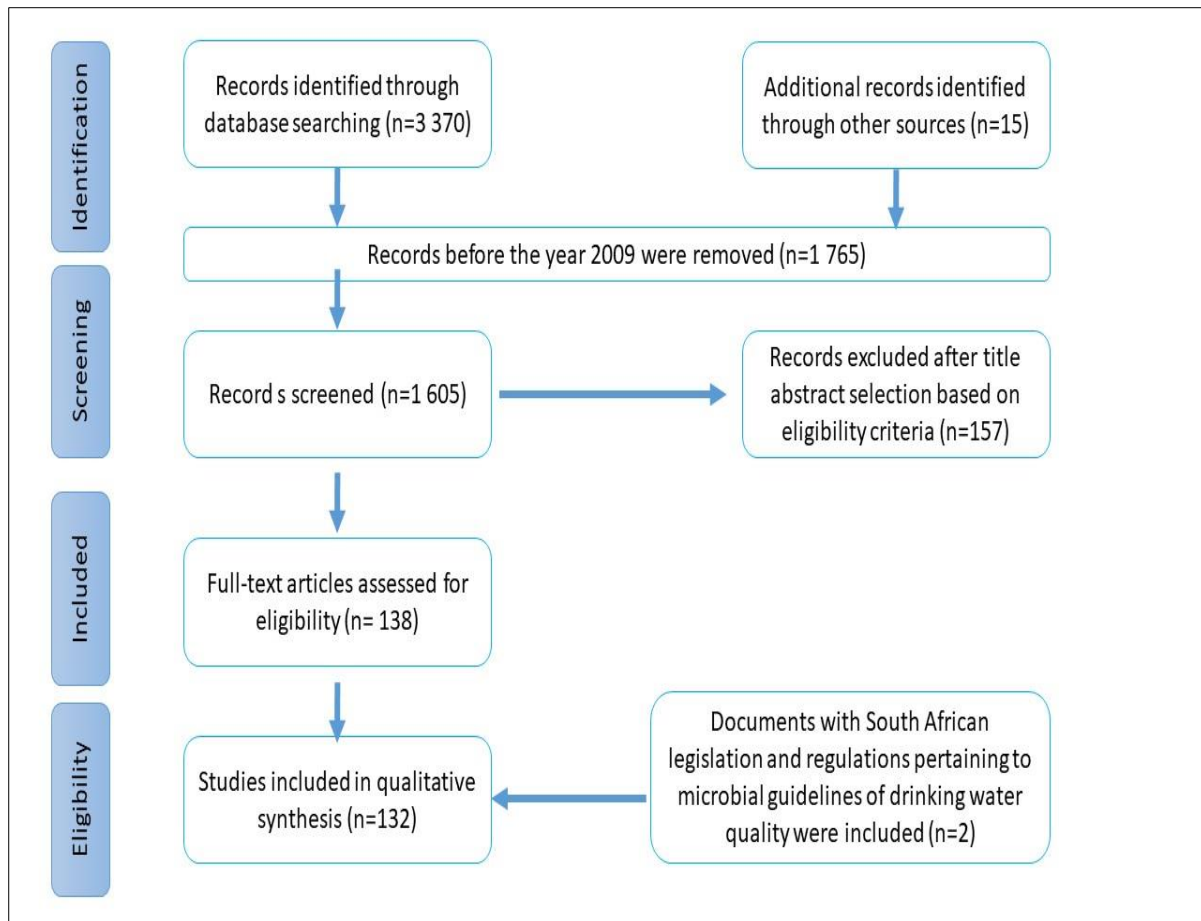


Figure 2.1: PRISMA flow diagram to illustrate the selection of articles included in the study (Moher et al., 2009).

2.3 Background

The dental chair unit (DCU) is an essential piece of equipment for performing routine oral healthcare procedures. Instruments that form part of the DCU, like high-speed rotating handpieces and ultrasonic scalers, generate heat during oral healthcare procedures, and this can cause tissue damage. Water is thus used to prevent the tooth from overheating during cavity preparations or finishing by irrigating the tooth surfaces and it is also used for the rinsing the oral cavity. Water is supplied to DCUs either directly from a municipal source or via tanks or bottles connected to the DCU. The water travels from the source to the various handpieces of the DCU via waterlines which form an interconnected network of narrow bore plastic tubing. Several studies have reported that DUWLs provide an optimal environment for the proliferation of both human and environmental pathogens and can accommodate the formation of biofilms.

The concentrations of bacteria in DUWLs can reach as high as $10^4 - 10^6$ colony forming units (CFU ml⁻¹), and these pose a risk to patients, oral healthcare providers and their personnel, and particularly patients with compromised immunity (Ji et al., 2019; -González, Harel and Guerrero-barrera, 2015).

2.3.1 Water contamination

The microbiological quality of water that is channelled through handpieces during oral healthcare procedures is dependent on the quality of the water that is supplied to the DCU (Siang et al., 2012). O'Donnell et al. (2011) and Szymańska and Sitkowska, (2013) argue that the quality of the water used for oral healthcare procedures is influenced by biofilm that is present on the walls of tubing that channels water through DUWLs. The water supplied to the DCU may be of optimal quality when sourced, but it can become compromised and thus deteriorate once it has been exposed to the biofilm that formed within the synthetic, narrow, plastic tubing of the DCU (Ma et al., 2012).

Bacteria levels in water channelled through DUWLs can often exceed 10^5 colony forming units per millilitre (CFU ml⁻¹) of water, which is approximately 200 times higher than the allowance for drinking water (Volgenant and Persoon, 2018). The available literature reported microbial counts from water exiting handpieces ranging from 10^4 CFU ml⁻¹ to more than 10^8 CFU ml⁻¹ (Costa et al., 2015). Nikaeen, Hatamzadeh and Zareh et al. (2009) demonstrated that heterotrophic plate counts in water from fast handpieces ranged between 7.1×10^2 to 3.68×10^4 CFU ml⁻¹. A study conducted on fifteen DUWLs in an oral healthcare facility in Brazil revealed similar microbial counts of 1×10^3 to 1.6×10^8 CFU ml⁻¹ (Szymańska and Sitkowska, 2012).

The most common cause of contamination of DCU source water and water used for oral healthcare procedures is biofilm formation on the lumens of DUWLs and the subsequent sloughing off of microbial biofilms (Salam, Mulamoottil and George, 2017). Biofilms in DUWLs can reportedly develop to a thickness of 30 to 50 micrometres (µm) (Rodrigues et al., 2017; Mamajiwala et al., 2018). DUWL biofilm formation and the contamination of water used to perform oral healthcare procedures are multifactorial, such as source water not being of optimal microbiological quality, the retraction of oral

fluids into the waterlines because of anti-retraction valve failure of DCU handpieces, water stagnation, heating of DCU water, improper handling and storage of reservoir bottles, and complications with the plumbing system of the DCU (O'Donnell et al., 2011).

2.3.2 Anti-retraction valve failure

DCU handpieces are fitted with anti-retraction valves (to avoid water from passively dripping from the handpieces), air-water syringes, and ultrasonic or electric scalers (to prevent retraction of oral material into the DUWL and the DCU). They have been designed to give handpieces a short 'terminal flush' of water to force out any retracted fluids and materials from the DUWL by creating a negative pressure (Lin, 2011). Water retraction occurs when anti-retraction valves do not function optimally, when the DCU is stopped, and when anti-retraction valves are incorrectly fitted or removed (Bowen et al., 2015). When anti-retraction valves do not function as they should, a negative pressure occurs within the DUWL causing retraction, and this results in water contamination by oral microorganisms (Barbot et al., 2012).

Bhadra et al. (2017) affirm that the colonisation and contamination of DCU water occur when anti-retraction valves fail to function optimally and water and oral material from the operating field are then retracted through the handpieces into the DUWL. A study conducted on the waterlines of 81 DCUs to investigate the presence of oral *Streptococci* in their DUWLs after therapy with the air-turbine handpiece found that oral *Streptococci* had a detection rate of 72%, which suggests that biological fluid that is retracted into the DUWLs during patient treatment may occur more frequently than anticipated (Petti et al., 2013).

The burs in the high-speed air-turbine handpiece uses air to rotate. This air supply is cut off when the air-turbine headpiece stops but it will continue to rotate for a few seconds while the bur is still in the patient's mouth. During this short period of rotation, outside air is drawn in through the exhaust tubing into the turbine handpiece as well as into the DUWL, which may cause a suck back of oral fluids, blood from the oral cavity, and cut shavings (Ozawa, Nakan and Arai, 2010). One millilitre of saliva that is sucked back into a DUWL may contain up to 6×10^9 CFU ml⁻¹ microorganisms

(Bhatnagar et al., 2013). Microbes can enter the aqueous environment of the DUWL and adhere to internal waterline surfaces to form biofilm colonies. The biofilm acts as a reservoir for continuous, long-term water contamination (Tilak, Geetha and Raja, 2012). Moreover, handpieces fitted with anti-retraction valves can become clogged and worn. Anti-retraction valves must therefore be inspected, changed, and maintained regularly to prevent the occurrence of flow back of bacteria and oral fluids from the patient's mouth into the DUWL (Barbot et al., 2012).

2.3.3 Water stagnation

Water stagnation can cause a build-up of high volumes of microorganisms and biofilm formation in DUWLs (Dillon et al., 2014). Microorganisms can enter the waterlines via water that is supplied to the DCU, and they can proliferate in the waterlines when water stagnates. Water stagnation occurs at intermittent periods such as when the DCU is not in use at night, over weekends, and during holiday periods (Costa et al., 2015). Alternatively, oral fluids can also be passively sucked back into the waterlines when DCU water is stagnant and the DCU is not in use, thus allowing colonisation of the DUWL by oral microorganisms as well as biofilm formation (Messano et al., 2013).

Water inside waterline tubing flows fastest in the middle of the water column. As organic matter forms and deposits on the lumen surfaces, the flow of water slows down near the lumen walls and the water becomes practically stagnant; the velocity of the water is then virtually zero at the biofilm-tubing interface. The narrow diameter of the tubing also creates a high surface area to volume ratio, facilitating the deposition and attachment of microorganisms to the waterline surface –which is optimal for biofilm growth (Choi and Nam, 2017). Shahabudin et al. (2018) affirm that the narrow diameter of the DUWL tubing provides an optimal environment for water stagnation.

In a quantitative study conducted in France on 8- to 12-year-old DCUs, samples were collected at two different periods in oral healthcare facilities. The bacterial quality of the water supplied to the DCUs and the water exiting the turbine handpieces after a 48-hour stagnation period (i.e., after a weekend) was examined. The qPCR investigations showed the colonisation of DUWLs by a wide range of potentially pathogenic microorganisms, including bacteria, fungi, and protozoa, despite the

flushing process and disinfecting treatment. Higher 16S and 18S rRNA gene copy numbers per 100 mm of water were quantified in water exiting handpieces than in the water supplied to the DCUs. The results highlight the critical influence of the water stagnation period as it is argued that it significantly increases microbial profusion inside DUWLs (Costa et al., 2016).

Similar observations were made in a study conducted on seven DCUs in France. The results of this study suggest that stagnation and DCU maintenance protocols are critical to the composition of DUWL bacterial communities. Potentially pathogenic organisms, including *Pseudomonas* and *Legionella* spp., were present in the water samples, while emerging and opportunistic pathogenic genera such as *Mycobacterium*, *Propionibacterium* and *Stenotrophomonas* were also recovered from the DUWLs (Costa et al., 2015).

2.3.4 Heating DCU water

Some DCU models have been designed in such a way that water used during oral healthcare procedures can be heated to near body temperature for patient comfort. This practice creates optimal conditions for the proliferation and amplification of human-derived organisms that may be resident within the DUWLs of DCUs (Britto and Muralidharan, 2013). The heating of DCU water creates optimal temperatures that mimic the body temperature of warm-blooded hosts for an assortment of bacteria (O'Donnell et al., 2011). It is also reported that heating DCU water for oral treatment promotes growth and proliferation of *Legionella* spp. at temperatures that exceed 20°C (Liaqat, 2017). Heating DCU water should thus be avoided to minimise the ability of organisms to multiply within the waterline of a DCU (O'Donnell et al., 2011).

2.3.5 Improper reservoir bottle handling

If DCUs are fitted with independent bottles to supply water to the handpiece instruments, the microbiological quality of the water inside the reservoir bottle can be compromised. Reservoir bottles are manually filled with water of OHCPs' choice, which means that municipal, distilled, or sterile water can be used. However, the reservoir bottle as well as its contents can easily become contaminated with skin

bacteria such as *Staphylococcus epidermis* and *Staphylococcus aureus*, which are human pathogens that are introduced as additional microorganisms into DUWLs (Barbot et al., 2012).

A Polish study that was conducted on 107 DCU water reservoir bottles in public oral healthcare facilities found that 54% of bacteria isolated from reservoir bottles were potentially pathogenic, with half of these bacteria capable of causing infectious and invasive diseases. The Polish study revealed that the mean concentration of mesophilic bacteria in DCU reservoir bottle water exceeded 1.1×10^5 CFU ml⁻¹. It was also found that that contamination of water in DCU reservoir bottles with aerobic and facultative anaerobic bacteria was commonplace (Szymańska and Sitkowska, 2013).

Reservoir bottles may become contaminated when they are removed from the DCU and filled with water from an alternative source. Source water may also be contaminated when it is being prepared, if containers are incorrectly handled, and/or if handlers do not use hygienic practices. All of these result in the transfer of organisms from one vesicle to another (Puttaiah et al., 2012). Enteric bacteria and skin organisms can be introduced to reservoir bottles and, consequently, to the DUWL if aseptic and handwashing protocols are not observed correctly (Lin, 2011). Reservoir bottles should therefore be cleaned and disinfected regularly, and they should ideally be sterilised in an autoclave before refilled with water to prevent contamination (Barbot et al., 2012).

2.4 Compromised DCU Water Supplies

The DCU is the single most essential piece of equipment that is used by OHCPs (Coleman et al., 2009). It consists of a complex system of interconnected narrow bore polyvinylchloride tubing that delivers water to various points (Lizzadro et al., 2019). A DCU consists of the dental chair, a pedestal unit that facilitates the supply and control of various instruments and ancillary attachments, and the oral evacuation or suction system. Ancillary attachments may consist of conventional handpieces, high-speed turbines, three-way air-water syringes, and ultrasonic scalers (Paramashivaiah, 2011).

Water is a crucial component of the modern DCU (O'Donnell et al., 2011) as all handpieces need water to function (Coleman et al., 2009). If sufficient water is not used, permanent damage may be caused during tooth preparation when using high-speed handpieces as friction between the tooth structure and the rotating bur creates excessive heat (Kwon et al., 2013). Without sufficient water spray to clear the working area and to cool tooth surfaces, heat can cause structural changes to the hard dental tissues and pathological changes to the dental pulp. It is therefore mandatory to use cooling water when performing oral healthcare procedures with high-speed handpieces. Furthermore, water cooling prevents clogging of the rotary instrument and acts as a lubricant between the cutting instrument and tooth structure (Farah, 2018). The water supplied to the DCU is also used for oral rinsing by patients and to wash out the DCU spittoon, or cuspidor (Coleman et al., 2010).

Dental chair unit water systems are classified as either open or closed based on the water source supplied to the DCU. The open system is directly connected to the municipal water supply while the closed system uses refillable storage tanks or bottled water reservoirs that are fitted to the DCU or that are placed nearby (Weissfeld, 2014). DUWLs are equipped with a dual water supply system that permits the supply of only municipal water or reservoir bottle water, or both. The use of different types of water sources is regulated by a bypass button on the control panel and is selected based on the water volume required or is used as a system of choice by the OHCP (Lizzadro et al., 2019).

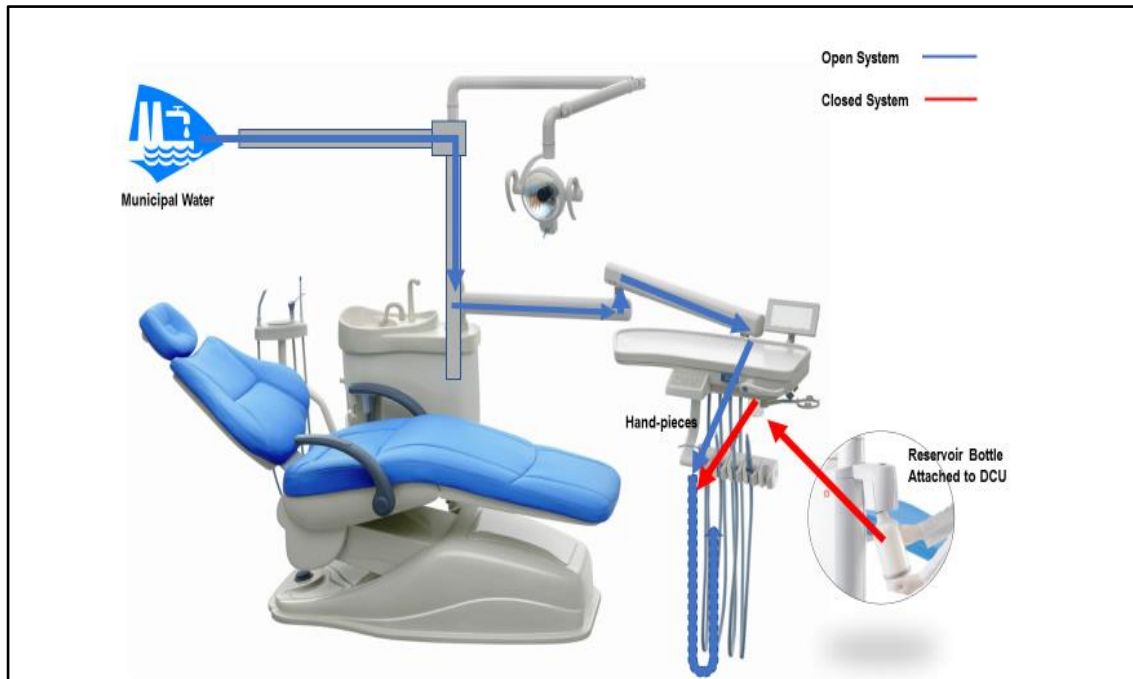


Figure 2.1: Water flow in the DCU in either the open or closed system (Figure accessed from https://www.alibaba.com/product-detail/yoshida-dental-chair_60281718383.html).

2.4.1 Municipal water supply

Historically, the majority of DUWLs were filled with municipal tap water, which is referred to as an open system (Valcina et al., 2014). The perception exists that municipal water supplies contain water of known microbiological quality directly to DCUs because the water is supplied directly from a treatment plant where acceptable protocols are followed to ensure water safety. It is also accepted that the chemical and microbial content of municipal water will vary according to geographical location and the degree of water treatment at municipal water plants. However, water supplied through the municipal water system may contain minerals, mainly calcium carbonates, that are deposited on DUWL surfaces where they promote the adhesion of bacteria (Barbot et al., 2012). Biofilm then forms as a result of these microscopically coarse deposits that colonise the internal surface of the narrow plastic tubing of dental waterlines, and this may eventually lead to waterline obstruction and contamination (Coleman, et al., 2009).

These observations were affirmed by a study that was conducted in Turkey, in which 100 water samples were collected from DUWLs belonging to nineteen private oral

healthcare facilities and thirty-one public oral healthcare facilities. Municipal water was supplied to forty-two out of fifty DCUs while eight DCUs used reservoir bottles. Following bacterial analyses, it was found that 36% of municipal water supplied DCUs contained microbial contamination and that 74% of water exiting these DCUs contained microbial contamination. The results also revealed that the number of microorganisms in the municipal output water was statistically significantly higher ($p < 0.01$) than in the water supplied by means of reservoir bottles, as the formation of biofilm occurred inside the DUWLs that used municipal water (Güngör, Kadaifçiler, and Peker, 2014).

Open DCU systems that receive their water directly from municipal water supplies often contain low numbers of diverse bacterial species. Municipal water with low bacterial counts enters DUWLs, the bacteria attach to the lumens of waterlines, and this eventually gives rise to multispecies biofilm. In Latvia it was observed that, within five days of DCU installation, microbial counts reached levels of 2.0×10^5 CFU ml⁻¹ in the water exiting handpieces. *Legionella spp.* was found in hot water samples from fifteen oral healthcare facilities, but not in the DUWLs. It was thus argued that the incoming municipal water posed a risk of infection and biofilm formation (Valcina et al., 2014).

It has also been stated that the water chlorination process leaves insufficient residual chlorine in municipal water supplies to kill colonised microorganisms, especially those that have built up a resistance to chlorine (Girones et al., 2010; Tilak, Geetha and Raja, 2012). A study by Bridier et al. (2011) found *Legionella spp.* in a municipal water source despite filtration and chlorination procedures.

2.4.2 Independent bottled water systems (reservoirs)

The majority of DCUs currently on the market come with independent reservoir bottles as a default option, and this system is classified as a closed-water system (Mills Porteous and Zawada, 2018). The reservoir isolates the DCU from the municipal water supply and draws fluid from a reservoir bottle holding the supplier's choice of water; or the OHCP can control the quality of the water that is being introduced into the DUWL system as independent reservoirs can be filled with sterile or distilled water or with

water directly from a tap (Ghosh and Mallick, 2012). Unfortunately, many systems using water reservoirs such as sinks, faucet aerators, showers, tub immersion, toilets, dialysis water, ice and ice machines, water baths, flower vases, eyewash stations, and dental-unit water stations have been linked to nosocomial outbreaks (Kanamori, Weber and Rutala, 2016).

A study on DCU water quality demonstrated unacceptably high levels of bacterial contamination in DCUs utilising municipal water and an independent reservoir bottle system (Karpay, Plamondon and Mills, 2010). Another study (Porteous et al., 2011) examined bacterial growth and type on biofilm-controlling DUWL tubing and control manufacturer's tubing in a laboratory DUWL model using ultrapure source water that was cycled through the waterlines, and the water in the reservoir bottles were examined. Bacterial counts measured directly from the purifier unit that supplied reservoir bottles with water were zero, but the counts increased exponentially when contained in a reservoir for 45 to 60 minutes during laboratory sampling procedures.

Szymańska and Sitkowska's (2013) study measured the water quality from six dental chair units and sampled water from various sites in these DCUs, including from the attached reservoir bottles. They found that the highest concentration of microorganisms in the reservoir bottles was 8.9×10^4 CFU ml⁻¹. Higher bacterial counts in reservoir bottles were found in another study (Szymańska and Sitkowska, 2013) that aimed to determine the rate of contamination of DCU reservoir water with aerobic and facultative anaerobic bacteria, and it aimed to investigate the health risks posed to oral healthcare personnel and patients should they be exposed to the isolated bacterial microflora. The average concentration of total bacteria isolated from the reservoir water was 1.1×10^5 CFU ml⁻¹, while the minimum concentration was 3.0×10^1 CFU ml⁻¹ and the maximum was 1.23×10^6 CFU ml⁻¹.

An independent water reservoir that isolates a DCU from the municipal water system can be used intermittently or continuously and cleaned using available cleaners or germicides. An independent reservoir bottle systems can also be used in conjunction with automated germicide metering or slow-release devices that may be fitted with filtration technology (Mills, Porteous and Zawada, 2018). Water reservoirs can easily be filled with disinfectants for regular treatment of dental unit waterlines. These

disinfectants can be flushed through the waterlines prior to and after patient treatments (O'Donnell et al., 2011). It is important that OHCPs employ aseptic and handwashing techniques and that they wear gloves when handling independent reservoir bottles to prevent improper handling that may result in contamination (Lin, 2011).

2.4.3 The formation of microbial biofilm in dental unit waterlines

Biofilms are multi-dimensional microbial aggregations that can form single and multispecies microbial communities. These communities can exhibit diverse features depending on their microbial composition and the microenvironment. Biofilms account for 80% of chronic microbial human infections, leading to increased hospitalisation, elevated healthcare costs, and an increase in mortality and morbidity rates (Khatoon et al., 2018). Biofilm has been associated with upper and lower respiratory tract diseases, native valve endocarditis, chronic otitis media, eye infections, chronic wounds, diabetic foot ulcers, urinary tract infections, and periodontitis (Magana et al., 2018).

Biofilms are highly dynamic microbial communities that can attach to both abiotic and biotic surfaces. They have been implicated in the development of wound infections, non-healing wounds, and medical device-related infections (Percival et al., 2015). It is evident that biofilm formation in man-made tubing attracts microorganisms from the water that flows through them, resulting in biofilm formation and water contamination (Lal et al., 2015). The architecture of DUWLs consists of plastic tubing with extremely narrow diameters (2 to 3 mm) and a system that allows very slow flow rates in handpieces (2-10 mm per minute). These measures favour the deposition of organic nutrients and the adhesion of microorganisms to the surface of the DCU tubing, promoting the formation and proliferation of biofilm on the lumens of waterlines (Yadav, Priyanka and Archana 2015; Lizzadro et al., 2019). The bacteria and other microorganisms that form a biofilm inside the DUWL tubing act as a primary reservoir for continuous contamination of the water used in oral healthcare procedures (Yada, Priyanka and Archana 2015).

DUWL surfaces and narrow tube diameters with their internal aqueous environment provide optimal conditions for the development of microbial biofilms. The source of

microorganisms for biofilm formation in DUWLs may be microbial load of source water piped into a DUWL and the retraction of patients' saliva into the waterline because of retraction valve failure (Nikaeen, Hatamzadeh and Zareh 2009). The source of bacterial contamination of water used in DUWLs is believed to be the accumulation of micro-colonies of proliferating bacteria, fungi, and protozoa on the inner surfaces of the waterlines (Shajahan et al., 2016). These organisms grow in aqueous environments in interwoven layers to form a cooperative ecosystem leading to the formation of biofilm (Tilak, Geetha and Raja, 2012). The narrow diameter of DUWL polyurethane and polyvinylchloride (PVC) tubing creates an optimal environment for rapid biofilm development, which consequently leads to the potential contamination of water used during oral healthcare procedures as well as the aerosols that are generated from handpieces (Wirthlin and Roth, 2015).

Biofilm can be defined as a sessile, heterogeneous congregation of surface-associated microbial cells encapsulated within a self-produced extracellular polymer matrix consisting of polysaccharide, protein, and DNA (Limoli, Jones and Wozniak, 2015). These microbial communities of biofilm produce glycocalyx polysaccharides that protect bacteria against desiccation, chemical treatments, and immunologic attack, while they also provide nutrition to the biofilm colony. Biofilm formation can occur very rapidly, especially within dental unit waterlines as it has been found that biofilm can form within hours after connecting a new DCU to a mains water supply (Perkins et al., 2009). These biofilms then develop into a robust community of micro-colonies embedded in a protective extracellular matrix (O'Donnell et al., 2011). Planktonic pathogens released from biofilm that contaminates DCU water can reach levels of 10^5 CFU ml⁻¹ of water within one week (Pan et al., 2017). This process contributes to an increase in microbial load in water that is channelled into and through the DCU (Singh and Mabe, 2009). The DCU is fitted with an interconnected network of plastic tubing that can extend up to 10 meters long with a diameter of approximately 0.5 mm. A thin, immobile layer of fluid (hydrodynamic boundary) exists at the boundary of the lumen wall (Figure. 2.3) and allows water to flow freely at the centre of the tubing (Abdouchakour et al., 2015). The plumbing system of the DCU is designed in such a way that the physics of laminar flow is promoted within the narrow diameters of the DUWL tubing (Merghni et al., 2017). Laminar flow of water occurs when the water in the waterlines flows in layers (Oleiwi, 2017).

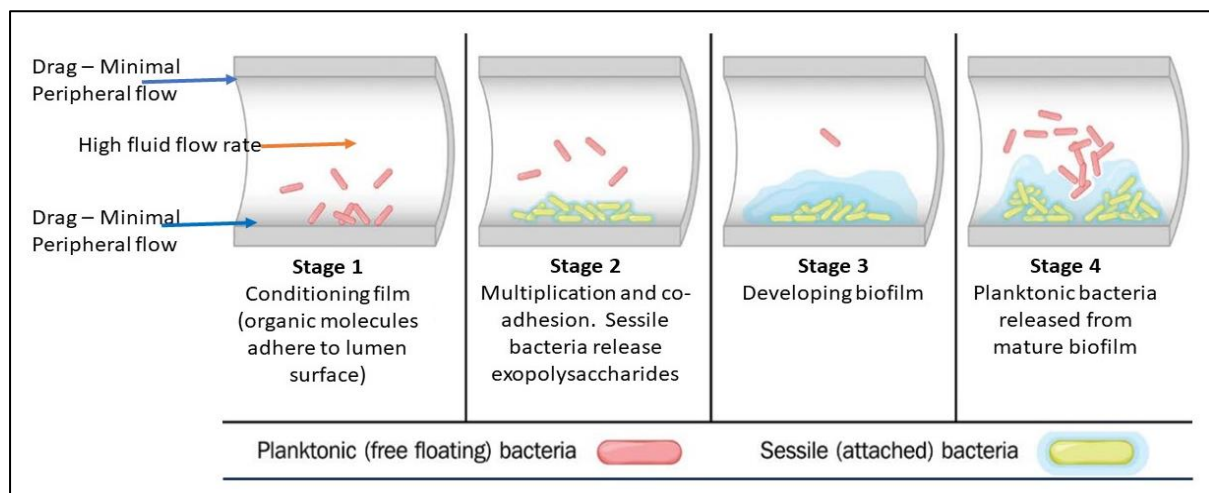


Figure 2.3: Biofilm formation in a dental unit waterline (adapted from Hird, 2016)

Due to the laminar flow pattern of water in the DUWL, a conditioning pellicle will form. Inorganic and organic molecules adhere to and are deposited on inner surfaces of the DUWL by both physical and chemical absorption to form the substratum of the conditioning pellicle that attracts other molecules protruding from the surface of the microorganisms (Mamajiwala et al., 2018). Over a very short time, the conditioning pellicle promotes the attachment of microorganisms, especially bacteria, that enter the hydrodynamic layer, adhere to the conditioning pellicle, and attach themselves more permanently by means of other cell attachment and adhesion mechanisms (Lal et al., 2014). A biofilm is reportedly able to grow inside a DUWL to a thickness of 30 μm after four weeks of growth (O'Donnell et al., 2011). The external surface layer of microorganisms in the biofilm grow rapidly, and some differentiate into robust planktonic cells that percolate in the aqueous environment of the waterline and initiate the formation of new biofilms (Gitipour et al., 2017).

The biofilm that is formed because of laminar flow in DUWLs appears as heterogeneous channels of rounded cell aggregates through which water can flow. These channels act as a communication pathway for the microbial community of biofilm through which percolating nutrients, dissolved oxygen, and metabolic products can flow (O'Donnell et al., 2011; Britto and Muralidharan, 2013). The sheer force that is exerted by handpiece operation facilitates the detachment of planktonic forms and pieces of biofilm that can be directly deposited into the mouth of a patient. Biofilm can

also be seeded elsewhere along the DUWL network or is aerosolised into the surrounding environment (Rodrigues et al., 2017). Aerosols pose an infection risk due to their gross contamination with microorganisms and blood. For instance, a fourfold increase in airborne bacteria was observed in areas where aerosol-producing equipment was used (Sawhney et al., 2015). The latter authors argue that aerosols can remain suspended in air for a considerable period of time before being aspirated by OHCPs and patients.

Biofilm that is resident in DUWLs serves as a continuous reservoir for the contamination of water used during oral healthcare procedures (Rodrigues et al., 2017). The high levels of microorganisms in dental unit waterline output water thus originate mainly from the microbial biofilms that form on the internal surfaces of waterlines (Lachachi et al., 2014). DWULs can be extensively colonised with microorganisms; so much so that at least forty different species have been isolated from the waterlines of DCUs (Szymańska and Sitkowska, 2013).

In a simulation study, a CDC biofilm reactor (CBR) with polyurethane tubing (2 mm internal diameter; Nitta Moore Corp., Gumi, South Korea) was used as a substratum for biofilm formation in the CBR system. Sections of the tubing were dissected longitudinally into two equal portions, and biofilm accumulation was measured at 1.3×10^5 CFU per cubic centimetre with a thickness of 10 to 14 μm after only four days. Bacteria in the biofilm consisted of cocci and rods of short to medium length. Additionally, 38 genera of bacteria were detected in the biofilm that had accumulated (Yoon and Lee, 2017). Coleman et al. (2009) argue that planktonic cells and by-products of colonised bacteria in DUWLs are released into output water and are aerosolised by DCU supplied instruments, and that this exposes patients and oral healthcare personnel to fragments of biofilm and bacterial endotoxins.

Endotoxin is a heat-stable toxin associated with Gram-negative bacterial cells and is released from the lipopolysaccharide layer of these cells into DCU water. Bacterial endotoxin is a significant source of DCU contamination and biofilm formation (Ghosh and Mallick, 2012). Endotoxins may be associated with Gram-negative bacteria that can cause a wide range of physiological responses in humans, including localised inflammation, fever, and even toxic shock in susceptible individuals. When

aerosolised, these endotoxins can have a harmful effect on dental personnel and patients because, when endotoxin is inhaled, it can precipitate reactive airway symptoms as well as asthma severity, which is directly correlated with the concentration of endotoxin (Coleman, O'Donnell et al., 2009).

A study conducted in the United States of America that measured the endotoxin levels in DCU water demonstrated that high concentrations of endotoxin of up to 15 000 endotoxin units per millilitre were present in DCU water (Huntington, Williams and Mackenzie 2015). In the latter study, eight samples of the air in the oral healthcare facility revealed that all aerosol samples contained detectable endotoxin. Considerably higher endotoxin concentrations were found in DUWL samples in a South African study conducted at five academic oral healthcare institutions (Luo, Porteous and Sun 2011). The results of the latter study revealed high endotoxin concentrations of 100 000 endotoxin units per millilitre of water in the DUWL, as well as airborne endotoxin concentrations of 555.9 units per cubic metre in certain areas of the oral healthcare environment (Singh et al., 2010). Once formed, biofilms are very difficult to destroy as they are protected by the polysaccharides and microbes living in a biofilm. The biofilm can be up to 1000 times more resistant to disinfection and as a result can cause serious complications including medical and dental device-related and health-related infections (Luo, Porteous and Sun, 2011).

2.4.4 The quality of water used in oral healthcare facilities

The term 'water quality' refers to the chemical, physical, and biological characteristics of water and is usually used in respect of water's suitability for an intended purpose. The characteristics of water are controlled or influenced by substances that can be either dissolved or suspended in water. Water quality is changed and affected by both natural processes and human activities (Bouslah, Djemili and Houichi, 2017). It therefore follows that water of good quality must be used in DCUs, especially to ensure the health of OHCPs and patients as they are regularly exposed to the water and aerosols generated by DCUs. Water quality has reportedly been of great concern recently because of an increased awareness of the potential occupational hazards it poses and the increasing numbers of immunocompromised patients seeking oral healthcare (Ma et al., 2015).

The quality of the water that is used during oral healthcare procedures should at least be within the stipulated regulations for human consumption because water comes into contact with oral cavities and respiratory tracts in the form of aerosols and may also be orally ingested or inoculated into open wounds (Tyagi, Kulkarni and Krisna Prasad, 2010). The water that flows through and from DCUs is not intended for human consumption, but should at least sensibly, reasonably, and responsibly approximate the acceptable standards and quality of potable water (Coleman et al., 2010).

2.5 Legislation and Standards Regulating the Quality of Drinking Water

2.5.1 An international perspective

Although DCUs are classified as medical devices, manufacturers provide limited guidelines in terms of microbiological water quality and infection control. The premise exists that the water used during oral healthcare procedures is not intended for human consumption as it is used to cool tooth surfaces and irrigate both handpieces and tooth surfaces. However, small quantities of water are often swallowed during oral healthcare procedures and aerosol aspiration (O'Donnell et al., 2011).

Infection prevention and control guidelines have been developed by the United States Centre for Disease Control and Prevention (CDC) through a careful, rigorous, evidence-based process overseen by the Health Infection Control Practice Advisory Committee (HICPAC) Division of Healthcare Quality Promotion of the National Centre for Infectious Diseases (Naik et al., 2014). The CDC recommends that water used for non-surgical oral healthcare procedures should be less than or equal to 500 CFU ml⁻¹ of aerobic heterotrophic bacteria (Michałkiewicz, Ginter-Kramarczyk and Kruszelnicka, 2015; CDC, 2016; Alkhulaifi et al., 2019). The CDC further states that no more than 5% of water samples that are tested when monitoring DCUs should be contaminated with total coliforms (Chate, 2010). The CDC Guidelines are considered the most definitive Standard of Care for Infection Control to prevent the transmission of diseases from the patient to the OHCP, from the OHCP to the patient, and from patient to patient during oral healthcare procedures (Hartshorne, 2010).

The standard required by the American Dental Association (ADA) for water delivered to patients for non-surgical oral healthcare procedures to stem aerobic mesophilic heterotrophic bacteria should be equal to, or not exceed, 200 CFU ml⁻¹ (Ma et al., 2012). However, a reviewed article reported that, over the past decade, virtually all standard dental chair units that were tested contained contaminated water (O'Donnell et al., 2011). Although the CDC and ADA standard for DUWLs is used as a regulatory guideline in the United States of America and in many countries globally, various countries have established their own regulatory guidelines and legislation on standards for water that is safe for human consumption. The drinking water quality standards set for the European Union, the USA and Japan are 100, 500, and 100 CFU ml⁻¹ respectively of aerobic heterotrophic bacteria (Coleman et al., 2009).

Poland and the European Union have no specific regulations for the water that is supplied to DCUs. There is a regulation in these countries for water quality from the Minister of Health of 29 March 2007 that was amended on 20 April 2010, and this can be used as a guideline to regulate the quality of water supplied to DCUs for oral procedures (Michałkiewicz, Ginter-Kramarczyk and Kruszelnicka, 2015). However, in France the quality of the water that is used during oral healthcare procedures through DUWLs is not subject to legislation. In fact, the guidelines published in 2006 by the Ministry of Health of this country refer to anti-retraction valves only by strongly recommending the presence of anti-retraction valves in DUWLs, although no recommendations on either their maintenance, inspection, or change are available (Barbot et al., 2012).

The Australian drinking water quality guidelines are similar to those recommended by the CDC and ADA. The Australian National Health and Medical Research Council implemented drinking water guidelines that state that the number of bacteria in water used as a coolant and irrigant for non-surgical oral healthcare procedures should be less than 500 CFU ml⁻¹ for tooth irrigation during oral cavity preparation, and for ultrasonic scaling it should be no less than the drinking water standard. When treating immunocompromised patients, it is recommended that water from DUWLs should contain less than 200 CFU ml⁻¹ (Australian Dental Association, 2015).

2.5.2 The South African perspective

Drinking water quality in South Africa is regulated by the Department of Water Affairs (DWA) in respect of the Water Services Act No. 108 of 1997 and related regulations promulgated under Section 9 of the Act: Norms and Oral Standards for Quality Water Services. The South African National Standard 241:1 of 2015 (SANS, 2015) Drinking Water Specification provides the minimum requirements for potable water to be considered safe for human consumption in this country.

The South African government currently has no explicit requirements for the quality of water which is supplied to DCUs, and neither has it issued an infection control policy that regulates or guides OHCPs to protect their patients and oral healthcare personnel. The National Health Policy also does not contain any regulations related to oral health aspects to curb the transmission of infectious diseases or for infection control issues related to the practice of oral healthcare (Hartshorne, 2010).

2.6 Microbiological determinants of water quality

2.6.1 Microbial quality measurements

Microbial water quality measures the microbiological conditions of water for human and animal health requirements. The detection, monitoring, prediction, and management of pathogenic contamination of water sources is critical in delivering water that is safe for human consumption and for its intended end use (Pachepsky et al., 2018). Due to the environmental increase of water-based opportunistic pathogens, procedures are required to monitor microbial water quality, particularly in healthcare settings (Ashbolt, 2015).

Microbial water quality measurement is a multi-dimensional science that involves the selection and evaluation of microbial water quality metrics, which currently include the prevalence and concentrations of pathogenic microorganisms, microbial indicators of potential pathogen contamination, and genetic material. It may also include the identification of the sites where organisms originated. Furthermore, microbial water quality may be used to derive metrics and elucidating processes that control the

release, transport, and survival of organisms and genetic materials that are of interest for the microbiological conditions of water (Pachepsky et al., 2018).

In South Africa, SANS 241:1 (SANS, 2015) specifies the quality of acceptable drinking water in terms of physical, chemical, microbiological and aesthetic parameters at the point of delivery. Safe drinking water that complies with the SANS 241:1 (SANS, 2015) requirement does not pose a significant risk to health over a lifetime of consumption, including life stages with increased sensitivity in babies, infants, the elderly, and the immunocompromised. SANS 241:1 (SANS, 2015) indicates that various properties of water need to be monitored to determine if water is safe for consumption. It categorises the properties of water into four primary risk categories and states the minimum values that water must conform with before being considered safe for drinking (SANS, 2011) (Table 2.1).

Table 2.1: SANS 241:1 - Microbiological determinants of drinking water

Determinant	Risk	Unit	Standard Limits
<i>E. coli</i> ^a or faecal coliforms ^b	Acute health - 1	Count per 100 ml	Not detected
Cytopathogenic viruses ^c	Acute health - 2	Count per 10 L	Not detected
Protozoan Parasites ^d	Acute Health – 2	Count per 10 L	Not detected
<i>Cryptosporidium spp</i>			
<i>Giardia spp</i>	Acute Health - 2	Count per 10 L	Not detected
Total coliforms ^e	Operational	Count per 100 ml	≤ 10
Heterotrophic plate count ^f	Operational	Count per ml	≤ 1000
Somatic Coliphages ^{c g}	Operational	Count per 10 ml	Not detected

(a) Definitive, preferred indicator of faecal pollution.

(b) Indicator of unacceptable microbial water quality could be tested instead of *E. coli* but is not the preferred indicator of faecal pollution. Also provides information on treatment efficiency and after-growth in distribution networks.

(c) Confirms the risk of human infection and faecal pollution and provides information on treatment efficiency. The detection of selected viruses confirms faecal pollution of human origin.

(d) Confirms the risk of infection and faecal pollution and provides information on treatment efficiency. The detection of selected protozoan parasites confirms a human health risk.

(e) Indicates potential faecal pollution and provides information on treatment efficiency and after-growth.

(f) Process indicator that provides information on treatment efficiency, after-growth in distribution networks, and adequacy of disinfectant residuals.

(g) Process indicator that provides information on treatment efficiency.

With reference to Table 2.1, microbial water quality should comply with the numerical limits for microbiological determinants. Where a microbiological value exceeds the numerical limit given in column 4 of Table 2.1, an unacceptable risk to human health is implied. Moreover, as the microbiological value increases, an increased risk to health is implied (SANS, 2015).

2.6.2 Indicators of microbial water quality

The quality of drinking water may be determined by microbiological examination. A significant risk is presented by microorganisms in water and this is associated with the consumption of drinking water that is contaminated with excrement from humans and animals, although other sources and routes of exposure may also be significant (Lachachi et al., 2014). Monitoring microbiological water quality is a prerequisite for risk benefit analysis to define practical safety limits for drinking water, human health impacts, and public health risks (Shakoor et al., 2018). Monitoring DCU water is also essential as almost all untreated DUWLs are colonised with aquatic biofilms that contaminate the water used during oral healthcare procedures. Bacterial levels as high as 160×10^7 CFU ml⁻¹ have been observed in dental unit water samples (Karpay, Plamondon and Mills, 2010).

Microbial indicators are used as a measure of possible water contamination for the purpose of water quality monitoring,. Four main indicators of microbial safety of drinking water are used: (i) heterotrophic plate counts (HPC), (ii) total coliform (TC), (iii) faecal coliform (FC), and (iv) *Escherichia coli* levels (Verhille, 2013). Globally, legislation to regulate water quality is measured in coliform counts. However, no safety standards exist for the quantification of opportunistic Gram-negative bacterial species or respiratory pathogens that can be present in the main water supply. These bacterial species numerically constitute the most critical organisms found in DUWLs (Tyagi, Kulkarni and Krisna Prasad 2010).

2.6.2.1 Heterotrophic plate counts (HPC)

The heterotrophic group of bacteria encompasses a broad spectrum of bacteria that metabolise organic carbon sources to grow. Heterotrophic bacteria are able to survive

in environments with deficient organic nutrient concentrations as their energy sources are present in water, air, soil, and food (Diduch, Polkowska, Namieśnik, 2016). Heterotrophic plate count (HPC) is conducted to estimate how much live heterotrophic bacteria may be present in a water sample. The enumeration of heterotrophic bacteria defines the parameters of this broad range of organisms as an indicator of food and water quality that poses a health risk to humans (Jaleilzadeh et al., 2016). Colony count of heterotrophic bacteria, referred to as HPC, provides an indication of the general load of aerobic and facultative anaerobic bacteria in a water sample (Verhille, 2013).

HPC bacteria are a subgroup of heterotrophic bacteria and can be used to detect bacteria but cannot be used as a faecal indicator of water contamination. Heterotrophic bacteria do not indicate pathogenic conditions, but opportunistic bacteria (like *Pseudomonas aeruginosa*) that cause skin and lung conditions and *Aeromonas spp.* (that could cause gastroenteritis) fall within this category of organisms. The enumeration of heterotrophic bacteria using the HPC test is a useful indicator for measuring coliform in water quality assessments (Amanidaz et al., 2015).

Numerous studies have demonstrated that heterotrophic bacteria persist in DUWLs as multispecies biofilm as well as in source water that is supplied to DCUs (Szymańska and Sitkowska, 2008.; Alkhulaifi, et al., 2008; Lal, et al., 2015; Spagnolo et al., 2020). The microbiological quality of water used to supply DCUs, as well as water exiting handpieces when used during oral healthcare procedures, therefore need to be constantly monitored (Türetgen, et al., 2009). For instance, in Istanbul 123 water samples were collected in a study that examined the DUWLs of 21 private oral healthcare facilities. The HPC results revealed high bacterial counts (2.00×10^5 , 3.66×10^5 and 9.7×10^4 CFU ml⁻¹ in air-water syringes, high-speed dental handpieces, and water supplying the DCUs respectively) (Dogruöz et al., 2012). In a quantitative bacterial analysis of samples collected from the DUWLs of twenty-five DCUs, 96% of the water samples did not meet the ADA standard for DCU water quality. This was observed through HPC analysis that indicated that 84% of air-water syringes had a bacterial count of 500 to 20 000 CFU ml⁻¹, while 96% of high-speed handpieces had a bacterial count of 710 to 36 (Nikaeen, Hatamzadeh and Zareh 2009). Bacterial contamination of DUWLs was also evaluated using ATP as well as heterotrophic

bacterial culture on R2A agar. Water from 44 DUWL samples revealed HPC results of 1.4×10^3 CFU ml⁻¹ to 2.7×10^5 CFU ml⁻¹. The quality of water from these HPC results did not meet the standard for HPC as determined by ADA or CDC (Watanabe et al., 2016).

In a study that assessed the efficacy of flushing DUWLs, HPC was observed. Samples were collected from the air-water syringe and the results demonstrated that HPC was high pre-flush. Although HPC decreased after the waterlines had been flushed for 2 minutes, the water did not meet the standards of the ADA and CDC. HPC counts ranged from 1.35×10^4 to 1.2×10^5 CFU ml⁻¹ in unflushed samples and 3×10^3 to 1.5×10^4 CFU ml⁻¹ in flushed samples (Shahabudin et al., 2018).

Several other studies have found high HPC counts that did not comply with ADA or CDC standards. In Göteborg, 75% (303 out of 405) of samples taken from air-water syringes did not meet acceptable drinking water quality standards (Dahlén, Alenäs-Jarl, Hjort, 2009). A study conducted in Switzerland showed that the microbiological quality of water from DUWLs was neither compliant with Swiss drinking water standards nor with the recommendations of the ADA and CDC. Samples were taken randomly from eighty DCUs in forty selected oral healthcare facilities from high-speed handpieces and the three-in-one syringe or micromotor. The results showed that 60% of the samples had HPC counts that did not meet Swiss, ADA, or CDC recommendations (Barben, Kuehni and Schmid, 2009). Identification of heterotrophic bacteria is a useful method in the quality assessment of drinking water in storage tanks and in water distribution networks (Amanidaz et al., 2015).

Although different bacteria pose different risks to public health, some of the main genera of heterotrophs found in water pose a high risk to those who are immunocompromised, such as the elderly, infants, and those with HIV. It is crucial that attention should be paid to the type and quantity of these organisms in order to prevent infection transmission via drinking water (Jaleilzadeh et al., 2016). With reference to SANS 241 (2011), Table 2.1 indicates that drinking water should contain <1000 CFU ml⁻¹ of heterotrophic organisms.

2.6.2.2 Total coliforms

Total coliforms are a group of bacteria commonly found in the aquatic environment in soil and vegetation as well as in the intestines of mammals, including humans. The coliform group of microorganisms belongs to an extensive diversity of organisms, most of which are members of the *Enterobacteriaceae* family that includes harmless *E. coli* and *Enterobacter*, common intestinal organisms, and pathogens such as *Klebsiella* and *Lectercia* genera and some members of the genus *Serratia*. Coliforms are single-celled bacteria that are classified as total and faecal coliforms, where faecal coliforms are indicative of severe water pollution. Despite reservations about their usefulness as indicators of faecal contamination, the total coliform group remains a water quality indicator in many countries and continues to be used to some extent as a regulatory parameter (Maheux et al., 2014).

Coliform bacteria are typically used as an indicator of faecal contamination in water because they inhabit the gastrointestinal tracts of homeothermic animals and are therefore of sanitary significance (Rochelle-Newall et al., 2015). The total coliform (TC) group is referred to as indicator organisms of faecal pollution as it indicates the presence of disease-causing bacteria in water that is hazardous to human health because of their association with the gut of homeothermic animals (Mahath and Mophinkani, 2016). To detect these waterborne pathogens at limited cost, faecal indicator bacteria are used as a proxy for pathogenic bacteria to assess human health risk (Weidhaas, Anderson and Jamal, 2018). The presence of coliform bacteria in water does not mean that drinking the water will cause an illness or pose a threat to health, but their presence indicates that a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply, and that pathogenic bacteria may use this pathway to enter the water supply. The presence of coliform bacteria may also indicate the presence of other potentially pathogenic organisms such as *E. coli*, viruses, and parasites (Gwimbi, 2011).

Establishing the rate of total coliform bacteria in a distribution system may enhance knowledge of water quality integrity throughout a water system and the overall condition of that water system. It is also posited that the total coliform bacteria in a

water distribution system will act as an operational indicator (Saxena et al., 2015). Moreover, the presence of total coliforms in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation or contamination through the gateway of foreign material (WHO, 2011). In instances where faecal contamination is detected, TC will occur in higher numbers than *E. coli*, therefore representing a more sensitive indicator. There are specific genera of TC that are more resistant to disinfection than *E. coli* and that are thus better indicators of inadequate disinfection protocols. The presence of TC in a water distribution system can also indicate a lack of integrity of that system (Maheux et al., 2014). The South African National Standard 241:1 (SANS, 2015), as presented in Table 2.1, indicates that drinking water should not contain >10 CFU 100 ml^{-1} of TC.

2.7 Clinical Impact and Presence of DUWL Pathogens

Water used during oral healthcare procedures is often contaminated with high concentrations of microorganisms, predominantly Gram-negative aerobic heterotrophic environmental bacteria. Maintaining an acceptable quality of the water that is channelled through DUWLs can therefore not be over-emphasised as the health status of patients and OHCPs is greatly influenced if the water is contaminated. Another significant influence on health status and infection risk is the challenge of biofilm formation in DUWLs and the prolonged persistence of biofilm-associated organisms that grow here (Naik et al., 2018). Untreated DUWLs host biofilms and facilitate the multiplication of microorganisms and their dispersion through waterlines. This water is then aerosolised by DCU instrument use, thus exposing patients and personnel to these microorganisms, to fragments of biofilm, and to bacterial endotoxins (Coleman et al., 2009). Microorganisms of at least forty different species have been identified as colonisers of DUWLs, including oral *Streptococci*, *Pseudomonas spp.*, *Enterobacteria*, *Candida albicans*, *Legionella pneumophila*, and nontuberculous *Mycobacterium spp.* (Pawar et al., 2016; Chate, 2010).

The origin of DUWL contamination is specifically related to the formation of biofilm, which is composed of microorganisms within water and is located on internal tubing surfaces. The most prominent bacterial species found in water used for oral healthcare procedures are Gram-negative aerobic heterotrophic environmental species (Chua,

Fathilah, and Himratul-Aznita, 2018) . These organisms exhibit very low pathogenicity but may be of concern when treating patients with systemic diseases and may cause morbidity in immune-compromised patients. Although the results of some epidemiologic studies suggest that contamination of DUWLs can be dangerous in patients with immune-deficiency or other immune system conditions, it can also pose a threat to pregnant women, the elderly, graft recipients, or even smokers. Similarly, the contamination of DUWLs can also impact patients and OHCPs who are in intimate contact with water and aerosols produced in the dental healthcare environment (Mansourian et al., 2011).

Human pathogens found in oral healthcare procedure water with the significant potential for infection include *Pseudomonas aeruginosa*, *Legionella pneumophila*, and the nontuberculous mycobacterium species. Only a few instances of cross-infection related to DUWLs and associated biofilms have been reported in the literature. However, it is still possible that infections caused by water that is used during oral healthcare procedures have gone undetected or unreported because of the failure to associate exposure to DUWL output water and aerosols generated from this water with the development of specific infections (Ditommaso et al., 2016).

2.7.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, rod-shaped and ubiquitous environmental bacterium. It is an opportunistic human pathogen capable of causing a wide range of life-threatening acute and chronic infections, particularly in immunocompromised individuals (Moradali, Ghods, Rehm, 2017). *P. aeruginosa* can thrive in low nutrient environments such as distilled water (Tilak, Geetha and Raja, 2012). It is a medically significant pathogen responsible for a wide range of diseases and nosocomial infections, especially in immunocompromised individuals. This organism frequently exhibits resistance to antibiotics and disinfectants in DUWLs, where it not only survives but proliferates, thus putting hosts with vulnerable immune defence at risk of infection (Fujitani, Moffett and Yu, 2017). It has been shown that *P. aeruginosa* derived from water used during oral healthcare causes infection. Amongst the Pseudomonads, *P. aeruginosa* is the most frequently found bacterium in DUWLs and has been isolated in up to 50% of samples of DCU water at concentrations of up

to 2×10^5 CFU ml⁻¹ with the ability to form resilient biofilms (D'Ovidio et al., 2011). In a pilot study conducted in five Italian cities that sampled DUWLs, *P. aeruginosa* was detected in 33% of the samples (Pasquarella et al., 2010).

Two postoperative cancer patients with solid tumours reportedly became ill due to infected oral wounds by DUWL water that was contaminated with *P. aeruginosa* found in waterlines in the oral healthcare facility where the patients had been treated. The patients subsequently developed oral abscesses and it was confirmed, through pyocine typing, that these were caused by the same strain isolated from the DUWL (Laheij et al., 2012). The latter study also reported that seventy-eight additional patients treated at the same DCU as the two cancer patients had oral colonisation of *P. aeruginosa* from the DCU water for four to ten weeks. It was presumed that these latter patients were not infected because they were not diagnosed with cancer or had weakened immune systems (Hird, 2016).

2.7.2 *Legionella* spp.

Legionella spp. are aerobic, pleomorphic, Gram-negative, motile, and non-spore-forming coccobacillus bacteria (Costa et al., 2015) and are ubiquitous inhabitants of natural and artificial aquatic environments. *Legionella* spp. bacteria are causative agents of Legionnaires' disease and Pontiac fever and have been associated with both community-acquired and nosocomial pneumonia. The major route of transmission of *Legionella*, namely Legionnaires' disease, is through inhalation of contaminated aerosols. Legionellosis outbreaks and deaths have been linked to *Legionella* bacteria that are aerosolised through devices such as shower heads, decorative fountains, cooling tower mists, and supermarket vegetable misting machines (Paridah, Moradba and Mohammed, 2016). *Legionella* spp. is responsible for claiming millions of lives annually worldwide, as *Legionella pneumophila* causes Legionnaires' disease which is a type of fatal pneumonia. *Legionella* spp. enters a susceptible host's respiratory system through small droplets of contaminated water that have been inhaled. All humans are susceptible to being infected by *Legionella*, but those at greatest risk of infection are smokers, drinkers, individuals with impaired immunity, and individuals who may be suffering from a kidney or respiratory disease (Deegan, 2015) *Legionella* infectious in immunocompromised individuals and 90% of nosocomial *Legionella*

bacterial infections can be found in immunocompromised individuals. *Legionella* bacteria can proliferate in multiple organs but commonly cause pneumonia, especially in immunocompromised individuals (Paridah, Moradba and Mohammed, et al., 2016). *Legionella* has the propensity to inhabit DUWLs as narrow plastic tubing provides an optimal environment for *Legionella* and other bacteria to colonise and facilitate biofilm formation (Islam et al., 2016). In one study that investigated the presence of *Legionella* in DUWLs to determine its mitigation by disinfection fifty DCUs were sampled, *Legionella* was found in 36% (18 out of 50) of the samples of water exiting the DUWLs that were tested (Sedlata et al., 2017).

Another study assessed the prevalence of *Legionella spp.* in DUWLs in an oral healthcare facility by verifying microbiological parameters used as indicators of water quality, and this study correlated *Legionella* with contamination. The study was carried out by monitoring sixty-three DCUs, and *Legionella* was isolated from 36.4%, 24.3% and 53.3% of samples from untreated, periodically treated, and continuously treated waterlines respectively. Based on the authors' observations, a risk management plan was implemented in this oral healthcare facility to verify whether the adopted disinfection protocols were effective in preventing *Legionella* colonisation in DUWLs (Leoni et al., 2015).

Aerosolisation of water from handpieces is an inevitable part of oral healthcare procedures. Aerosols from handpieces is a not only a recognised mode of infection exposure of patients and OHCPs to pathogenic bacteria, but also increases the microbial load in oral healthcare facilities (Barben et al., 2009). High concentrations of *Legionella* biofilm can form within DUWLs and aerosolisation of this biofilm can cause infection in humans. Infections are spread by inhaling the aerosols containing *Legionella* or by aspiration of contaminated water. All these aspects present a potential risk for patients and especially for OHCPs and oral healthcare facility personnel (Kevorkyan et al., 2017).

The prevalence of Legionellosis has escalated, which might indicate a greater awareness and reporting of the disease. Three cases reported fatal *Legionella* incidents linked to DUWLs. One is the fatal case of an 82-year-old Italian woman who contracted Legionnaires' disease that could be traced to contaminated DUWLs (Ricci

et al., 2012; Costa et al., 2015). The second case is of a Californian dentist who was exposed to *Legionella* contaminated water from the DUWL aerosols of the chair he used during oral healthcare procedures (Carinci et al., 2018). After the dentist's death, water samples were taken from his home water supply and his oral health facility. *Legionella* was found in high levels ($>1 \times 10^4$ organisms per millilitre) in his DCU but in low levels (<100 organisms per millilitre) in his home water. Although not definitive, the evidence linking his death to his DCU waterline was circumstantial, but there is a high likelihood that the contamination of his dental unit waterlines and aerosols may have been the source of his infection (Barbot et al., 2012).

In 2017, a Swedish report described fatal Legionellosis in a 67-year-old man with leukaemia who had received oral treatment at a healthcare facility (Mills, Porteous and Zawada, 2018). In this case, the results of analyses of clinical specimens and isolates from the dental unit cup-filler used for oral rinsing strongly suggested that they were from a common source. *Legionella pneumophila* serogroup 1 was detected, and serological testing determined that the *Legionella* strain isolated from the patient was the same strain detected in the dental unit water. This case has been validated as one of the first that verified cases of Legionellosis acquired through a dental unit (Schönning et al., 2017).

A case was investigated in Cape Town, South Africa, where a 35-year-old dental receptionist was hospitalised and diagnosed with the pneumonic Legionnaires' disease. A research team, led by the head of the Division of Specialised Environmental Health in City Health in Cape Town, investigated this case. The investigation revealed that the cause of the infection was inhalation of contaminated aerosolised water droplets containing *Legionella spp.* from DUWLs (Chikte et al., 2011).

It has been suggested that *Legionella spp.* that lurks in DUWLs may contribute to respiratory illnesses among OHCPs, oral health personnel, and patients, but that it often remains undiagnosed. Higher rates of seropositivity for *Legionella* antibodies have been found among dental personnel than among the general public, which suggests that aerosols generated during oral healthcare procedures are a source of exposure to *Legionella spp.*, and this is clearly an occupational health risk (Spagnolo, Sartini and Cristina, 2020). OHCPs and oral health personnel may also contract

infection or mild Pontiac fever due to persistent exposure to aerosols from handpieces (Arvand and Hack, 2013). An increase in the number of immunosuppressed or chronic disease patients receiving oral treatment parallels an increase in the number of patients that are susceptible to opportunistic pathogens found in DUWLs. This affirms the notion that the presence of high concentrations of microorganisms in DCU water is a potential risk of infection for oral healthcare patients and personnel and is incompatible with good cross-infection control practices (Tilak, Geetha and Raja, 2012).

2.7.3 Nontuberculous mycobacterium (NTM)

Mycobacteria are aerobic, non-motile bacteria that appear positive with acid-fast alcohol stains. They have a lipid-rich, hydrophobic cell wall that is significantly thicker than that of most other bacteria. Because of the thickness and composition of their cell walls, mycobacteria are impermeable to hydrophilic nutrients and are resistant to heavy metals, disinfectants, and antibiotics. Nontuberculous mycobacterium (NTM) is environmentally ubiquitous, can be found in high concentrations in soil and water, and are associated with biofilm formation. NTM hydrophobicity favours water aerosolisation and many of these organisms are resistant to high temperatures and low pH (Johnson and Odell, 2014).

NTM has been isolated from water systems in hospitals and hemodialysis centres, and it has been reported that oral healthcare facilities experience unusually high rates of mycobacterium colonisation (Kanamori, Weber and Rutala, 2016). Exposure to these atypical *Mycobacterium spp.* through contact, aspiration, or ingestion of contaminated water can cause a range of diseases involving the skeleton, lymph nodes, skin, and soft tissues as well as the respiratory, gastrointestinal, and genito-urinary tracts (Pawar et al., 2016). NTM is a diverse family of microorganism defined as mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Infections in humans by NTM pulmonary disease is possibly acquired through environmental exposure although, in most cases, specific sources of infection could not be identified. Acquisition of NTM pulmonary infection likely occurs through the inhalation of aerosolised organisms and the aspiration of aerosols generated by DCU handpieces. There is a concern that NTM can be swallowed or inoculated into open

oral wounds when oral healthcare procedures are performed (Pankhurst and Coulter, 2007).

The mycobacterium has been found to be 400 times higher in water used during oral healthcare procedures than in general municipal water. These high concentrations of NTM and the continuous exposure to aerosols containing NTM by OHCPs and patients may contribute to respiratory system diseases like pneumonia, inflammation or ulceration of the skin, bone system diseases, as well as hypersensitivity to the organism. It is known that diseases caused in humans by atypical mycobacteria are not transmitted through direct contact, but that infection may occur due to exposure to water aerosol through the respiratory tract and damaged skin. High numbers of NTM may be swallowed, inhaled, or inoculated into oral wounds during dental treatment with the potential for colonisation or infection (Szymańska and Sitkowska 2012). Hruska and Kaevska (2012) affirm that NTM is potentially pathogenic and can cause diseases in humans, especially in individuals who are immunocompromised.

Two cases related to NTM species have been reported that are of public concern. In 2015, twenty children were admitted to hospital after they had been treated at a dental clinic in Georgia by receiving pulpotomy treatments. The CDC investigated the reason for the infections in the Georgia clinic, and it was determined that the source of the bacterial contamination was an untreated DUWL with biofilm that had become colonised with *Mycobacterium abscessus*. In another case that occurred in 2016, fifty-eight children contracted *M. abscessus* infection after they had received pulpotomy treatment at a dental clinic in California. The infections were linked to contaminated DUWLs (Cuny and Eklund, 2017).

2.8 Conclusion

Studies conducted over the past four decades have revealed that an extensive list of organisms that were isolated from DUWLs exists. Although there are few documented cases of disease-related outbreaks due to exposure to DUWL organisms, some organisms detected in oral healthcare facilities have raised a red flag and are reason for caution and concern for patients, OHCPs, and oral healthcare personnel.

DCUs are used for the treatment of most dental patients on a daily basis across the world. Microbial colonisation of DCUs may not be a dire public health risk to healthy individuals, but patients and oral healthcare personnel who fall in the medically compromised, immunocompromised, the very young or elderly categories may be at risk, especially if environmental opportunistic pathogens are present in the waterlines of DCUs or if they are aerosolised during treatment into the surrounding environment.

Infection prevention and control practices are of critical importance in the routine operation of oral healthcare procedures. Water quality guidelines and standard operating procedures should be followed to ensure the safety of patients, OHCPs, and oral healthcare personnel. With the escalating body of evidence related to DUWL-linked illnesses and infections, it is of critical importance that oral healthcare providers adhere strictly to infection control practices and regulations related to water quality. Water should be monitored continuously and regularly tested to ensure that it is of optimal quality during routine oral healthcare procedures involving patients (Mills, Porteous and Zawada, 2018).

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CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

The water quality and biofilm-associated organisms that occur in dental unit waterlines in oral healthcare facilities in Mangaung, Free State Province, South Africa was the focus of this study. The study involved six private and government oral healthcare facilities to examine and evaluate the water quality of the dental chair units (DCUs) from both private and government oral healthcare facilities in the study area.

This study measured microbial parameters of drinking water at source and at the water's corresponding point-of-use. The source of water can be defined as the origin or starting point from which water was supplied to a specific DCU, or from which it was sourced to fill distiller bottles or reservoirs of water attached to DCUs. A sample of water taken from that point in the dental unit waterline system represented treated municipal (tap) water. The point-of-use was defined as the point at which water exited the fast handpiece and was used to perform oral healthcare procedures (Singh et al., 2013). Water quality was measured by analysing heterotrophic plate counts, total coliform counts, and the presence of *Pseudomonas aeruginosa* and *Legionella spp.*

The study was prompted by the problem that microorganisms found in the water supplied to DCUs, as well as in oral fluids that may be sucked back into dental unit waterlines (DUWLs), can attach to the inner surfaces of dental unit waterlines as microcolonies, and this may result in the formation of biofilm in a waterline. Moreover, planktonic forms percolating in DUWLs, as well as biofilm that slough off from DUWLs, can be transferred to patients' mouths from handpiece water, or they may be aerosolised into the environment by rotating handpieces (de Figueiredo-Filho et al., 2019). Swab samples were therefore collected from internal surfaces of tubes that channel source water as well as from surfaces at point of use (distiller bottles, reservoir bottles, and fast handpieces). The swab samples were analysed for the presence of biofilm-associated organisms by determining heterotrophic plate counts, total coliform counts, and the presence of *Pseudomonas aeruginosa*.

3.2 Study Area

The Free State Province lies in the heart of South Africa. It is the third largest province in South Africa comprising an approximate area of 129 825 km². The population of about 2 834 714 is the second smallest population and the second lowest population density in South Africa. Mangaung, which includes the city of Bloemfontein and the Botshabelo and Thaba Nchu areas, became South Africa's newest metropolitan authority in May 2011. The Mangaung Metropolitan Municipality has a variety of oral healthcare facilities ranging for urban to peri-urban based facilities. Various oral healthcare facilities in Mangaung were approached to voluntarily participate in this study (Figure 3.1).

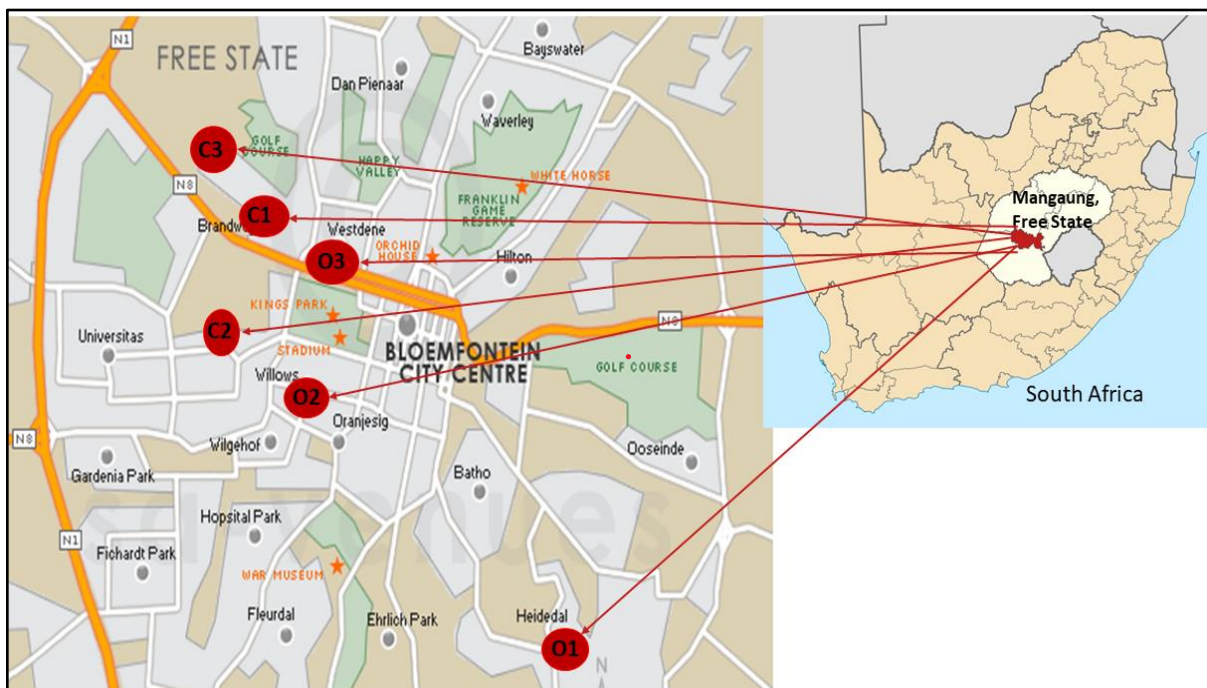


Figure 3.1: Map of Mangaung in the Free State. The image shows the locations of oral healthcare facilities that voluntarily participated in the study. Oral healthcare facilities with open system DCUs are indicated as O1, O2, and O3. Oral healthcare facilities with closed system DCUs are indicated as C1, C2, and C3.

A total of six oral healthcare facilities were ultimately recruited to participate in the study. Three oral healthcare facilities used an open-water system. Two DCUs were investigated at oral healthcare facility O1, four DCUs were investigated at O2, and four DCUs were investigated at O3. Water was supplied directly from the municipal mains to these oral healthcare facilities' DCUs. At the C-facilities, the DCUs used a closed system. Three DCUs were sampled at C1, five DCUs were sampled at C2, and three

DCUs were sampled at C3. At these facilities, the reservoir bottles were filled with distilled water and attached to the DCUs, or fitted at a designated location within the oral healthcare facility to ensure water supply (Figure 3.2).

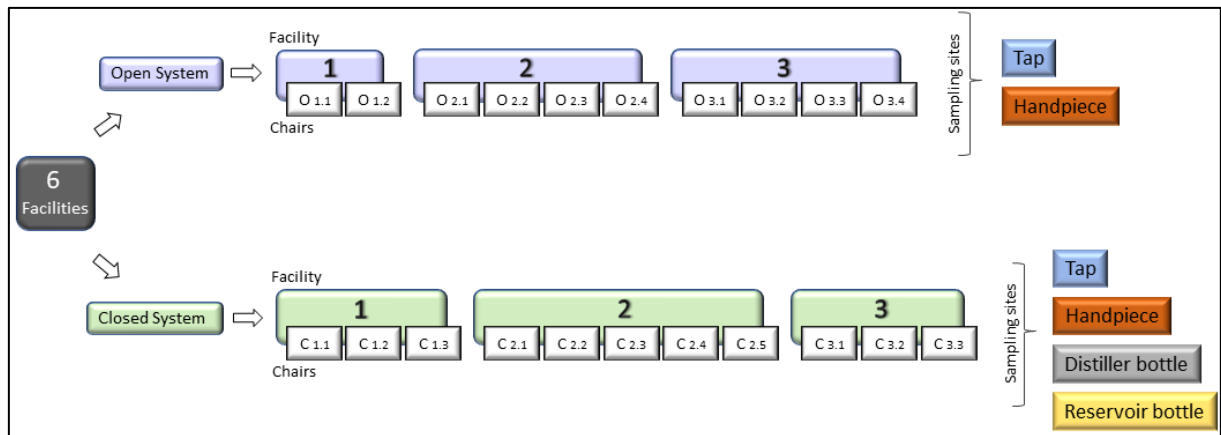


Figure 3.2: Schematic representation of the layout of the participating oral healthcare facilities indicating the number of open and closed system DCUs that were sampled at each site. The figure also illustrates the sampling points for open and closed system dental chair units.

The study followed a quantitative research approach, and a purposeful method of sampling was executed (Figure 3.2). Oral healthcare providers (OHCPs) of an already existing network in Mangaung were approached as part of the process of informed consent to carry out this study at their oral healthcare facilities. The OHCPs received a letter (Annexure A) informing them of the research project and describing the aims and objectives of the study. They were assured of their privacy and anonymity throughout the study and in subsequent reports and articles.

3.3 Study Design

The water quality variables used to measure the environmental health risk during this study was the South African National Standard 241:1 (SANS, 2015) for microbial water quality parameters for heterotrophic plate counts (HPC) and total coliforms. Heterotrophic plate count (HPC) measures a broad range of the total bacterial load in a substance and is typically assessed to determine the general hygiene condition or safety of drinking water (Verhille, 2013). The measurement of total coliform bacteria is a primary indicator of 'potability'; i.e., how suitable the water is for drinking. It may also

be an indicator of the degree of water contamination due to treatment deficiencies or lack of water system integrity (Wingender and Flemming, 2011). It measures the concentration of all (total) of the coliform bacteria in a water sample with the reasonable assumption that higher concentrations are associated with the likely presence of disease-causing organisms as well as those that are more benign. Total coliforms found in water samples can indicate the presence of biofilm or serve as an indicator of treatment efficiency because of their sensitivity to chlorine (Verhille, 2013). To further assess the impact of environmental health risk of contaminated dental unit waterlines, the presence of pathogens (*Pseudomonas aeruginosa* and *Legionella spp.*) were measured to determine the water quality. *P. aeruginosa* and *Legionella spp.* were identified in several studies and their presence was highlighted as a potential health and infection control risks in DUWLs (Perkins et al., 2009; Arvand and Hack, 2013; Ouellet et al., 2015; Schönning et al., 2017).

To determine the presence of biofilm-associated organisms, swab samples were taken of the interior surfaces of taps and fast handpieces of the open system DCUs as well those of the taps, distiller bottles, reservoir bottles, and fast handpieces used in the closed system DCUs. To determine the presence of biofilm-associated organisms, the fast handpieces were uncoupled and swabs were taken of the internal surfaces of the waterlines. Dental unit waterlines were thus not detached from the DCUs. Some sections of the tubing could not be removed or cut to analyse them for biofilm on the internal surfaces of the waterlines due to the high cost of replacing a DCU's waterline tubing.

The variables for biofilm-associated organism detection to assess environmental health risks by this study was the South African National Standard 241:1 (SANS, 2015), with particular reference to microbial water quality parameters. The SANS 241:1 (SANS, 2015) standard was used to assess the results of surface swab samples that ascertained the presence of biofilm-associated organisms for HPC and total coliforms (SANS, 2015). *Pseudomonas aeruginosa* (*P. aeruginosa*) is known to be present in the output water of DUWLs and that it commonly colonises DUWLs to form biofilm (O'Donnell et al., 2011; Costa, et al., 2016; Girardot, et al., 2016; Gawish, Abbass and Abza, 2019). Swab samples that were obtained by from various sampling sites on the

DCUs (both open and closed systems) were analysed for the presence of *P. aeruginosa*.

The water quality and surface swab data are presented and discussed in the following sections with the purpose of assessing the overall environmental and personal health risks posed by DUWLs in the identified oral healthcare facilities in Mangaung. This was achieved by calculating the overall bacterial counts for microbial water quality and biofilm-associated organisms of surface swab samples using descriptive statistical methods.

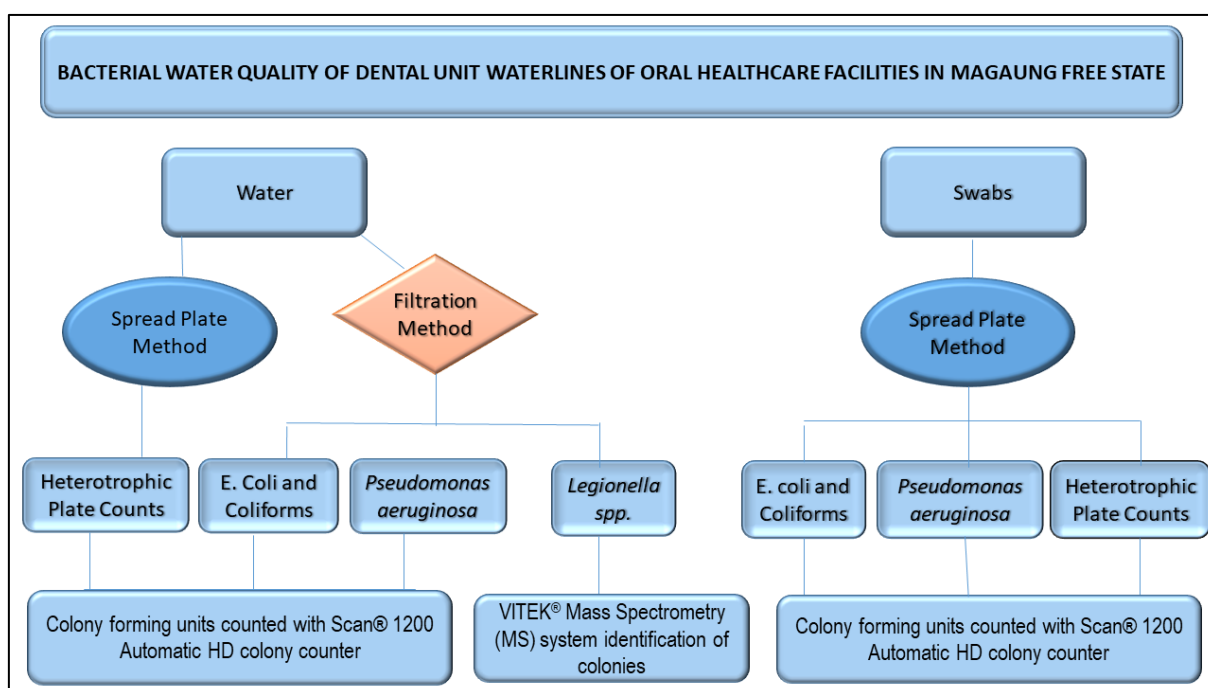


Figure 3.3: Schematic representation of the experimental design for the collection of water and swab samples from open and closed system DCUs. The figure also illustrates the method of bacterial enumeration for the measurement of water quality and the detection of biofilm-associated microorganisms in dental unit waterlines.

3.4 Sampling Protocols

3.4.1 Letter of consent

Oral healthcare facilities participating in the study had to sign a letter of consent and complete a set of questions (Annexure A). The consent letter explained that the study would test the water that was used within the oral healthcare facility and selected DCUs and, more specifically, that the bacterial water quality of the water in the waterlines

would be tested for biofilm-associated organisms DCUs be used and that biofilm swabs would be taken of the municipal water supply, distiller tanks, reservoir bottles, and fast handpiece to test for the presence of biofilm-associated organisms. It was also explained that a set of questions will be asked about the age of the DCU being sampled as well as any cleaning regimes and chemicals that are used for disinfection.

3.4.2 Collection of water samples

The exterior surfaces of taps, fast handpieces and DCU tubing were disinfected with an alcohol swab to avoid any other contamination before sampling commenced (Rodrigues et al., 2017). Water samples were collected aseptically in wide-mouthed sterile glass bottles with a volume of 500 ml and these were labelled with a unique number. Water samples were collected from both open and closed system DCUs. The tap water and water exiting the fast handpieces was allowed to run for ten seconds before a water sample was collected (Lisboa et al., 2014). One sample was collected from the municipal water source and a fast handpiece in each of the DCU systems under study. It was noted that distilled water was used to fill reservoir bottles that were attached to the closed-water system DCUs to supply water to the handpieces. Water samples were additionally collected from distiller bottles as well as from reservoir bottles that were used for closed-water system DCUs. The disinfectant property of the residual chlorine in the water samples was neutralised with the addition of 0.5 ml of sodium thiosulphate (Bedada, et al., 2018).

The water samples were placed in a cooler box on ice and sustained at a temperature not exceeding 4°C (Chua, Fathilah and Himratul-Aznita, 2018) and transported to the Centre for Applied Food Sustainability and Biotechnology (CAFSaB) laboratory at the Central University of Technology (CUT) and processed within twenty-four hours.

Legionella falls within the Animal Biosafety Level-2 (ABSL-2) category. Routine processing of environmental water samples for *Legionella* may only be performed in laboratories accredited to carry out standard ABSL-2 practices. Water samples to test for the presence of *Legionella spp.* were therefore sent to Aspirita Laboratories in Pretoria that has SANAS accreditation for *Legionella* testing.

3.4.3 Collection of biofilm samples

The conventional swabbing method of field testing with a cotton swab has been recommended on surfaces like stainless steel, wood, and plastic and is the recommended method of field testing to detect pathogenic bacteria (Ismail et al., 2013; Jansson et al., 2020). When a cotton swab bud is applied on a material surface it recovers bacteria, fungi, and spores. When placed into an extracting solution, microorganisms collected on the swab are released into an extracting solution during vortexing. The solution is then directly plated or diluted for plating. The swab can also directly be rubbed onto an agar plate with a selective or non-selective growth medium (Ismail et al., 2013).

The exterior surfaces of taps, fast handpieces and DCU tubing were disinfected with an alcohol swab to avoid any other contamination before sampling commenced (Rodrigues et al., 2017). Swab samples were collected from the internal surfaces of distal outlets of taps and the fast handpieces of open and closed system DCUs (Figure 3.4). Distilled water was used to fill the reservoir bottles that were attached to the closed-water DCUs to supply the handpieces with water for use during oral healthcare procedures. Swab samples were therefore additionally collected from distiller bottles and reservoir bottles in the case of closed-water system DCUs.

Swabs of internal surfaces of sampling sites were collected aseptically using a pre-moistened cotton swab (COPAN SRK) containing sodium thiosulphate using a perpendicular back and forth motion (Figure 3.4). Each swab sample was labelled with a unique number. The tip of the swab was broken off and suspended in 1 ml of sterile buffered saline containing sodium thiosulphate to neutralise residual chlorine (Chate, 2010). The swab samples were placed in a cooler box on ice with a temperature not exceeding 4°C. The samples were transported to the CAFSaB laboratory at the CUT and processed within twenty-four hours. The spread plate method was used for the propagation and enumeration of swab samples for heterotrophic bacteria, total coliforms, and *Pseudomonas aeruginosa* for swab samples from both open and closed system DCUs (Porteous et al., 2013).

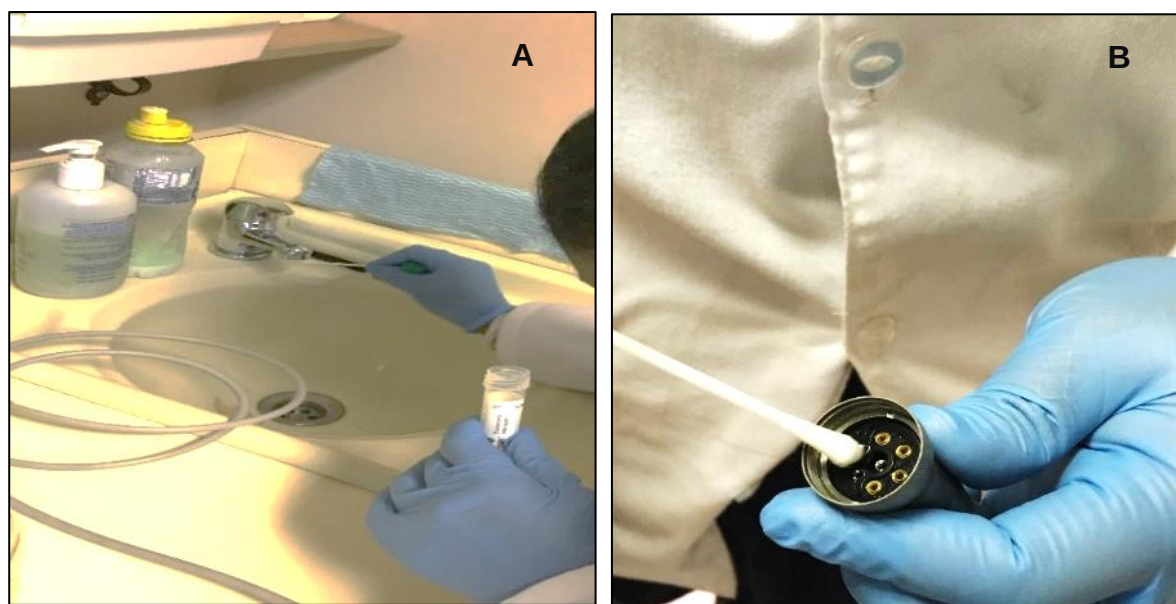


Figure 3.4: Swab samples that were collected from the distal outlets of taps (A) and the waterlines (B) supplying water to fast handpieces. The latter was done after the handpiece had been removed.

3.5 Microbial Analyses

3.5.1 Heterotrophic plate counts

Heterotrophic microorganisms can broadly be defined as all microorganisms that require organic carbon for growth. These bacteria are universally present in all types of water, food, soil, vegetation, and air. Primary and secondary bacterial pathogens are included under this broad definition, as are coliforms. Heterotrophic bacteria are enumerated by applying a variety of methods that are all referred to as heterotrophic plate counts (HPC) that are commonly used as indicator organisms of microbiological water quality (Chowdhury, 2012).

A 1 ml sample of water in an Eppendorf tube was agitated by vortexing for 15 seconds at the highest speed (Nikaeen, Hatamzadeh and Zareh, 2009). A volume of 0,1 ml of the water sample was plated onto R2A agar using the constant function of the easySpiral Pro[®] automatic plater (interscience) and spreading it evenly over the agar (Figure 3.4) (Costa, Mercier, et al., 2016). This procedure was performed in duplicate for all water samples. Plates were incubated at a temperature of 27°C for 5 days (Abdallah and Khalil, 2011). Colonies were counted using the Scan[®] 1200 Automatic

HD colony counter (interscience) and reported as colony forming units per millilitre (CFU ml⁻¹), as stipulated in SANS 241:1 (SANS, 2015).



Figure 3.5: EasySpiral Pro® automatic plater (interscience). The easySpiral Pro® patented technology allows the automatic plating of samples on a Petri dish in only 25 seconds.

The spread plate method was similarly followed for the propagation and enumeration of heterotrophic bacteria for swab samples from the open and closed system DCUs (Porteous et al., 2013). Swab samples were collected from the internal surfaces of distal outlets of the taps and fast handpieces of open and closed system DCUs. As distilled water is used to fill reservoir bottles that are attached to closed system DCUs to supply the handpieces with water for use during oral healthcare procedures, swab samples were therefore additionally collected from distiller bottles and reservoir bottles in the case of closed system DCUs.

3.5.2 Coliforms and *Escherichia coli*

Coliforms can be analysed using a variety of bacteriological media that contain lactose as the primary fermentable sugar. For the detection of coliforms in low level contaminated water, the membrane filtration method is mainly used. Membrane filtration is a very sensitive technique and can detect one (culturable) cell in 500 ml or even 1 000 ml of water (Cabral, 2010).

The membrane filtration technique was performed for the propagation and enumeration of total coliform bacteria (Figure 3.6). A volume of 100 ml of each water sample was filtered through a Millipore Filtration assembly with a sterile membrane nitrocellulose grid filter with a 0.45 μm (microliter) pore size. The water was filtered using a vacuum venturi system attached to a tap which, when opened, created a vacuum to filter the water. Bacteria present in the water sample were retained on the membrane filter (Chua, Fathilah and Himratul-Aznita, 2018). Membrane filters were placed grid side facing up on RAPID'*E. coli* 2 agar (BIO-RAD Laboratories). RAPID'*E. coli* 2 agar for water testing is composed of the RAPID'*E. coli* 2 chromogenic medium and a selective supplement. Agar plates were incubated at 37°C for 24 hours. This procedure was duplicated for all water samples. Following the incubation period, colonies on the surface of the membrane filter were counted. Purple to violet colonies represented *E. coli* colonies while and green to blue colonies represented coliform colonies. Colonies were counted collectively to obtain total coliform counts. Colonies were counted with the Scan® 1200 Automatic HD colony counter (interscience) and reported as colony forming units per 100 millilitre (CFU ml⁻¹) as stipulated in SANS 241:1 (SANS, 2015).

The spread plate method was used for the propagation and enumeration of total coliforms swab samples. Swabs of internal surfaces of sampling sites in open and closed system DCUs were collected aseptically with a pre-moistened cotton swab COPAN SRK solution (which is a non-nutrient phosphate buffered neutralising solution) using a perpendicular back and forth motion. Each swab sample was labelled with a unique number. The tip of the swab was broken off and suspended in 1 ml of sterile buffered saline in an Eppendorf tube containing sodium thiosulphate to neutralise residual chlorine.

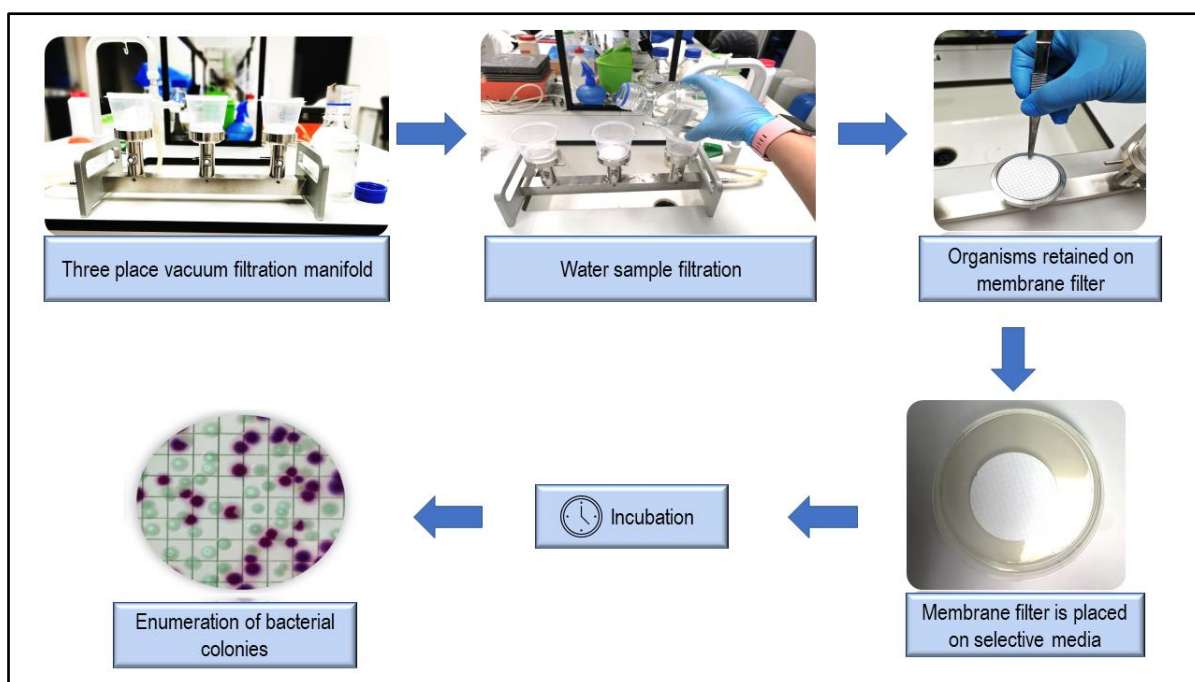


Figure 3.6: Schematic representation of the membrane filtration technique for microbiological analysis of water samples. A 100 ml water sample was passed through a sterile membrane filter housed on a 3-place vacuum filtration manifold. Microorganisms were concentrated onto the micro filter following filtration. The filter containing the suspended organisms were aseptically transferred onto the selected medium for the targeted organism. The passage of nutrients through the filter during incubation facilitates the growth of organisms in the form of colonies on the upper surface of the membrane.

The 1 ml swab suspension in the Eppendorf tube was vortexed at the highest speed for fifteen seconds (Yoon and Lee, 2017). A volume of 0.1 ml of the sample was plated and spread evenly onto RAPID'E. coli 2 agar using the constant function of the easySpiral Pro® automatic plater (interscience). Plates were inoculated in duplicate and incubated at 30°C for 24 hours. Following incubation, colonies on the surface of the agar were counted.

3.5.3 *Pseudomonas aeruginosa*

Propagation and enumeration of *P. aeruginosa* were completed using the membrane filtration technique. A volume of 100 ml of each water sample from the respective sampling sights of open and closed system DCUs was filtered through a Millipore filtration assembly with a sterile membrane nitrocellulose grid filter with a 0.45 µm pore size. Bacteria present in the water sample were retained on the membrane filter. The

membrane filter was placed grid side up onto Cephalothin-Sodium Fusidate-Cetrimide agar (CFC). Cetrimide, fucidin and cephalothin inhibit Gram-positive bacteria and support the growth of *P. aeruginosa*, while inhibiting most other Gram-negative bacteria. This procedure was duplicated for all water samples. The plates were incubated at 30°C for 48 hours. The plates were placed under an ultraviolet (UV) light after their incubation period for the detection of *P. aeruginosa* colonies. *P. aeruginosa* colonies fluoresced under the ultraviolet light and no other colonies were observed as the medium inhibits growth of most other Gram-negative bacteria. Colonies were counted with the Scan® 1200 Automatic HD colony counter (interscience). The number of CFUs that produced fluorescence under UV illumination was recorded as the number of *P. aeruginosa* per 100 ml present in the sample.

P. aeruginosa was propagated and enumerated from swab samples from the respective sampling sites for open and closed system DCUs using the spread plate method. The 1 ml swab suspension in the Eppendorf tube was vortexed at the highest speed for fifteen seconds (Yoon and Lee, 2017). A volume of 0.1 ml of the swab sample was plated and spread evenly onto CFC agar using the constant function of the easySpiral Pro® automatic plater (interscience). The plates were incubated at 30°C for 48 hours. Plates were placed under an ultraviolet (UV) light after the incubation period for the detection of *Pseudomonas aeruginosa* colonies. *P. aeruginosa* colonies fluoresced under the ultraviolet light, and no other colonies were detected as none fluoresced. Colonies were counted with the Scan® 1200 Automatic HD colony counter (interscience). The number of CFUs that produced fluorescence under UV illumination was recorded as the number of *P. aeruginosa* per 100 ml present in the sample.

3.5.4 *Legionella* spp.

The membrane filtration technique was used for the propagation and enumeration of *Legionella* spp. using SANS 7218, which presents the general requirements for and guides microbiological examinations, and ISO 11731 of 2017 (water quality enumeration of *Legionella*). Water samples were collected aseptically in wide-mouthed sterile glass bottles with a volume of 200 ml and each was labelled with a unique number. Water samples were collected from open and closed system fast handpieces.

Analysis for the presence of *Legionella spp.* was conducted by Aspirata Laboratories (Pretoria).

Legionella spp. was isolated using the membrane filtration method. A volume of 100 ml of each water sample was filtered through a Millipore Filtration assembly with a sterile membrane nitrocellulose grid filter with a 0.45 µm pore size. The filter was subject to acid treatment, and then plated onto non-selective charcoal yeast extract (CYE) agar, followed by the addition of a selective GVPC (glycine, vancomycin, polymyxinB, cycloheximide) agar. Plates were incubated for up to 10 days at 36°C. Positive colonies fluoresced when placed under a UV lamp at 360 nm. *Legionella* was further confirmed using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology in the VITEK MS® (BioMerieux). An automated mass spectrometry microbial identification system was used for the identification of bacterial species.

3.6 Questionnaire Administration

A list of questions was administered to the manager of each oral healthcare facility, or his/her representative, to determine the number of DCUs in each facility and to gather information about the cleaning procedures each facility employed to ensure the health safety of patients and personnel. The questionnaires had been prepared in advance, adhered to ethical procedures, and were completed voluntarily. The information that emerged from the questionnaire responses was used to augment the findings regarding the cleaning and decontamination protocols, if any, that were followed at each facility.

3.7 Data Analysis

Descriptive statistics were conducted. Levels of microbial contamination were summarised using descriptive statistics (mean, standard deviation, minimum, median, maximum) separately by type, location, and system. SAS procedure TABULATE (SAS, 2017) software as well as IBM® SPSS® Statistics Version 25 were used for statistical analyses.

3.7.1 Comparison of levels of contamination of heterotrophic plate count (HPC) samples

The levels of contamination with HPC were compared between open and closed systems using a mixed model. SAS procedure MIXED (SAS, 2017) software was used for mixed model analysis. The average count between duplicate samples per chair was used as a dependant variable and not the SANS 241:1 (SANS, 2015). From the mixed model, the mean contamination levels were estimated for the two systems, as well as the mean difference between the two systems and the associated 95% confidence interval (CI) and p -value. Where the p -value was less than 0.05 (<0.05), the null hypothesis was rejected. It could be concluded that there was a difference in the mean count between the two systems.

3.7.2 Comparison of levels of contamination of total coliforms and *Pseudomonas aeruginosa*

Fisher's Exact test was used to analyse the comparison of the proportions of compliant samples between the two systems (refs). SAS procedure SUMMARY (SAS, 2017) software was used for mixed model analysis. The null hypothesis was that the relative proportions of one variable (closed system) were independent of the second variable (open system).

The null hypothesis was that the probability of getting the same number of chairs meeting the SANS 241 (SANS, 2015) standard was the same whether the open or closed system was used. Therefore, if the p -value was <0.05 , the null hypothesis was in favour of the alternative, which would suggest a difference between the two variables; i.e., there was a difference between the two systems that met the SANS 241:1 (SANS, 2015) standard.

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CHAPTER 4: BACTERIAL WATER QUALITY OF DENTAL CHAIR UNIT WATERLINES

4.1 Introduction

Over the past forty years, numerous studies have reported on the high levels of microbial contamination in dental unit waterlines. In this context, it is disturbing that contamination levels ranging from 10^2 to 10^8 colony forming units per millilitre have regularly been reported (O'Donnell et al., 2006; Fujita et al., 2015; Costa et al., 2016; Spagnolo et al., 2019). The water quality of dental unit waterlines may be affected by various factors such as the source water not being of optimum microbiological quality, anti-retraction valve failure, suck back of oral fluids into waterlines, water stagnation, heating of dental chair unit water, and improper handling of reservoir bottles (Spagnolo et al., 2019). Contaminated dental unit water poses a significant risk of infection to oral healthcare providers as well as their personnel and patients, especially those who are immune- or medically compromised (Patel et al., 2016).

The narrow diameters of plastic dental unit waterlines provide an optimal environment for the growth of environmental microorganisms, pathogens, and opportunistic pathogens. Biofilm fragments and planktonic forms can detach from the distal surfaces of dental unit waterlines, and this biofilm contributes to the microbial load of water flowing from the distal outlets of handpieces of DCUs. While performing oral healthcare procedures, the microbial quality of the water exiting the distal outlets of handpieces is dependent on the composition of the water that is supplied to the dental chair unit. Moreover, the quality of the water flowing from the distal outlets of the handpieces will also be affected by any biofilm that is present on the distal surface of dental unit waterlines (Szymańska and Sitkowska, 2013). Dental unit waterline contamination puts immunocompromised patients, all other patients, OHCPs, and personnel at risk of possible infection (Patel et al., 2016).

Dental chair units and their ancillary equipment need a substantial amount of water to function. The use of a dental turbine handpiece and a three-in-one syringe or ultrasonic scaling instrument generates aerosols from the water exiting distal outlets during oral healthcare procedures (Hallier et al., 2010). A study conducted at six clinics at the University of the Witwatersrand Oral and Dental Hospital in South Africa found

that the water samples taken from DCUs contained *Legionella spp.*, which is a Gram-negative organism that was present in 40% of the tested DUWLs. The study argues that even though *Legionella* was present in the water from handpieces in low concentrations, prolonged dental treatment with a high-speed turbine drill could create an aerosol that would release these microorganisms into the air (Singh and Coogan, 2005). Singh et al. (2010), who conducted a study across all dental schools in South Africa, demonstrated the importance of dental institutional characteristics that determine endotoxin exposure levels in dental clinic settings. Their findings demonstrated that considerably high concentrations ($100\,000\text{ EU ml}^{-1}$) of endotoxin were present in water samples taken from three-in-one handpieces. They argued that the presence of endotoxin in the water was indicative of the presence of Gram-negative bacteria in the waterlines, which would be found in biofilm that releases endotoxins during the lysis phase of bacterial growth (Singh et al., 2010). It has been affirmed that biofilm development is encouraged by the environmental conditions within DUWLs and that this poses a risk to patients, OHCPs, and their personnel when they are exposed to water and aerosols generated during oral healthcare procedures, particularly if they are immunocompromised (Robert et al., 2013).

The aim of the current study was to establish the microbial quality of water in oral healthcare facilities in the Mangaung district in the Free State to assess the potential contamination of waterlines and to determine the levels of contamination on distal surfaces (taps, handpieces, and reservoir and distiller bottles).

4.2 Results and Discussion

4.2.1 Heterotrophic bacteria

Dental chair units and their ancillary equipment use water to function when OHCPs perform dental procedures. The water flowing from DCU handpieces and the water used for oral rinsing comes into contact with open wounds during oral procedures and is often swallowed. Water from rotating dental chair instruments can furthermore be aerosolised into the environment and inhaled by patients, OHCPs, and their personnel (Cicciù, 2020). The quality of this water is therefore of critical importance and can be measured through various parameters and the characterisation of their physical,

chemical, and biological properties. Heterotrophic plate counts (HPCs) are practical biological indicators that can be used when assessing water quality (Mukate et al., 2019). HPC analysis is therefore a useful method of monitoring water quality for effective treatment processes as well as the detection of regrowth of bacterial microorganisms in various water distribution systems (Allen, et al., 2004), such as DCUs.

Water quality results of both open and closed system DCUs were obtained and characterised by analysing the concentration of heterotrophic bacteria in the water at various points of use (Appendix A). These HPCs were enumerated on R2A agar which were specifically developed with a low nutrient formulation for the enumeration of heterotrophic organisms in potable water (Porteous et al., 2013). The R2A formulation enhances the recovery of stressed organisms that have a low nutrient acquisition that would not otherwise grow in higher nutrient concentrations (Lal et al., 2014). The results from the HPCs were compared to the SANS 241:1 (SANS, 2015) standard for the presence of heterotrophic bacteria. SANS 241:1 (SANS, 2015)¹ specifies that water should not contain more than 1×10^3 CFU ml⁻¹ of heterotrophic bacteria to be considered safe for human consumption. Tap water as a water source in open and closed systems is supplied by municipalities and is expected to have a low microbial load due to chemical treatment and subsequent microbiological testing of the water before it is distributed to the public to ensure that it is safe for human consumption.

The current study found that the tap water used to supply both the open and closed system DCUs exceeded the required limit for safe drinking water with mean counts of 1.48×10^4 CFU ml⁻¹ and 1.71×10^4 CFU ml⁻¹ respectively (Appendix A). The tap water supplying open system DCUs, particularly O 3.3, only peripherally complied with the SANS 241:1 (SANS, 2015) limit for heterotrophic bacteria with a mean count of 8.5×10^2 CFU ml⁻¹ (Figure 4.1). Zhang et al. (2018) argue that elevated HPCs in tap water may serve as a precursor for further bacterial growth within DUWLs where they propagate the formation of biofilm. According to the SANS 241:1 (SANS, 2015) standard for potable water quality, a heterotrophic bacteria level of above 1×10^3 CFU

¹ Hereafter only referred to as SANS 241:1

ml⁻¹ indicates post treatment contamination or after-growth in a water distribution system.

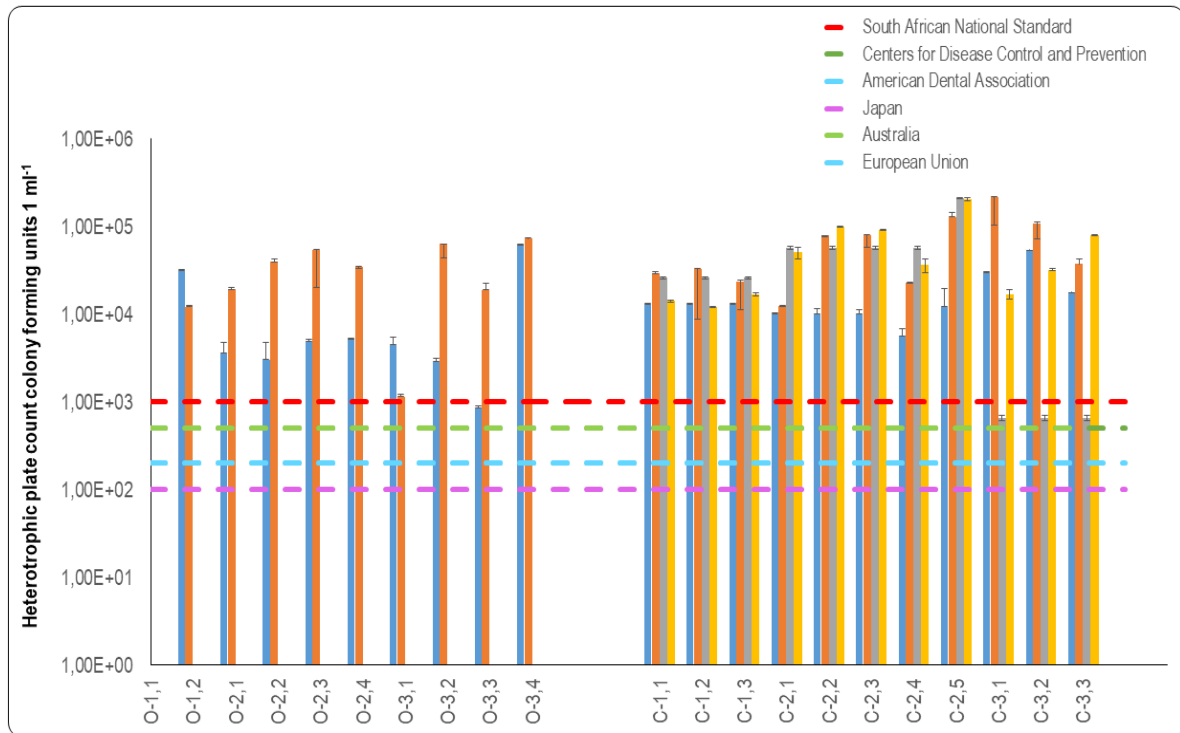


Figure 4.1: Graphic representation of data from the oral healthcare facilities for heterotrophic plate counts for water quality of open and closed system dental units. Water samples were taken from taps (blue) and fast handpieces (orange) in open system DCUs (O1.1 to O3.4). Water samples were taken from taps (blue), distiller bottles (grey), reservoir bottles (yellow) and fast handpieces (orange) used in closed system DCUs (C1.1 to C3.3). The dashed lines represent recommendations by the American Dental Association and the Centre for Disease Control and Prevention for HPCs for DUWLs as well as the drinking water quality limitations for various countries for heterotrophic bacteria per millilitre.

4.2.2 HPCs of water quality for open system DCUs

The tap (source) water that supplies dental chair units with water for functioning of fast handpieces during oral healthcare procedures contained high heterotrophic bacterial counts. It was therefore reasonable to assume that the HPCs from the fast handpieces fed by this water would also exceed the SANS 241:1 (SANS, 2015) limit for heterotrophic bacteria. This was indeed the case, as the HPC values obtained from the fast handpieces of open system DCUs ranged from 1.15×10^3 CFU ml⁻¹ to 7.20×10^4 CFU ml⁻¹, and those from the closed system DCUs ranged from 1.24×10^4 CFU

ml⁻¹ to 2.11×10^5 CFU ml⁻¹ (Figure 4.2). There was a significant difference between the microbial load between tap water and water exiting the distal outlets of fast handpieces in open system DCUs ($p = 0.015$). This significant increase in HPCs in water exiting the fast handpieces of open system DCUs was not unexpected. Similar to the range of HPCs in this study, a study conducted on the water of distal outlets of the three-in-one syringes of DCUs at the oral health centre of Sefako Makgatho Health Sciences University (a dental school and a comprehensive care referral hospital on the outskirts of Pretoria) found that the baseline mean bacterial count was 1.8×10^4 CFU ml⁻¹ in the distal outlet water (Kgabi and Mthethwa, 2020).

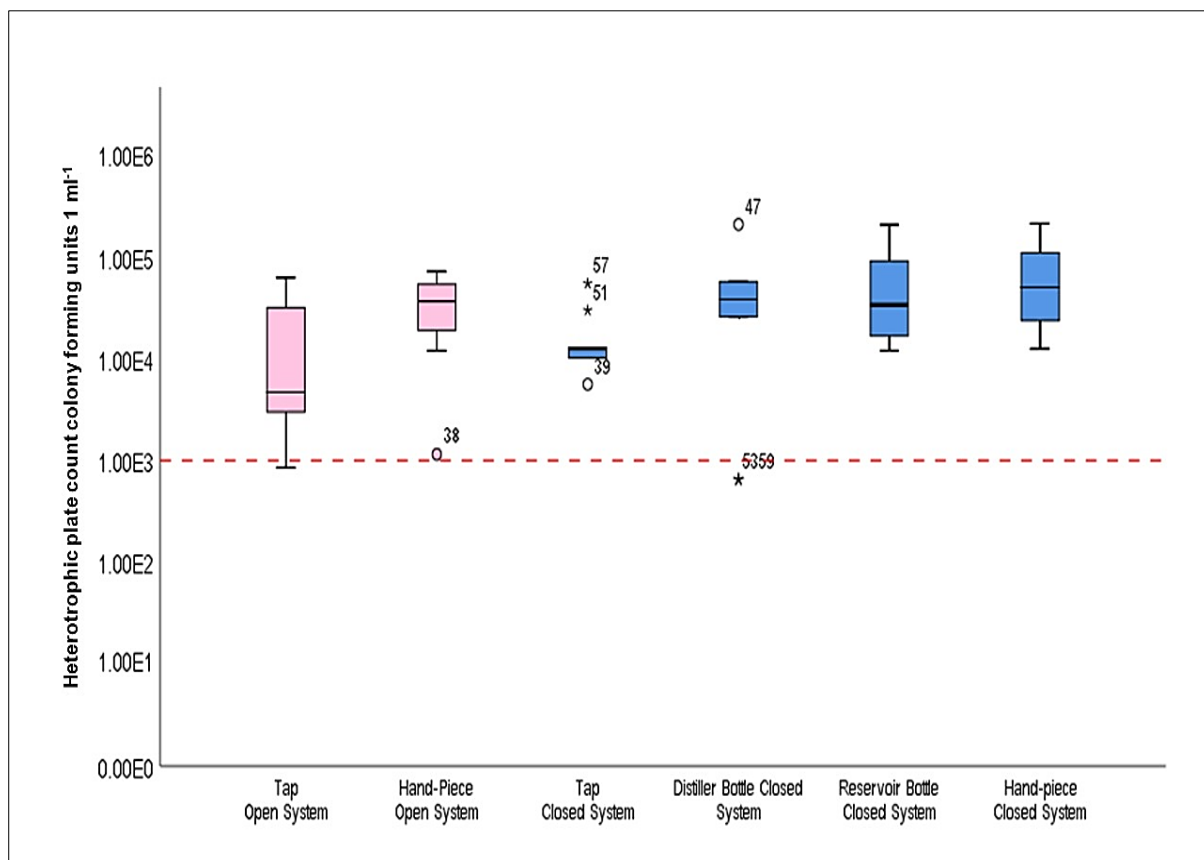


Figure 4.2: ‘Box and whisker’ plots and descriptive statistics of heterotrophic plate counts of water quality in open and closed systems. The bottom and top of each box indicate the 25th and 75th percentiles, where the height of the box therefore indicates the interquartile range. The line dissecting the box is the median value. The ‘whiskers’ indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1 (SANS, 2015) limitation for heterotrophic bacteria per millilitre.

Based on information obtained from the oral healthcare practitioner at each of the facilities, differences in microbial load among facilities were expected (Table 4.1). Facility O2 reported daily flushing of DUWLs as well as autoclaving and cold sterilisation of handpieces. It was therefore expected that water exiting the fast handpieces at facility O2 should have at least a similar biological load in the supply water. This was not the case as can be seen in Figure 4.1, and the observed difference in HPCs between source water and water exiting the handpieces was statistically significant ($p = 0.025$). This result suggests that either the sterilisation methods were inadequate or there could have been biofilm present in the DUWLs. Similar findings were reported by Türetgen, Göksay and Cotuk (2009), who obtained 123 water samples from high-speed handpieces, three-in-one syringes, and source (incoming) water supplies from 41 DCUs in Istanbul. The mean bacterial counts of source water complied with the American Dental Association recommendation. However, 39 out of 41 (91%) DCUs (112 out of 123 water samples) that were analysed at distal outlets (handpieces) were not able to meet the ADA's limit of 200 CFU ml⁻¹ in DU water and were higher than that of the source water supplying these DCUs (Türetgen, Göksay and Cotuk, 2009).



Table 4.1: Results of questionnaire administered to OHCPs

Facility	Chair Number	Age of dental unit (in years)	Is water distilled	What water source is used to fill reservoir bottles	Do you use a cleaning regime to clean DUWLs	If yes, list the chemicals used to clean DUWLs	Indicate the frequency of cleaning regime for the DCU (hours/days/months)	Areas of concern or comments by OHCPs
Open System DCUs								
1	O-1.1	5+	N/A	N/A	Yes	Sodium hypochlorite (bleach)	Daily at the beginning and end of each working day	There are times when the water coming from the taps in the clinical areas are not clean. Sometimes the water is brownish.
1	O-1.2	5+	N/A	N/A	Yes	Sodium hypochlorite (bleach)	Daily at the beginning and end of each working day	There are times when the water coming from the taps in the clinical areas are not clean. Sometimes the water is brownish.
2	O-2.1	6+	N/A	N/A	Yes	Qulaliclean Sodium hypochlorite	Daily at the beginning and end of each working day	Handpieces are autoclaved along with cold sterilisation
2	O-2.2	6+	N/A	N/A	Yes	Qulaliclean Sodium hypochlorite	Daily at the beginning and end of each working day	Handpieces are autoclaved along with cold sterilisation
2	O-2.3	15	N/A	N/A	Yes	Qulaliclean Sodium hypochlorite	Daily at the beginning and end of each working day	Chair is not used as frequently as other chairs in the clinic. Chair is on the 'older' side of the clinic. Handpieces are autoclaved along with cold sterilisation
2	O-2.4	6+	N/A	N/A	Yes	Qulaliclean Sodium hypochlorite	Daily at the beginning and end of each working day	Handpieces are autoclaved along with cold sterilisation
3	O-3.1	7	N/A	N/A	Yes	Steri201 Quaternary Ammonium Compound/ Biguanide blend (99,3%)	Oral healthcare provider did not comment	None
3	O-3.2	7	N/A	N/A	Yes	Steri201 Quaternary Ammonium Compound/ Biguanide blend (99,3%)	Oral healthcare provider did not comment	None
3	O-3.3	7	N/A	N/A	Yes	Steri201 Quaternary Ammonium Compound/ Biguanide blend (99,3%)	Oral healthcare provider did not comment	None
3	O-3.4	7	N/A	N/A	Yes	Steri201 Quaternary Ammonium Compound/ Biguanide blend (99,3%)	Oral healthcare provider did not comment	None
Closed System DCUs								
4	C-1.1	30	Yes	Distilled	Yes	Diluted Milton (Active ingredient hypo chloride)	Every three months	None
4	C-1.2	30	Yes	Distilled	Yes	Diluted Milton (Active ingredient hypochlorite)	Every three months	None
4	C-1.3	30	Yes	Distilled	Yes	Diluted Milton (Active ingredient hypochlorite)	Every three months	None

Table 4.1: Results of questionnaire administered to OHCPs at the various dental healthcare facilities (continued)

5	C-2.1	5+	Yes	Distilled	Yes	E20 waterline treatment. Hydrogen peroxide 1-2%	Daily at the beginning and end of each day. Monthly shock treatment	There is a distilled water tank in each clinical area from which the reservoir bottles are filled.
5	C-2.2	5+	Yes	Distilled	Yes	E20 waterline treatment. Hydrogen peroxide 1-2%	Daily at the beginning and end of each day. Monthly shock treatment	There is a distilled water tank in each clinical area from which the reservoir bottles are filled.
5	C-2.3	5+	Yes	Distilled	Yes	E20 waterline treatment. Hydrogen peroxide 1-2%	Daily at the beginning and end of each day. Monthly shock treatment	There is a distilled water tank in each clinical area from which the reservoir bottles are filled.
5	C-2.4	5+	Yes	Distilled	Yes	E20 waterline treatment. Hydrogen peroxide 1-2%	Daily at the beginning and end of each day. Monthly shock treatment	There is a distilled water tank in each clinical area from which the reservoir bottles are filled.
5	C-2.5	5+	Yes	Distilled	Yes	E20 waterline treatment. Hydrogen peroxide 1-2%	Daily at the beginning and end of each day. Monthly shock treatment	This chair uses a different distiller than the other chairs in the oral healthcare facility. Water is poured into the reservoir bottle.
6	C-3.1	6	Yes	Distilled	Yes	San-O-Dent (Water, sodium chloride, HOCl - Hypochlorous Acid)	Flush beginning and end of day. Weekly clean/shock	It was observed that the protocol for filling reservoir bottles from distiller bottles was that the distiller bottle cap was placed face down on the counter. The entire contents of the distiller bottle were not emptied into the reservoir bottle and the distiller bottle was closed for later use. The RB was removed from O3.1, and dental assistant did not wear gloves when removing or replacing the RB to the DCU
6	C-3.2	6	Yes	Distilled	Yes	San-O-Dent (Water, sodium chloride, HOCl - Hypochlorous Acid)	Flush beginning and end of day. Weekly clean/shock	
6	C-3.3	6	Yes	Distilled	Yes	San-O-Dent (Water, sodium chloride, HOCl - Hypochlorous Acid)	Flush beginning and end of day. Weekly clean/shock	

4.2.3 HPC water quality of closed system DCUs

The water used to supply closed system DCUs was subject to a distillation process. Distilled water was used to fill reservoir bottles that were attached to the DCUs to supply them with water. The microbial load of distilled water should be the same as, if not lower, at its point of that of the source (tap) water when reservoir bottles are filled. This was not the case (see Figure 4.1) at facility C1 and C2 where the HPCs of water in the distilled bottles were higher than those of the source water, and this finding was thus statistically significant ($p = 0,004$). This finding may be an indication that the distillation process was ineffective and that the distillers were not functioning optimally. Additionally, the HPCs of the water distilled at facility C1 and C2 may also have been elevated as a result of the initial microbial load in the source water, thus rendering the distillation process ineffective in eliminating or reducing the microbial load in the water that would be used in the reservoir bottles.

This finding affirms that it is important to inspect and service distillers regularly to ensure that they are working optimally. The elevated HPCs may have occurred as a result of biofilm present in the distiller bottles due to ineffective disinfection, as proposed by Mills, Porteous and Zawada (2018). The distillation process at facility C3 resulted in a reduction of HPCs after source water treatment, as observed in Figure 4.1. However, this process does not necessarily guarantee the elimination of potentially pathogenic organisms.

The quality of the water distilled at facility C3 was within the SANS 241:1 (SANS, 2015) limit for HPCs. Distilled water was used in reservoir bottles to supply water to the closed system DCUs. The microbial load in distiller and reservoir bottles should be different, as reservoir bottles should have an exceptionally low to no microbial load at all. The HPCs for the water in the distiller bottles at facility C3 all complied with SANS 241:1(SANS, 2015) for HPCs after the distillation process. However, the water in all the reservoir bottles of the DCUs at facility C3 displayed elevated HPC values compared to those of the water in the distiller bottles (Figure 4.1). Although there was an observed difference in HPCs between the water in distilled and reservoir bottles, this difference was not statistically significant ($p = 0.052$). Based on the information received from the oral healthcare practitioner at facility C3 (Table 4.1), the DUWLs

were flushed daily, and shock treated weekly. However, regardless of measures to disinfect the waterlines, the reservoir bottles were contaminated and therefore most likely contained biofilm. It was observed that reservoir bottles were disconnected from DCUs and filled at facility C3 without using gloves, which could have contributed to the contamination of the reservoir bottles. Skin organisms can easily contaminate reservoir bottles and can introduce human pathogens and other human organisms into DUWLs. To prevent contamination, reservoir bottles should be handled aseptically and cleaned and disinfected regularly. Reservoir bottles should preferably be sterilised by autoclaving (Coleman, 2009). In a study conducted on the water quality of reservoir bottles, Szymańska and Sitkowska (2007) found that the water in all the reservoir bottles sampled in their study were contaminated. The bacterial counts in reservoir bottle water in the current study had a minimum count of $2.23 \times 10^4 \text{ ml}^{-1}$ and a maximum count of $5.83 \times 10^5 \text{ CFU ml}^{-1}$. The results of this study thus revealed a similar range of HPCs in DCUs with a minimum of $1.20 \times 10^4 \text{ CFU ml}^{-1}$ and a maximum of $2.06 \times 10^5 \text{ CFU ml}^{-1}$ (Appendix A and Figure 4.2).

The tap water used to fill the distiller bottles for DCUs C3.1 ($2.98 \times 10^4 \text{ CFU ml}^{-1}$), C3.2 ($5.46 \times 10^4 \text{ CFU ml}^{-1}$), and C3.3 ($1.80 \times 10^4 \text{ CFU ml}^{-1}$) did not comply with the SANS 241:1(SANS, 2015) standard (Figure 4.1). The water from the distiller bottles of DCUs C3.1, C3.2, and C3.3 was only marginally compliant with the SANS 241:1(SANS, 2015) limit for heterotrophic bacteria at a count of $6.50 \times 10^2 \text{ CFU ml}^{-1}$ respectively for all three distiller bottles (Figure 4.1). The reservoir bottles were filled with water from the same distiller bottle on the day samples were taken from these DCUs (researcher's observation). The distillation process itself may also have caused a decrease in the number of HPCs in these DCUs as the distillation process can remove waterborne biological contaminants such as bacteria, viruses, and others (Niveda and Malaiappan, 2019).

Although an increase in HPCs is evident in Figure 4.1 when looking at all three closed system DCUs, the one order of magnitude differences between distiller and reservoir bottles were not statistically significant ($p = 0.21$). The HPCs of closed system reservoir bottles and fast handpieces also showed no statistical difference ($p = 0.476$). HPCs for reservoir bottles ranged between a minimum count of $1.20 \times 10^4 \text{ CFU ml}^{-1}$ to a maximum count of $2.05 \times 10^5 \text{ CFU ml}^{-1}$. Counts for handpieces displayed a similar

range with a minimum of 1.24×10^4 CFU ml⁻¹ and a maximum 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, perhaps because the initial microbial load was too high for the distillation process to have eliminated all microbes in the water supply; or the reservoir bottles may have been contaminated and possibly contained biofilm.

4.2.4 Comparison of HPC water quality between open and closed system DCUs

It was evident that the HPCs for tap water quality and the water used to perform oral healthcare procedures did not meet the SANS 241:1 (SANS, 2015) standard in either the open or closed system DCUs. There was a statistically significant difference ($p = 0.040$) in the HPCs of tap water between the open and closed system DCUs. The count for the tap water of closed system DCUs (1.71×10^6 CFU ml⁻¹) was higher than that of the open system DCUs (1.50×10^6 CFU ml⁻¹). There was no statistically significant difference between the counts for the water exiting distal outlets of the fast handpieces used in the open and closed system DCUs ($p = 0.099$). However, the HPCs for fast handpieces in both the open and closed system DCUs were high and exceeded the SANS 241:1 (SANS, 2015) limitation, which suggests that further contamination could potentially have occurred in the DCUs of both systems. The average microbial load HPC in water from the fast handpieces of the open system DCUs was 3.53×10^6 CFU ml⁻¹, while that of the closed system DCUs was 6.94×10^6 CFU ml⁻¹ in. For DUWLs, earlier studies reported an average viable microbial count for water exiting dental handpieces of 1×10^5 CFU ml⁻¹ (Walker et al., 2000; Pederson et al., 2002, Walker & Marsh, 2004).

HPCs have been used to monitor the effectiveness of disinfection treatments and to measure the extent of bacterial colonisation found in water distribution systems. Considering the questionnaire information supplied by the oral healthcare providers of the various oral healthcare facilities (Table 4.1), it appears that different cleaning and sanitising protocols did not make a significant difference to the microbial load exiting fast handpieces. This was evident even though disinfectants and sanitisers with different active ingredients were used.

The fact that the HPCs for the surveyed DCUs did not comply with the SANS 241:1 (SANS, 2015) regulation for drinking water does not necessarily imply that there was (or will be) an imminent public health risk. The monitoring of water quality using HPCs as indicators, which are not themselves indicative of the cause of an infection or disease, must be realistically interpreted as organisms that *could* predict potential risks associated with pathogens (Duda et al., 2015). Therefore, the presence of heterotrophic bacteria in water that is supplied to DCUs serves as a potential and not actual initiator of biofilm formation in DUWLs. The biofilm formed in DUWLs excretes an extracellular matrix that provides protection for the growth of more pathogenic organisms (O'Donnell et al., 2011). High HPCs can thus serve as an early warning system for potential health risk and should not be used exclusively as a contamination indicator in DCUs (Bristela et al., 2012). Bristela (2012) recommends that distribution pipework and/or output water exiting DCUs be further examined for pathogenic organisms such as *Pseudomonas aeruginosa* and *Legionella spp.*

4.2.5 Total coliforms

Infection burden and risk to human health can greatly be attributed to the microbial contamination of water, and therefore the monitoring and testing of water quality for microbial contamination is a significant element of drinking water safety (Bain et al., 2012). The analysis of HPCs for microbial water quality indicates the effectiveness of water purification or disinfection processes. Water that measures high HPCs suggests the presence of coliforms and may indicate that faecal contamination is present in a water distribution system. However, the presence of coliforms in water does not necessarily indicate a threat to human health (Mulamattathil, Bezuidenhout, and Mbewe, 2015), but it could indicate that pathogens associated with intestinal infections, dysentery, hepatitis, typhoid fever, cholera, and other diseases could be present in the affected water system (Emmanuel, Pierre and Perrodin, 2009). If total coliforms are present in a water distribution system, it may additionally indicate a lack of system integrity. It is for these reasons that total coliform bacteria are commonly used to evaluate the general sanitary quality of water (Maheux et al., 2014).

In the current study, the water quality results of the open and closed system DCUs were analysed by determining the concentration of total coliform bacteria present in

the water at various points of use (Appendix A). The results from the total coliforms were compared to the SANS 241:1 (SANS, 2015) requirement for the presence of total coliform bacteria. SANS 241:1 (SANS, 2015) specifies that water should not contain more than 1×10^1 CFU ml⁻¹ of total coliform bacteria to be considered safe for human consumption.

It was found that the mean total coliform counts recorded for the tap water supplied to the open system DCUs were: O2.1 = 2.35×10^1 CFU 100 ml⁻¹, O2.2 = 1.40×10^1 CFU 100 ml⁻¹, O2.3 = 2.95×10^1 CFU 100 ml⁻¹, and O2.4 = 2.25×10^1 CFU 100 ml⁻¹. These levels did not comply with the SANS 241:1 (SANS, 2015) limit for total coliform bacteria in water (Figure 4.3). The presence of coliforms in the source water that is channelled to DCUs can serve as a precursor for the formation of biofilm in DCUs' DUWLs, where these organisms can attach to waterline lumens and form biofilm colonies that further multiply in DUWLs.

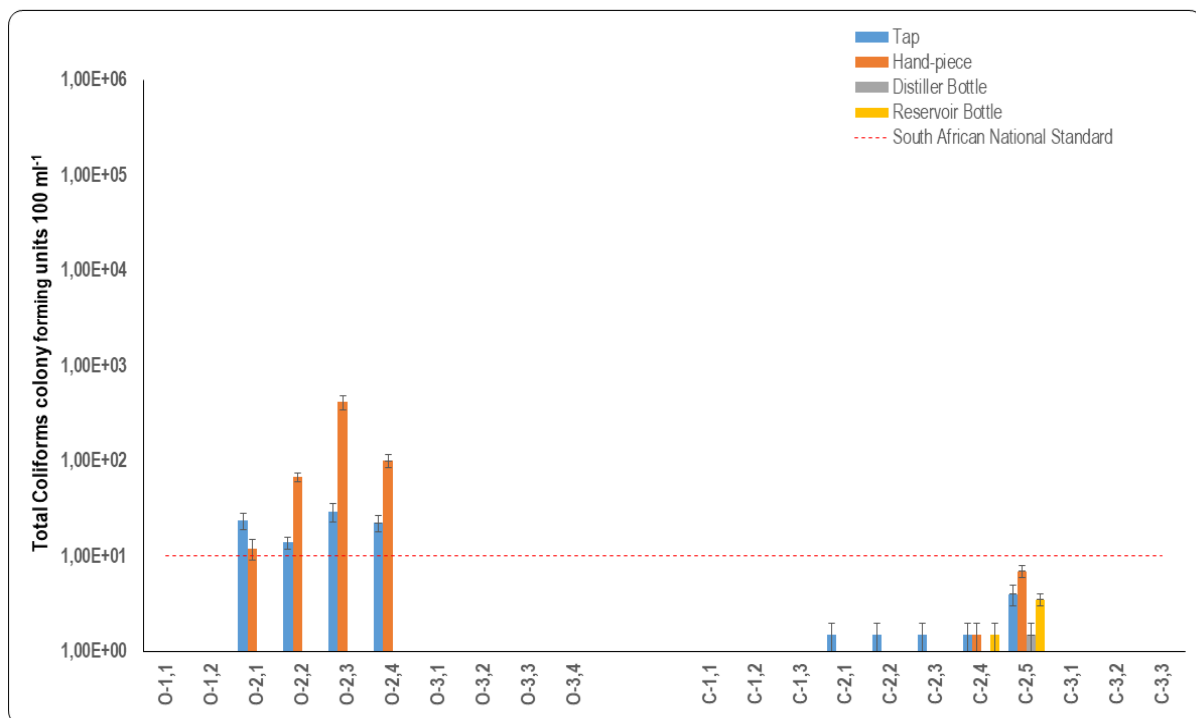


Figure 4.3: Graphic representation of data from oral healthcare facilities for total coliform counts for water quality of open and closed system dental units. Water samples were taken from taps and fast handpieces in open system dental chair units (O1.1 to O3.4). Water samples were also taken from taps, distiller bottles, reservoir bottles, and fast handpieces of closed system dental chair units (C1.1 to C3.3). The

dashed red line represents the SANS 241:1(SANS, 2015) limit for drinking water quality for total coliforms per 100 millilitres.

A fast handpiece relies on water to function effectively and for the OHCP to perform oral healthcare procedures. The results of the water supplied to the fast handpieces of the open system DCUs indicated that it would be reasonable to assume that the water exiting the fast handpieces would similarly display mean coliform counts that would not meet the SANS 241:1 (SANS, 2015) limit. The mean coliform counts for the fast handpieces of the DCUs were: O2.1 = 1.20×10^1 CFU 100 ml⁻¹, O2.2 = 6.75×10^1 CFU 100 ml⁻¹, O2.3 = 4.19×10^2 CFU 100 ml⁻¹, and O2.4 = 1.01×10^2 CFU 100 ml. These results indeed displayed total coliform counts exceeding the limitation as specified by SANS 241:1 (SANS, 2015) for total coliforms (Figure 4.3). The mean total coliform count for the fast handpiece of DCU O2.1 (1.20×10^1 CFU 100 ml⁻¹) was lower than that of the source water supplied to this DCU (2.35×10^1 CFU 100 ml⁻¹) (Figure 4.3). The lower total coliform count in this fast handpiece could be attributed to flushing of the DUWL system before sampling, or the decontamination of the waterline prior to sampling. There was no statistical difference ($p = 0.329$) between the total coliform counts of tap water and fast handpieces in open system DCUs. In a study of sixteen private and ten government dental healthcare facilities in Bushehr, water samples were taken from the municipal water distribution system and two sites of DUWLs, including the three-in one syringes and high-speed handpieces used in twenty-six DCUs. The results revealed that total coliform samples were negative in all municipal water samples, while 25% of high-speed handpieces and 25.8% of air-water syringe samples tested positive for total coliforms (Dobaradaran et al., 2014). In this instance, water contamination may have been caused by total coliforms that were present in the source water (Figure 4.3).

In closed system DCUs, tap water is used to fill distiller bottles; once distilled, this water is used to fill reservoir bottles attached to the DCU and to supply the fast handpiece. In the current study, total coliform counts that were recorded for the closed system DCUs were all within the limits as regulated by SANS 241:1(SANS, 2015) for total coliforms in drinking water (Figure 4.4). However, despite these counts falling below this limitation, total coliforms were detected in the tap water of the closed system DCUs: C2.1 = 1.5×10^1 C FU 100 ml⁻¹, C2.2 = 1.50×10^1 CFU 100 ml⁻¹, C2.3 = $1.5 \times$

10^1 CFU 100 ml⁻¹, C2.4 = 1.5×10^1 CFU 100 ml⁻¹, and C2.5 = 4.0×10^1 CFU 100 ml⁻¹ (Figure 4.3).

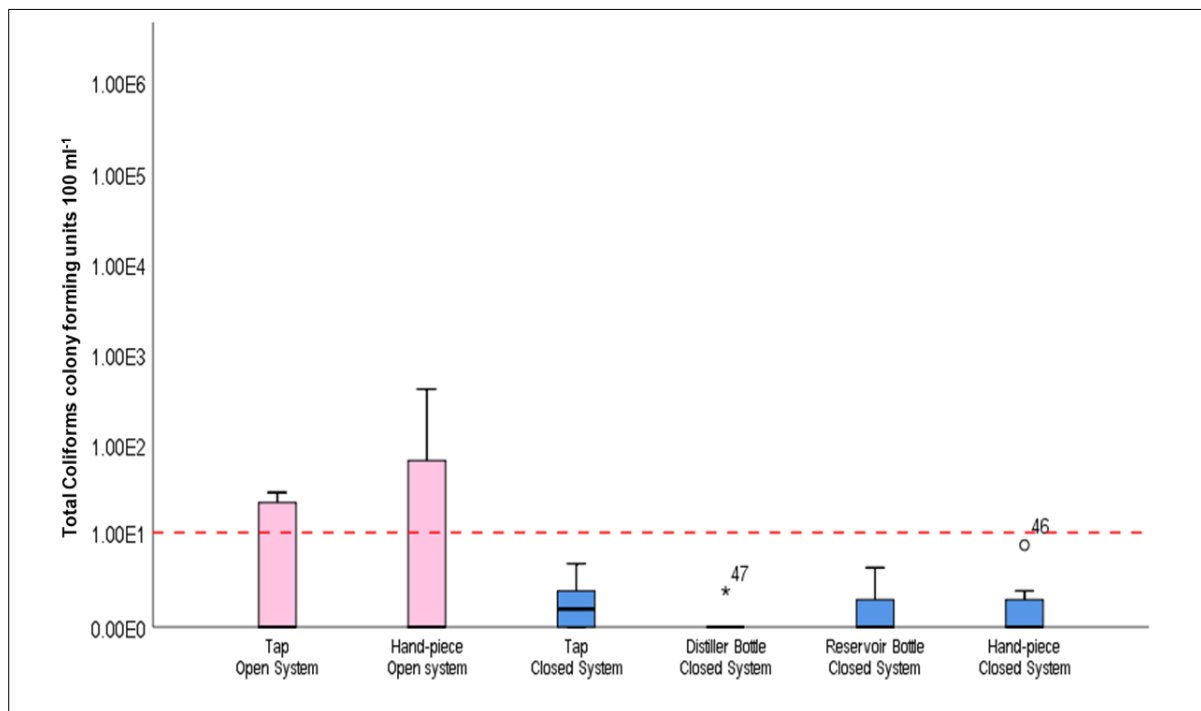


Figure 4.4: ‘Box and whisker’ plots and descriptive statistics of total coliform counts of water quality for the open and closed DCU systems. The bottom and top of each box indicate the 25th and 75th percentiles, and the height of the box therefore indicates the interquartile range. The line dissecting the box is the median value. The ‘whiskers’ indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1 (SANS, 2015) limitation for total coliform bacteria per 100 millilitre.

It was found that the water in most of the distiller bottles was compliant with SANS 241:1(SANS, 2015) (Figure 4.4). Total coliforms were present in the water of one DCU (C2.5 = 1.5×10^1 CFU 100 ml⁻¹), but this level was below the SANS 241:1(SANS, 2015) limit (Figure 4.3).

There was a noticeable increase in the mean count of total coliforms in the reservoir bottle water of DCU C2.5 (3.50×10^0 CFU 100 ml⁻¹) (Figure 4.3). It is reasonable to assume that the presence of the total coliforms in this reservoir bottle was because both the source water and the distiller bottle contained total coliforms. According to information presented in Table 4.1, an oral healthcare practitioner reported daily

flushing and weekly shock treatment of the DUWLs. However, despite these protocols there was still contamination by total coliforms in the reservoir bottles originating from the source water. The total coliform count in the reservoir bottle of DCU C2.5 ($3,50 \times 10^0$ CFU 100 ml⁻¹) was within the SANS 241:1(SANS, 2015) limit. Total coliforms were also measured in the reservoir bottle attached to DCU C2.4 (1.5×10^0 CFU 100 ml⁻¹) (Figure 4.3). There was no statistical significance ($p = 0.295$) between the reservoir bottles and the fast handpieces of the closed system DCUs. The contamination in this instance may have resulted because of lax hand hygiene protocols and improper handling of the reservoir bottle when it was removed from the DCU to refill it with distilled water and when reattached to the DCU. Enteric or skin microorganisms can be introduced into a bottled water source if reservoir bottles are carelessly handled. The opportunity for such contamination can occur when the reservoir bottle on the dental unit is being filled or removed, or when the water source bottles are handled or being prepared (Mills, 2001; Syzemanska, 2013; Mills, Porteous and Zawada, 2018).

The water emitted from all the fast handpieces in the open system DCUs complied with the SANS 241:1(SANS, 2015) limitation for total coliforms (Figure 4.3 and Figure 4.4). Total coliforms within the SANS 241:1(SANS, 2015) limitation were present in fast handpieces in DCUs C2.4 and C2.5 (Figure 4.3). The presence of these coliforms may be attributed to the introduction of coliform bacteria by the contaminated reservoir bottles that were supplying these fast handpieces with water, and also by inappropriate handling procedures. Dreyer and Hauman (2001) demonstrated that internal surfaces of dental handpieces became contaminated during normal oral procedures, with waterlines attached to the handpiece displaying the densest contamination. They concluded that autoclaving handpieces could possibly be the only effective way to sterilise both internal and external surfaces. A study that was conducted in Brazil on thirty DCUs collected samples from three-in-one syringes, high-speed handpieces coupled to tubing, the tubing of high-speed handpieces without them being coupled, water reservoir bottles, and the source water supplying these reservoir bottles, and the findings revealed that total coliforms were detected in nine out of thirty (30%) water samples (Lisboa et al., 2014).

The one order magnitude of difference between tap water used in open system DCUs and in closed system DCUs was statistically significant ($p = 0.043$). When comparing

the two DCU systems, the tap water supplying open system DCUs had higher total coliform counts than those of the closed system DCUs. However, there was no statistically significant difference between the tap water samples of the two DCU systems ($p = 0.669$).

Total coliforms are a group of bacteria that are used as a water quality indicator and, to a certain extent, as a regulatory parameter. Where faecal contamination may be present, total coliforms represent a more sensitive indicator. Furthermore, total coliforms are more resistant to disinfection and their presence in analysed water samples is a clear indication of inadequate disinfection. Additionally, where total coliforms are present in a water distribution system such as a DUWL, it may indicate a lack of system integrity. Total coliforms are therefore optimal for monitoring the sanitary quality of water (Maheux, 2014). DUWLs create an ideal environment for the development of microbial biofilms. Microbial contamination of water in DUWLs is thus thought to be the result of biofilm formation as it could serve as a haven for pathogens. These results in the current study for DCUs were within the parameters set by SANS 241:1(SANS, 2015), as will be discussed in the following sections.

4.2.5 1 *Pseudomonas aeruginosa*

Water is an essential component of almost all dental procedures performed by oral healthcare providers. Microbial monitoring of water used during oral healthcare procedures using indicator organisms can give timeous insight regarding potential environmental health risks. The microbial quality of the water that is used is of vital importance to ensure that there is no health risk to patients, OHCPs, and/or their personnel when exposed to the water used to carry out oral healthcare procedures. The majority of DUWL contaminants are Gram-negative aerobic environmental species. Although not necessarily harmful to healthy individuals, these species may be of concern regarding patients who are treated and are immunocompromised or suffer from respiratory conditions (Lizzadro et al., 2019).

These opportunistic respiratory pathogens that can be found resident in DUWLs include *Pseudomonas aeruginosa*, *Legionella spp.*, and non-tuberculosis mycobacteria infection (Pankhurst and Coulter, 2007). The most prevalent water-

related infections in DUWLs are of bacterial origin because bacteria represent the dominant part of the microflora resident in these tubes. Dental infections can be triggered when patients are directly exposed to pathogens from oral cavities. These pathogens are spread by the transfer of contaminated DCU handpieces and instruments and/or the aspiration of oral fluids (Barbot et al., 2012).

In the current study, the water quality of both the open and closed system DCUs were characterised by analysing the concentration of *Pseudomonas aeruginosa* in water samples at various points of use (Appendix A). There is no legislation that regulates the quality of DCU water used for oral healthcare procedures, but in most countries the recommendations of water standards for microbial indicators are used, or the recommendations by the American Dental Association or the Centers for Disease Control and Prevention are considered. However, for many potential bacterial pathogens, such as *P. aeruginosa*, no special recommendations can be found in most water quality guidelines as these are not considered bacteriological indicators of contamination and, in general, are not subject to drinking water regulations although their presence have been widely reported (Castellano Realpe et al., 2020).

The mean *P. aeruginosa* count measured in the tap water that supplied the open system DCUs was 3.32×10^1 CFU 100 ml⁻¹ (Appendix A). *P. aeruginosa* was present in this tap water at mean counts of O2.1 = 2.05×10^1 CFU 100 ml⁻¹, O2.2 = 1.40×10^1 CFU 100 ml⁻¹, O2.3 = 2.95×10^1 CFU 100 ml⁻¹, O2.4 = 2.90×10^1 CFU 100 ml⁻¹, and O3.4 = 3.35×10^1 CFU 100 ml⁻¹ (Figure 4.5). Generally, tap water that is sourced from a municipality supply is assumed to be safe for consumption as all such water is subject to water quality testing before it is supplied to the public. However, tap water is not monitored for *P. aeruginosa* before distribution to the public and may contain minerals that deposit on internal DUWL surfaces. These minerals allow molecules to concentrate on their surfaces, thus promoting adhesion of bacteria that are suspended in the municipal water supply. Over time, individual cells are able to attach to the surface and multiply to form biofilm (Mills, 2001). The presence of *P. aeruginosa* in tap water supplying open system DCUs can act as an initiator for colonisation and subsequent biofilm formation in open system DCUs by means of an attachment mechanism. Moreover, the safety of the water that exists the fast handpiece can be

greatly compromised if the tap water supplied to the DCUs is contaminated from the onset.

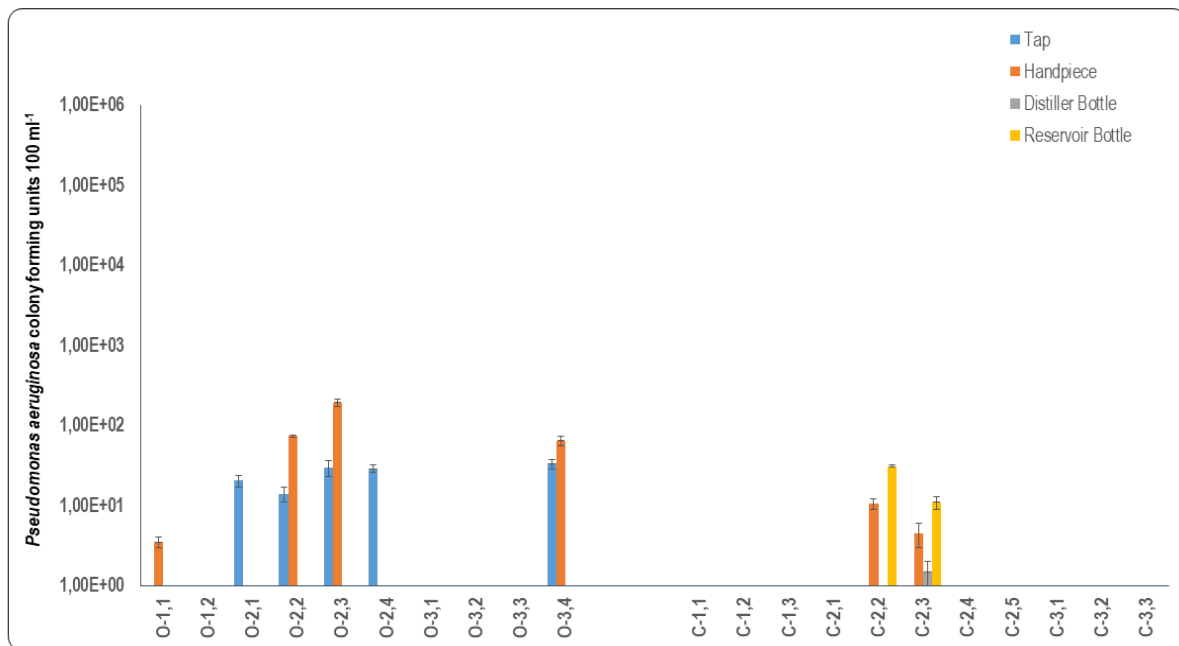


Figure 4.5: Graphic representation of data from oral healthcare facilities for *Pseudomonas aeruginosa* counts to determine the water quality of open and closed system DCUs. Water samples were taken from taps and fast handpieces for open system dental DCUs (O1.1 to O3.4). Water samples were taken from taps, distiller bottles, reservoir bottles, and fast handpieces for closed system DCUs (C1.1 to C3.3).

In the current study, water that exited the distal outlets of fast handpieces of open system DCUs were analysed for the presence of *P. aeruginosa*. The mean *P. aeruginosa* count for these fast handpieces was 3.35×10^1 CFU 100 ml⁻¹ (Appendix A). The water supplied to the open system DCUs for chairs O2.1, O2.2, O2.3 and O3.4 all contained *P. aeruginosa* (Figure 4.5). It can be assumed that, because the water supplying these fast handpieces was contaminated with *P. aeruginosa*, the water exiting the distal outlets of the fast handpieces would be similarly contaminated. The *P. aeruginosa* mean counts measured for the handpieces of the open system DCUs revealed that *P. aeruginosa* was present in DCUs O1.1 (3.5×10^0 CFU 100 ml⁻¹), O.2 (7.45×10^1 CFU 100 ml⁻¹), O2.3 (1.93×10^2 CFU 100 ml⁻¹), and O3.4 (6.45×10^1 CFU 100 ml⁻¹) (Figure 4.5). The *P. aeruginosa* results for these fast handpieces were similar to those reported by a study that assessed three-in-one syringes (5.29×10^1 CFU 100

ml⁻¹) and high-speed handpieces (6.78×10^1 CFU 100 ml⁻¹) that were supplied by water collected from twenty DCUs (Freitas, Sand and Simonetti, 2010).

In the current study, no *P. aeruginosa* was detected in the water sampled at the distal outlet of the fast handpieces of DCU O2.1 and O2.4, even though the tap water supplying these DCUs contained *P. aeruginosa* (Figure 4.5). However, the tap water supplying DCUs O2.2 and O2.3 contained *P. aeruginosa* and the fast handpieces of these DCUs exhibited elevated *P. aeruginosa* mean counts (O2.2 = 2.95×10^1 CFU 100 ml⁻¹ and O2.3 = 2.90×10^1 CFU 100 ml⁻¹) (Figure 4.5). It could be assumed that the increase in *P. aeruginosa* in the waterlines of DCUs O2.2 and O2.3 was colonised by *P. aeruginosa* to form biofilm colonies. The biofilm was possibly sloughed off and was fed into the water supply of the fast handpieces where it contaminated the water at the distal outlets of these instruments. The mean *P. aeruginosa* count in DCU O1.1 (3.5×10^0 CFU 100 ml⁻¹) was attributed to a biofilm that may have been resident within the waterlines of this DCU (Figure 4.5). No *P. aeruginosa* was detected in the source water supplying this DCU. Moreover, no statistical difference ($p = 0.21$) was measured between the *P. aeruginosa* counts of tap water and the water exiting the distal outlet of the fast handpiece of this open system DCU. The *P. aeruginosa* in the water from the fast handpiece may have become contaminated by oral bacteria sucked back into the waterline because of a faulty anti-retraction valve in the fast handpiece and/or the colonised waterlines of this DCU.

The *P. aeruginosa* counts in closed system DCUs revealed that none was present in the tap water that was used to fill the distiller bottles. Distilled water samples were also generally free from *P. aeruginosa* bacteria (Figure 4.6). The only distilled water that contained *P. aeruginosa* was found in DCU C2.3 (1.5×10^1 CFU 100 ml⁻¹). There was no statistical significance ($p = 0.156$) between the tap water and distiller bottles of the open system DCUs. Distilled water could have become contaminated due to improper storage of the distilled water and ineffective decontamination of the distiller bottles.

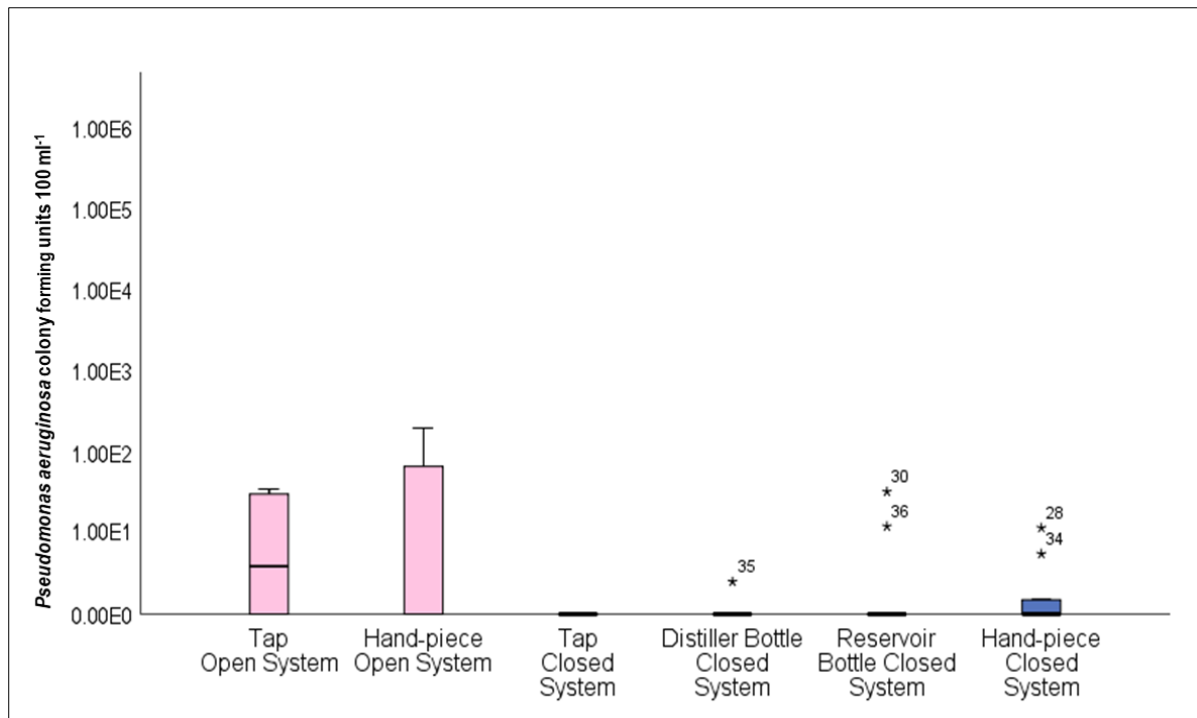


Figure 4.6: ‘Box and whisker’ plots and descriptive statistics of *Pseudomonas aeruginosa* counts of water quality for open and closed systems. The bottom and top of each box indicate the 25th and 75th percentiles, and the height of the box therefore indicates the interquartile range. The line dissecting the box is the median value. The ‘whiskers’ indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1(SANS, 2015) limitation for total coliform bacteria per 100 millilitre.

The reservoir bottles that were attached to the closed system DCUs that supplied the fast handpieces with water generally did not contain *P. aeruginosa* counts. The water contained in the two reservoir bottles attached to DCU C2.2 and C2.3 had mean *P. aeruginosa* counts of 3.1×10^1 CFU 100 ml⁻¹ and 1.1×10^1 CFU 100 ml⁻¹ respectively (Figure 4.5). No *P. aeruginosa* was detected in the distilled water samples of the water that was used to fill these reservoirs. Reservoir bottles could have become contaminated due to improper disinfection of reservoir bottles and water stagnation. There was no statistically significant difference ($p = 0.151$) between the *P. aeruginosa* counts of the water of distiller bottles and those of the reservoir bottles of the closed system DCUs.

The fast handpieces of closed system DCUs C2.2 (1.05×10^1 CFU 100 ml⁻¹) and C2.3 (4.50×10^0 CFU 100 ml⁻¹) showed higher levels of *P. aeruginosa* than the reservoir bottles that supplied these DCUs (Figure 4.5). Contamination of the water exiting the fast handpieces in this instance could have resulted from the DUWLs being contaminated by *P. aeruginosa* in contaminated reservoir bottles. It can be assumed that, because the *P. aeruginosa* counts were higher in the water at the distal outlets of these two fast handpieces, *P. aeruginosa* colonised and formed biofilm within the DUWLs and would have been present in the water exiting the fast handpiece distal outlets. There was no statistical difference ($p = 0.272$) between the reservoir bottles and the fast handpieces of the closed system DCUs.

The origin of bacteria that contaminate DUWLs can be attributed to two factors: (i) contaminated municipal water that is used in DCUs, and (ii) the suck back of patients' saliva into the DUWL because of ineffective or faulty anti-retraction valves in handpieces (Montebugnoli and Dolci, 2002). When comparing the two DCU systems, no statistical significance ($p = 0.19$) was observed between the tap water supplied to the DCUs and the water exiting the fast handpieces ($p = 0.12$). The internal architecture of DUWLs comprises a network of small bores and narrow lumens that create an optimal environment for *P. aeruginosa* colonisation when even a small amount is present in a municipal water system. *P. aeruginosa* has been recovered from oral cavities of approximately 4% of healthy individuals (Barben and Schmid, 2008). It is therefore possible that some of these bacteria are aspirated into DUWLs. However, if water is monitored and maintained according to hygiene guidelines, the prevalence of *P. aeruginosa* in the municipal water supply will be extremely low (Barben and Schmid, 2008).

4.2.5.2 *Legionella* spp.

Legionella spp. are common inhabitants of water and soil and may also be present in artificial aquatic environments (Leoni et al., 2015). Dental unit waterlines provide a favourable environment for the growth of *Legionella* spp. bacteria where they can reproduce to high concentrations and can cause illness in humans. The disease they cause is spread by inhaling aerosols containing *Legionella* spp. or aspiration (Kevorkyan et al., 2017). *Legionella* has been isolated from and found present in

biofilm in DUWLs. It can be aerosolised into the environment by handpieces where it can be aspirated and will then pose a risk to both patients, OHCPs, and their personnel. The fast rotation of handpieces used during oral healthcare procedures produces aerosols with a diameter of less than 5 micrometres. These can be inhaled and will easily reach the alveoli in the lungs (Leoni et al., 2015).

Water samples sourced at the distal outlets of fast handpieces used in both open and closed system DCUs were analysed for the presence of *Legionella spp.*, and it was detected in the water that exited the distal outlet of fast handpiece O2.1 (2.2×10^1 CFU 100 ml⁻¹). No *Legionella spp.* was detected in the water samples from fast handpieces in closed water system DCUs. The presence of *Legionella spp.* in the water of fast handpiece O2.1 was considered a high risk, especially if this water would be aerosolised and aspirated by patients who were immunocompromised.

Legionella spp. was confirmed using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology in the VITEK MS® (BioMerieux) and was reported to the environmental health officer at the dental healthcare facility where it was detected and the Free State Department of Health. The current study was put on hold and the DCU was suspended for use of oral procedures for a period of 14 days until a suitable intervention could be agreed upon and executed. The waterlines of DCU O2.1 were flushed daily with 50 ml of 0.2% chlorhexidine mouthwash. The mouthwash was run through the waterlines until its blue colour was observed at the distal outlet of the fast handpiece and other ancillary attachments.

Following the fourteen days of suspension of use of the DCU, the water at the distal outlet of the fast handpiece of DCU O2.1 was sampled and analysed for the presence of *Legionella spp.* Upon examination, no *Legionella spp.* was detected in the water of this fast handpiece, and hence the daily flushing with 0.2% chlorhexidine mouthwash was assumed to have eliminated the *Legionella spp.* from the water. Agahi et al. (2014), demonstrated similar results with 50 ml of 0.2% chlorhexidine to reduce microbial counts in DUWLs. In the latter study, the water exiting the high-speed handpiece and three-in-one syringe of thirty-five DCUs was analysed. The DUWL water from all DCUs was reported to be contaminated before chlorohexidine use, but no further contamination was detected after flushing with chlorohexidine. Twenty-eight

of the thirty treated DUWLs had microbial values of less than 2×10^2 CFU ml⁻¹ after flushing daily for one week.

Several other studies have also reported the presence of *Legionella spp.* in dental unit water. In one case study Chikte et al. (2011) reported a case of Legionnaires' disease in a 35-year-old dental receptionist in Cape Town, South Africa. It was found that she had inhaled contaminated aerosols in the oral healthcare facility where she worked. *Legionella spp.* concentrations at two sites in the oral healthcare facility were reported at concentrations of 4.00×10^2 CFU L⁻¹ for *Legionella pneumophila* serogroup 1 and 8.8×10^3 CFU L⁻¹ for *Legionella pneumophila* serogroup 2-14 (Chikte et al., 2011).

4.2.6 Conclusions

Water is an important commodity for the daily functioning of dental chair units and in the execution of oral healthcare procedures. It is therefore vital that water used to perform oral healthcare procedures is of a standard that is safe for patient use. The results of the water quality of open and closed system DCUs highlighted that high levels of heterotrophic bacteria were present in the water that was supplied to some open and closed system DCUs at all sampling sites. The heterotrophic bacteria present in source water can serve as an initiators of biofilm formation in DUWLs. They are usually Gram-negative species, which indicate that opportunistic pathogens may be present in the water and in DUWLs. HPCs of the equipment in nearly all the sites that were sampled (taps, fast handpieces, distiller and reservoir bottles) did not comply with the SANS 241:1(SANS, 2015) recommendation for heterotrophic bacteria in drinking water.

The tap water that was supplied to the open system DCUs and the water that was distilled for use in the closed system DCUs had high mean HPCs. This could have compromised the entire DCU system as, in such conditions, bacteria could colonise DUWLs, distilled water, water in reservoir bottles, and water exiting the distal outlets of fast handpieces. The high HPCs that have been reported by several other studies (O'Donnell et al., 2009; Pasquarella et al., 2012; Szymańska & Sitkowska, 2013) were validated by the results observed for mean HPCs at the various sampling sites of both the open and closed system DCUs in the current study.

Total coliforms are also used to determine water quality and the water quality of both the open and closed system DCUs was thus assessed. The mean total coliform results for both tap water and the fast handpieces used in open system DCUs did not comply with the SANS 241:1 (SANS, 2015) requirement. The total coliform counts in the closed system DCUs were all within the SANS 241:1 (SANS, 2015) limit of less than 1×10^0 CFU ml⁻¹. Total coliform counts as an indicator of organisms in water and on surfaces can provide evidence of contamination and/or ineffective decontamination or sterilisation methods used in DCUs and DUWLs.

The water used in DCU systems may contain opportunistic pathogens such as *Pseudomonas aeruginosa* and *Legionella spp.* and monitoring this water for oral healthcare purposes is thus essential. These organisms may be present in low concentrations, but because of their opportunistic nature they can pose a health risk to immunocompromised patients. The results revealed that *P. aeruginosa* was present in 50% of the tap water samples and in 30% of the fast handpiece water in the open system DCUs. *P. aeruginosa* was detected in one distiller bottle and 20% of reservoir bottles and the corresponding fast handpieces of the closed system DCUs. The presence of *P. aeruginosa* in the distiller bottle as well as the reservoir bottle was attributed to ineffective autoclaving and/or improper bottle handling when the bottle was filled with water. The *P. aeruginosa* contamination of the fast handpieces of the closed system DCUs may have been due to biofilm formation on the internal surfaces of the DUWLs, or the retraction of oral or other fluids into the waterline during oral healthcare procedures.

Legionella spp. was also detected in the water at the distal outlet of one fast handpiece in an open system DCU. Although it was detected only in the water at the distal outlet of the fast handpiece of one open system DCU, this organism could have infected patients with underlying conditions or diseases who were treated in this dental chair. *Legionella spp.* was successfully eliminated from this chair after decontamination intervention for two weeks. Had it not been for the present study, the presence of *Legionella spp.* would have remained undetected, and this could have had dire implications for patients. This highlights the need for water monitoring programmes in oral healthcare facilities.

Based on the results from the water samples that were tested in this study, and even by acknowledging the limitations of this study, it is undeniable that OHCPs cannot rely on the fact that water coming into an oral healthcare facility is safe for oral healthcare procedures on the premise that the water was treated and tested by the municipality. The onus thus rests on OHCPs to continuously monitor the pipelines that supply tap water to the facility as well as the water entering and exiting their DCUs. Decontamination of and sterilisation protocols for DCUs and all their ancillary equipment should also be enforced and adhered to for the safety of patients and personnel.

4.3 Biofilm-Associated Organisms

4.3.1 Introduction

DCUs form an integral part of oral healthcare facilities and the procedures related to oral healthcare. These units rely greatly on the use of water for the execution of oral healthcare procedures. Water is used daily for oral rinsing, cooling of handpieces, and other procedures. The microbial water quality of water exiting the distal outlets of handpieces while oral healthcare procedures are performed is dependent on the composition of the water that is supplied to the DCU. Moreover, the quality of the water flowing from the distal outlets of handpieces attached to a DCU will also be affected by biofilm if it is present on the distal surface of dental unit waterlines (Szymańska and Sitkowska, 2013). Infections and diseases caused by waterborne organisms remain a major source of global morbidity and mortality. The phenomenon of nosocomial infections acquired from oral healthcare facilities related to the water quality and biofilm formation in dental unit waterlines has also gained major concern worldwide (Spagnolo, Sartini and Cristina, 2020).

While it is important to monitor the water quality of DUWLs for the assessment of possible bacterial contamination, the detection and analysis of biofilms in DUWLs is equally important (de Figueiredo-Filho et al., 2019). Dental chair units consist of a network of interconnected small bores and tubing with narrow diameters. The narrow diameter and smooth plastic surface of DUWLs provide a high surface to volume ratio

that, together with the fluid dynamics and characteristics of waterlines, present an ideal environment for microbial colonisation and the proliferation of environmental microorganisms, pathogens, and opportunistic pathogens such as *Legionella* spp. and *Pseudomonas* spp. (Göksay, Cotuk and Zeybek, 2008). Water supplied to DCUs contain inorganic and organic materials that settle on the inner surfaces of DUWLs where they form conditioning pellicles that are sites for microorganisms to attach themselves. The source water entering DUWLs contain microorganisms that adhere to the conditioning pellicles and attach themselves more permanently by other cell attachment and adhesion mechanisms over time.

Adherent, early colonisers in the DCU water supply system provide diverse adhesion sites for other microorganisms, called secondary colonisers, that also commence growth themselves, and this results in the establishment of microcolonies. Attached cells and microcolonies almost instantly begin to secrete complex polymers into the surrounding environment. This phenomenon is characteristic of and essential for the formation of biofilm (Rai, 2016). A diverse range of bacteria, particularly environmental species, secrete exopolysaccharides (EPS) during biofilm formation. This assists with cell protection against adverse environmental conditions, aids with attachment to surfaces, and supports the acquisition of nutrients. Biofilms in DUWLs are quite fastidious and can resist penetration by a wide range of chemical agents such as detergents, disinfectants, antibiotics, and other antimicrobial agents (O'Donnell et al., 2011). Biofilm fragments and planktonic forms can also detach from the distal surfaces of dental unit waterlines and are shed to seed biofilm formation elsewhere in the waterline network, or to percolate in the DCU water from where they are directly transferred into the mouths of patients during oral healthcare procedures. Handpieces also produce fine aerosols that are transferred into the surrounding environment of oral healthcare facilities. The aerosols are inhaled as fine droplets and can enter the lungs of patients as well as those of OHCPs and their personnel (Pankhurst and Coulter, 2007).

Biofilm formation serves as a continuous reservoir for microbial contamination and contributes to the microbial load of water flowing from the distal outlets of handpieces of DCUs. Dental unit waterline contamination places immunocompromised patients as well as oral healthcare providers and their personnel at risk of possible infection (Patel

et al., 2016). The water quality of samples taken from DCUs in this study revealed that water going into the DCUs and exiting from the distal outlets of handpieces largely measured high bacterial counts that could possibly indicate the presence of biofilm in the DUWLs and in DCU water.

Microbial monitoring is vital in ensuring the safety of patients, OHCPs, and their personnel. To further analyse the microbial load and the presence of biofilm-associated organisms in DCUs, surface swabs were taken at the distal outlets of open and closed DCUs as well as from the inner surfaces of distiller and reservoir bottles.

4.3.2 Heterotrophic plate counts associated with biofilm

Dental unit waterlines provide an optimal environment for the formation and proliferation of biofilms. Biofilm formation is believed to be the result of microbial contamination of DCU water and serves as a harbour for pathogens (Nikaeen, Hatamzadeh and Zareh, 2009). DCUs that are connected to a municipal water supply usually contain low levels of several bacterial species, and these microorganisms attach to DUWL tubing and over time give rise to the colonisation of multispecies biofilms in these lines (Tuttlebee et al., 2002). Most microbial species found in DUWL output water are Gram-negative aerobic heterotrophic environmental bacterial species of very low pathogenicity, although they may be of concern in the treatment of immunocompromised and medically compromised patients (Lauritano et al., 2017).

The biofilm results of samples that were taken from both open and closed system DCUs were characterised by analysing the concentration of heterotrophic bacteria from swab samples taken at various points of use (Appendix B). The HPC results were compared to the SANS 241:1 (SANS, 2015) standard for the presence of heterotrophic bacteria. SANS 241:1 (SANS, 2015) specifies that water should not contain more than 1×10^3 CFU ml⁻¹ of heterotrophic bacteria to be considered safe for human consumption.

Swab samples were taken of the inner distal surfaces of taps supplying water to open and closed DCUs. A mean count of 1.47×10^4 CFU ml⁻¹ was obtained by HPC measuring of open system DCU samples, and the results indicated that they were not

in compliance with the SANS 241:1 (SANS, 2015) specification (Appendix B). It is generally assumed that water provided by a local municipality have undergone microbiological testing and complies with standards as regulated by SANS 241:1 (SANS, 2015). However, it has been reported that drinking water distribution systems have a propensity to form biofilm on the pipelines that distribute this water, and this can enhance the proliferation of microorganisms on the inner surfaces of taps, which contributes to the microbial loading of organisms that are transferred from water supplied to open system DCUs to their DUWLs (Simões and Simões, 2013; Pareek et al., 2013).

4.3.2.1 Biofilm-associated HPCs of open system DCUs

It can be assumed that, because water flows through the taps that supply open system DCUs with water, that the water will also contain high HPCs (Figure 4.1). Moreover, the microbial load on the inner surfaces of DUWLs could affect the water exiting from fast handpieces as this water passes over tap surfaces.

To assess the impact of this on the DCUs under study, surface swabs of handpieces were taken and a mean count of 1.39×10^4 CFU ml⁻¹ was achieved (Appendix B). As was expected, this mean HPC value exceeded the SANS 241:1 (SANS, 2015) limit due to the elevated HPCs of the tap water supplied to the open system DCUs and the distal inner surfaces of these taps (Figure 4.8). The microorganisms use attachment mechanisms to attach to DUWLs where they form a biofilm and, when they are detached, they are transferred to patients' mouths via fast handpiece water or aerosolised water that reaches the environment. The only open system DCU that was compliant with SANS 241:1 (SANS, 2015) was O3.1 with an HPC of 6.50×10^2 CFU ml⁻¹ (Figure 4.7).

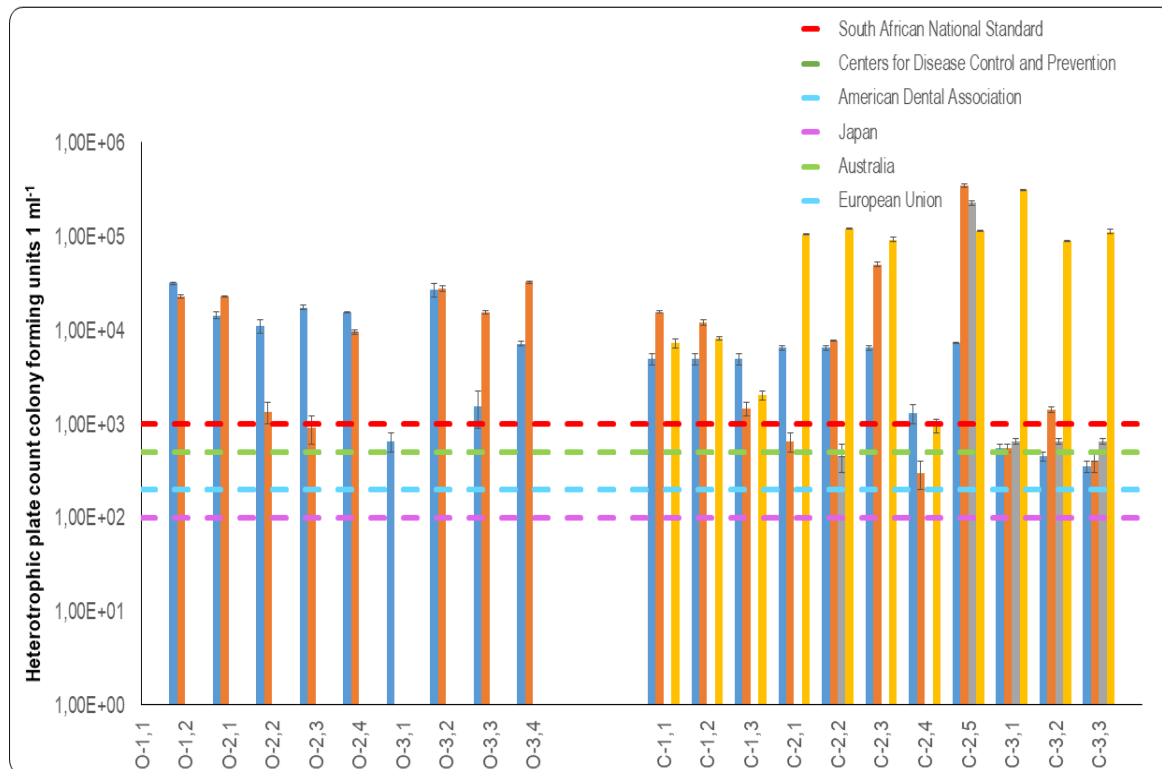


Figure 4.7: Graphic representation of data from oral healthcare facilities for heterotrophic plate counts from swab samples of open and closed system dental units. Samples were taken from taps (blue) and fast handpieces (orange) in open system DCUs (O1.1 to O3.4). Water samples were taken from taps (blue), distiller bottles (grey), reservoir bottles (yellow) and fast handpieces (orange) of closed system DCUs (C1.1 to C3.3). The dashed red line represents the American Dental Association and the Centers for Disease Control and Prevention recommendations for HPC for DUWLs as well as drinking water quality limitations for various countries for heterotrophic bacteria per millilitre.

Based on the information received from oral healthcare providers (Table 4.1), it was expected that there would be a difference in the microbial load on the surface swab samples taken at the various facilities. To swab the fast handpieces, the coupling was removed to expose the surface of the waterline that supplied the fast handpiece with water. The results indicated that the surfaces of the taps at facility O1 had a high microbial load, but the microbial load on the surfaces of the fast handpieces was lower than that of the taps (Figure 4.7). As reported, this oral healthcare facility flushed the DUWL of DCUs daily; however, the HPCs of the surfaces of the fast handpieces were still above the SANS 241:1 (SANS, 2015) recommendation. Flushing may have had an effect by reducing the microbial load, but it was evidently not effective enough to eliminate the biofilm that was likely to be present in the DUWL. Shahabudin et al.

(2018) conducted a study to determine the efficacy of flushing waterlines on the quality of water, and obtained mean HPC counts of 6.33×10^4 CFU ml⁻¹ before flushing and 8.36 CFU ml⁻¹ post flushing. Reduced HPCs were observed after flushing the DUWLs, but HPC counts were not reduced to a level where the mean count adhered to the standard stipulated by the Environmental Protection Agency. The reason for this is the use of the DCUs over time and biofilm that is already resident within DUWLs.

The internal surfaces of the taps at facility O2 had HPCs that were above the acceptable limit (Figure 4.7), which indicated the possible presence of biofilm within the pipelines of these taps. The oral healthcare practitioner reported (see Table 4.1) that waterlines were flushed daily and shocked weekly with sodium hypochlorite. Dental chair units O2.2 and O2.3 were the only chairs at facility O2 where the internal surfaces of the fast handpieces recorded lower HPCs than the internal surfaces of the taps, as displayed in Figure 4.7. However, the HPCs of tap and fast handpieces were not statistically significant ($p = 0.06$).

The oral healthcare practitioner at facility O3 did not comment on the frequency of decontamination procedures but indicated the use of a chemical treatment to disinfect the DUWLs. It was found that the internal tap surfaces of DCU O3.1 was within the prescribed limit for HPCs, while the fast handpiece had no HPC recorded (Figure 4.7). The absence of HPC in the fast handpiece could not be explained with the data available, indicating that the questionnaire may have to be expanded to include chair unit history and frequency of usage. The internal surfaces of the fast handpieces of DCUs O3.2, O3.3, and O3.4 all displayed a high microbial load (Figure 4.7) which was higher than that of the internal surfaces of the taps that supplied water to these DCUs. Similar to a study conducted at a dental school in Tehran University of Medical Sciences, the results showed that the contamination of the three-in-one syringe was higher than that of the tap water supplying the dental units (Mansourian, 2011). There was no statistical significance between the internal tap surfaces and the fast handpieces in facility O3 ($p = 0.24$).

It is thus evident that the protocols and disinfectants that were used in DCU for O3.2, O3.3, and O3.4 were ineffective to control the growth of biofilm on the internal surfaces of the DUWLs. This suggests that the pipelines supplying water to the DCUs, as well

as the internal surfaces of the fast handpieces, should be monitored regularly. Upon reflection of the findings, it may be argued that the presence of biofilms may be one of the predominant causes of high microbial loads, which was in all probability the case for the fast handpieces at this oral healthcare facility. Studies that were conducted previously indicated that flushing and disinfection led to only to a 9.1% reduction in viable counts and 0.5% improvement in biofilm coverage. Chemical germicides have been recommended for the removal of biofilm, but should be safe and noncorrosive (Agarwal et al., 2008; Fotedar and Ganju, 2015; Salam, Mulamoottil and George, 2017; Alkhualifi et al., 2020). The one order magnitude of difference between inner tap surfaces and the surfaces of the distal outlets of the fast handpieces in open system DCUs was not statistically significant ($p = 0.337$).

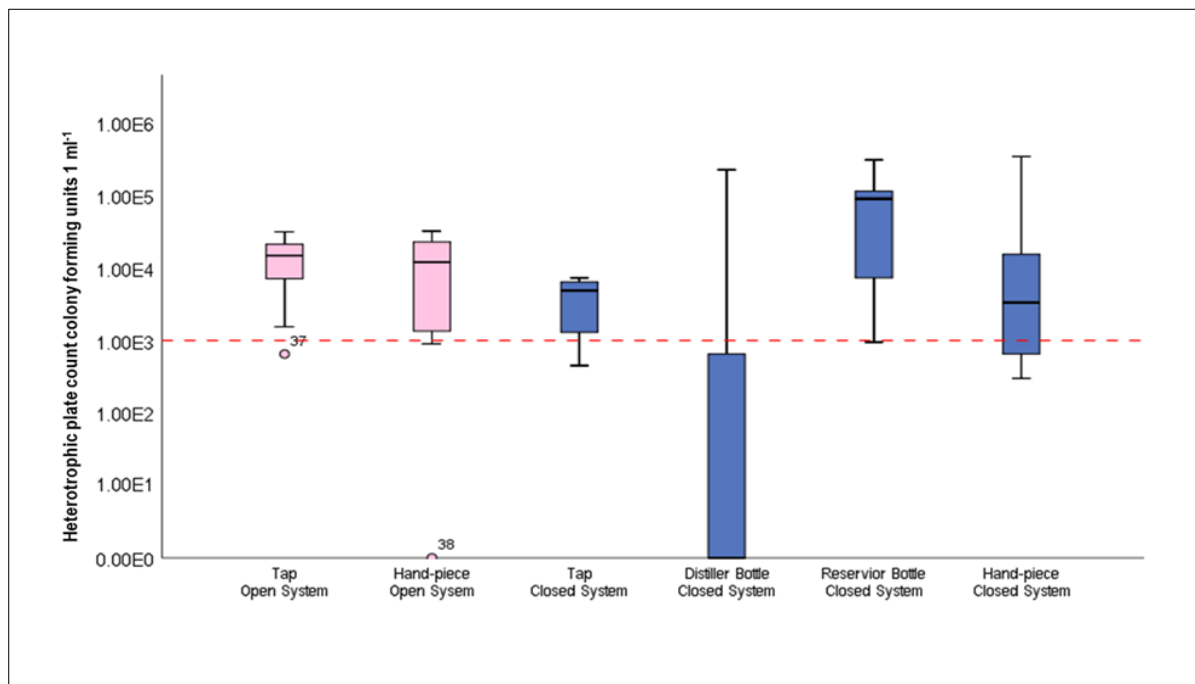


Figure 4.8: ‘Box and whisker’ plots and descriptive statistics of heterotrophic plate counts of swab samples for open and closed systems. The bottom and top of each box indicate the 25th and 75th percentiles, with the height of the box therefore indicating the interquartile range. The line dissecting the box is the median value. The ‘whiskers’ indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1 (SANS, 2015) limitation for heterotrophic bacteria per millilitre.

4.3.2.2 Biofilm-associated HPCs of closed system DCUs

The inner distal surfaces of taps used in closed system DCUs were swabbed for analysis to detect heterotrophic bacteria. It was found that these inner surfaces had a mean count of 4×10^3 CFU ml⁻¹, which did not comply with the prescribed SANS 241:1 (SANS, 2015) limit (Appendix B). However, there were some tap inner surfaces that did meet the SANS 241:1 (SANS, 2015) limit: O.3.1 (5.50×10^2 CFU ml⁻¹), O3.2 (4.50×10^2 CFU ml⁻¹), and O3.3 (3.50×10^2 CFU ml⁻¹) (Figure 4.7). This was the case even though the water flowing from the distal outlet of these taps was not compliant with SANS 241:1 (SANS, 2015). The internal surfaces of the taps at facility C1 indicated elevated microbial loads as can be seen in Figure 4.7. When considering the age of the DCUs and the frequency of disinfection reported by the oral healthcare practitioner (Table 4.1), it was unsurprising that biofilm would be present in the DUWLs, as can be observed in Figure 4.7. The HPCs of the internal surfaces of the fast handpieces are also presented.

When the HPCs are considered, it appears that the microbial load on the inner surfaces of the taps suggests that biofilm may have been present in the pipeline supplying water to the taps, as observed in Figure 4.7. The disinfection protocol for C2.4 seemed to have an effect on the microbial load in that the HPCs were marginally within the limit for the internal tap surface and within the limit for the fast handpiece. The oral healthcare practitioner utilising C2.5 reported (Table 4.1) that the water used for this chair was not the same as that used for the others in the facility, and this may explain why the HPC for the internal surface of the fast handpiece of this unit was extremely high (Figure 4.7). There may have been biofilm present in the DUWLs of this unit as the internal surface of the fast handpiece displayed a much higher HPC than the count for the internal surface of the tap.

Tap water was used to fill the distiller bottles of the closed system DCUs, and therefore the organisms that may have been present on the inner surfaces of the taps as well as in the tap water may have influenced the HPCs of the distilled water and the surfaces of the distiller bottles. The internal surfaces of the distiller bottles at site C1 showed no HPC, however, the reservoir bottles' HPCs were high (Figure 4.7). This may indicate that the reservoir bottles were contaminated or could have contained

biofilm. Conversely, the internal surfaces of distiller bottles at facility C2 were all compliant with the HPC limitation (Figure 4.7), except C2.5. The oral healthcare practitioner reported (Table 4.1) that a separate distillation room was used to distil water and fill the reservoir bottle of this DCU. The internal surface results varied for DCUs at facility C2 in terms of distiller bottles and reservoir bottles, and no statistical significance was evident ($p = 0.33$) when they were compared. It is important to observe that the internal surface of the distiller bottle of C2.5 had a high microbial load and was contaminated from the onset, which appeared to have influenced the HPC and microbial load of the reservoir bottle where biofilm may have formed. The condition and cleanliness of the distiller used to fill the reservoir bottle of this chair may not have been optimal for the distillation of water, and the distiller bottle may have contained biofilm that could have been transferred to the reservoir bottle. The microbiological quality of the water used in the reservoir bottles may thus have been compromised by the storage conditions and the condition of the plastic container that was used repeatedly (O'Donnell et al, 2011).

The oral healthcare providers at facility C3 reported (Table 4.1) that they flushed the waterlines daily and did a weekly shock treatment as part of their disinfection protocol. The internal surfaces of the distiller bottles that were used at the facility were within the limit for HPC (Figure 4.7), but the reservoir bottles showed elevated levels of HPC. The internal surfaces of the distiller and reservoir bottles showed statistical significance ($p = 0.26$). The elevated HPCs for the internal surfaces of the reservoir bottles at facility C3 may have been an indication of contamination or the presence of biofilm in the reservoir bottles.

The distiller bottle surfaces of the closed system DCUs were not affected by the heterotrophic bacteria in the tap water, or the distilled water contained in the bottles. When compared, the HPCs of the internal surfaces of the distiller bottles and the handpieces showed no statistical significance ($p = 0.300$), indicating that the microbial load on the inner surfaces of the distiller bottles had no effect on the microbial load of the inner surfaces of the fast handpieces. The internal surface swabs of the reservoir bottles had a mean HPC of 8.74×10^4 CFU ml⁻¹ (Appendix B). This measurement exceeded the HPC limit as stipulated by SANS 241:1 (SANS, 2015). The surface swabs of reservoir bottles C1.1, C1.2, C1.3, C2.3, and C2.4 did not indicate any

heterotrophic bacteria. The inner surfaces of the distiller bottles also did not indicate high HPCs; however, the distilled water contained high HPCs. The mean count for the surface swabs indicated that the distilled water quality may have affected the HPCs of the surfaces of the reservoir bottles, or that the reservoir bottles could have become contaminated due to inadequate disinfection or improper handling when they were removed from the DCUs, filled with distilled water, and attached to the units again. Biofilm growth on the inner surfaces of reservoir bottles can cause rapid deterioration of the microbiological quality of the water in these bottles that supply water to closed system DCUs (O'Donnell et al., 2011). The oral healthcare providers did not report any cleaning or disinfection protocols for the reservoir bottles.

The coupling of the fast handpieces was removed to expose the surface of the waterline supplying it so that a swab sample could be taken of this tool in closed system DCUs (Figure 3.4B). The HPCs of the inner surfaces of the waterline attached to fast handpieces of the closed system DCUs measured a mean count of 3.96×10^4 CFU ml⁻¹, which exceeded the limit prescribed by SANS 241:1 (SANS, 2015) for heterotrophic bacteria (Appendix B). However, some waterline surfaces of the fast handpieces of the closed system DCUs did comply with the SANS limitation, namely C2.1 (6.50×10^2 CFU ml⁻¹), C2.4 (3.00×10^2 CFU ml⁻¹), C3.1 (5.50×10^2 CFU ml⁻¹), and C3.3 (4.00×10^2 CFU ml⁻¹) (Figure 4.7). These fast handpiece waterlines could have been decontaminated or flushed prior to sampling, thus resulting in low microbial load counts on the inner surfaces of the waterlines supplying these fast handpieces. In a study conducted on three-in-one syringes, high-speed air turbine hand pieces, an ultrasonic scalar, and oral rinse sources, higher HPCs were observed prior to flushing than after flushing. This suggested that flushing these instruments for a period of three minutes did not completely eliminate heterotrophic bacteria, but it caused a noticeable reduction in their number (Zahir et al., 2016). The one order magnitude of difference between internal reservoir bottle surfaces and the inner surfaces of fast handpiece waterlines in the closed system DCUs was not statistically significant ($p = 0.356$).

4.3.2.3 Comparison of open system and closed system DCUs

Results for the two DCU systems were compared, and it was found that HPCs for the inner tap surfaces of the closed system were lower than that of the inner tap surfaces

of open system DCUs, and that the one order magnitude of difference between the two systems was statistically significant ($p = 0.013$). When the HPCs for the inner surfaces of fast handpieces used in the two DCU systems were compared, the result was not statistically significant ($p = 0.270$). This indicates that the distillation processes used in the closed system were flawed and resulted in increased biofilm formation at the distal outlets of the inner surfaces of the fast handpieces. Flushing water through a DCU is simple and should be a routine procedure because it is able to reduce the level of total aerobic bacteria in water in both old and new dental units (Watanabe et al., 2008).

4.3.3 Total coliforms associated with biofilm

The biofilm results pertaining to open and closed system DCUs were characterised by analysing the concentration of total coliform bacteria from swab samples taken at various points of use (Appendix B) and comparing the total coliform results to the SANS 241:1 (SANS, 2015) requirement for the presence of total coliform bacteria. SANS 241:1 (SANS, 2015) specifies that water should not contain more than 1×10^1 CFU ml⁻¹ of total coliform bacteria to be considered safe for human consumption.

Swabs were taken of the inner distal surfaces of taps supplying water to open and closed DCUs and total coliform counts were obtained. The total coliform counts of the inner distal tap surfaces of open system DCUs presented a mean count of 5.09×10^3 CFU 100 ml⁻¹, which was not in compliance with SANS 241:1 (SANS, 2015) (Appendix B). The open system DCUs that did not comply with SANS 241:1 (SANS, 2015) for total coliforms had total coliform counts of O2.1 = 1.66×10^4 CFU 100 ml⁻¹, O2.2 = 1.84×10^4 CFU 100 ml⁻¹, and O2.3 = 1.61×10^4 CFU 100 ml⁻¹ (Figure 4.9). All the taps at facility O2 contained total coliforms except O2.4, which evidently did not contain biofilm such as the other DCUs in this facility. No coliform bacteria were detected on any other tap surfaces of open system DCUs.

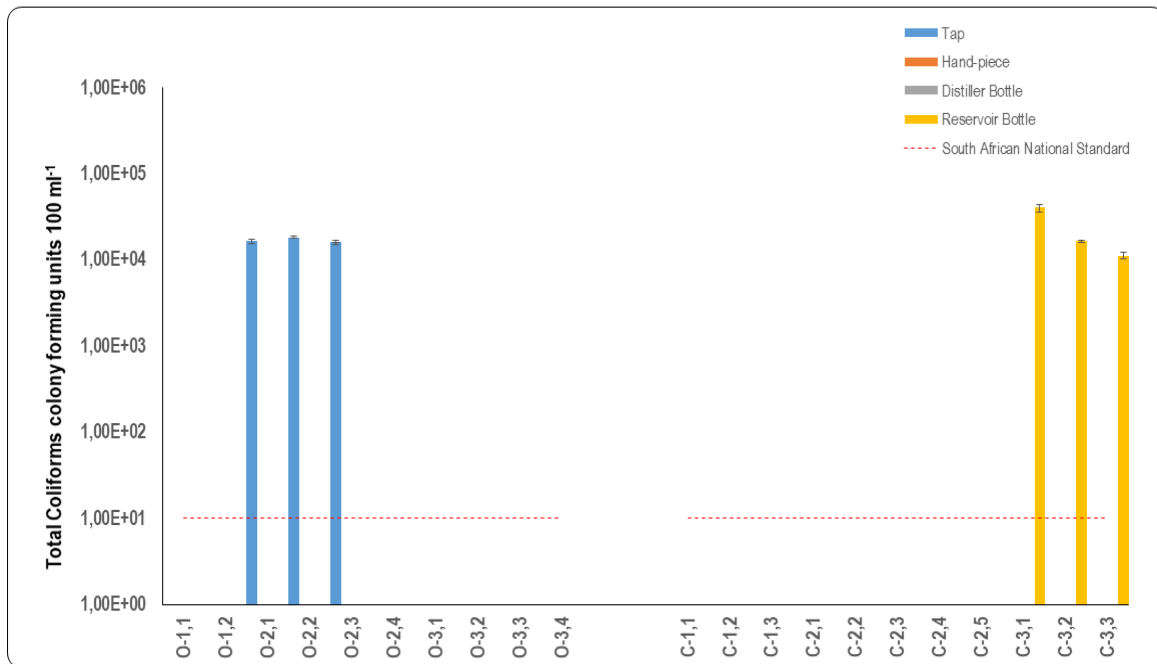


Figure 4.9: Graphic representation of data from oral healthcare facilities for biofilm-associated total coliform counts for swab samples of open and closed system DCUs. Water samples were taken from taps and fast handpieces in open system DCUs (O1.1 to O3.4) and taps, distiller bottles, reservoir bottles, and fast handpieces of closed system DCUs (C1.1 to C3.3). The dashed red line represents the SANS 241:1(SANS, 2015) for drinking water quality for total coliforms per 100 millilitres.

No coliforms were present on the inner tap surfaces of taps that supplied water to distiller bottles in open system DCUs. The surfaces of the handpieces of the closed system DCUs all complied with SANS for total coliforms as no coliforms were detected (Figure 4.9 and Figure 4.10). Total coliforms measured in closed system DCUs showed a total absence of this microbial group on the inner surfaces of taps, the inner surfaces of distiller bottles, and the inner surfaces of reservoir bottles (Figure 4.10). Total coliforms were present in reservoir bottles of closed system DCUs at a mean count of 6.23×10^3 CFU 100 ml⁻¹ (Figure 4.9). Total coliforms were also present in reservoir bottles in open system DCUs, and specifically in C3.1 (4.07×10^4 CFU 100 ml⁻¹), C3.2 (1.66×10^4 CFU 100 ml⁻¹), and C3.3 (1.13×10^4 CFU ml⁻¹) (Figure 4.9).

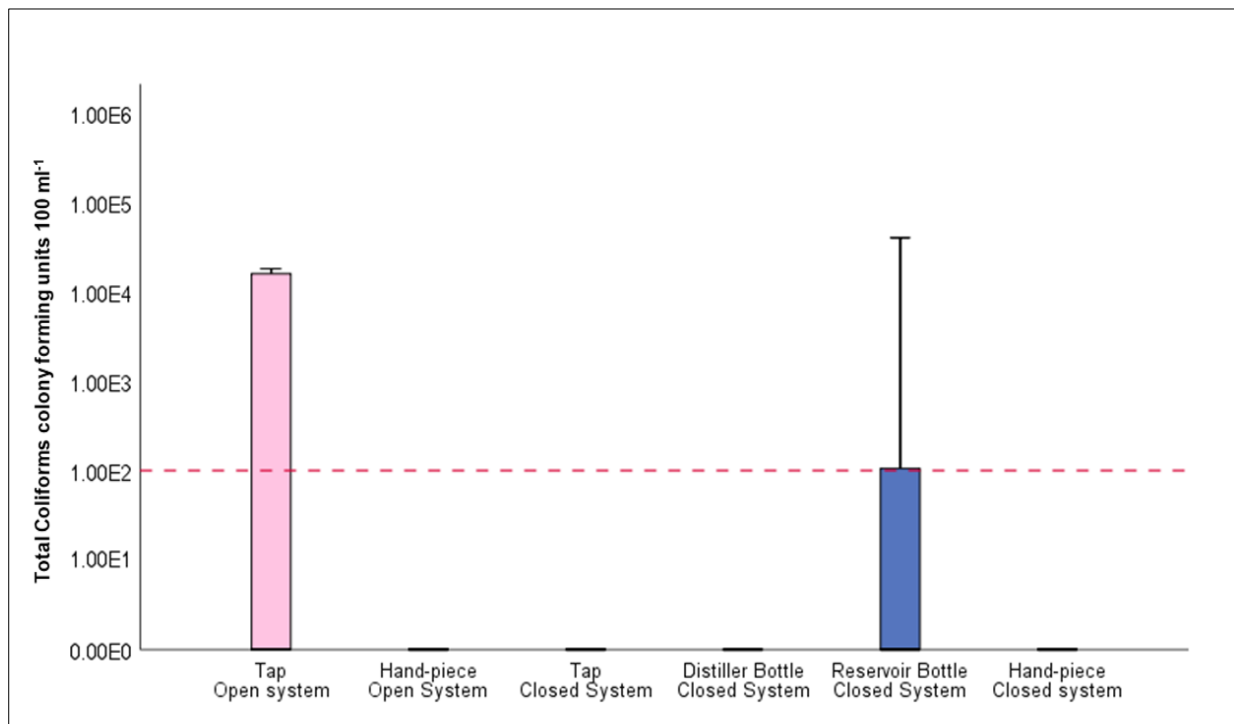


Figure 4.10: 'Box and whisker' plot and descriptive statistics of total coliform counts of swab samples for open and closed systems. The bottom and top of each box indicate the 25th and 75th percentiles, with the height of the box therefore indicating the interquartile range. The line dissecting the box is the median value. The 'whiskers' indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1 (SANS, 2015) limitation for total coliform bacteria per 100 millilitres.

The coupling of the fast handpieces of the closed system DCUs was removed to expose the surface of the waterline supplying them so that a swab sample could be taken. No total coliforms were present on the inner surfaces of the fast handpieces of the closed system DCUs. There was also no statistical significance ($p = 0.101$) between the inner surfaces of the reservoir bottles and the fast handpieces.

The total coliform mean counts of the open and closed system DCUs were compared for the inner surfaces of taps supplying source water. The results for the inner tap water samples were statistically significant ($p = 0.032$). It was found that the inner surfaces of the taps of open system DCUs contained total coliforms, while the inner tap surfaces of closed system DCUs did not indicate any total coliforms. Biofilm was

found on the inner surfaces of taps of open system DCUs, but the inner surfaces of the fast handpieces of both the open and closed system DCUs did not have any total coliforms on inner surfaces.

4.4 *Pseudomonas aeruginosa* associated with biofilm

Biofilm is a universal dilemma found in dental unit waterlines that provide an optimal environment for ubiquitous organisms that, even if they are present in low numbers, flourish as biofilm on the lumen surfaces of the narrow tubing of DUWLs. Opportunistic pathogens like *Pseudomonas aeruginosa* are widely reported to be present in hospital water systems and for the fact that they contaminate DUWLs. Their presence as biofilm colonisers in DUWLs is of significance in the nosocomial infection of patients who may be immunocompromised, while they may also affect OHCPs and their personnel. These individuals are at risk of being infected with these opportunistic pathogens by cross-infection or when aerosols are dispersed into the environment by handpieces (Spagnolo, Sartini and Cristina, 2021).

The biofilm results of the open and closed system DCUs were obtained by analysing the concentration of *P. aeruginosa* bacteria from swab samples taken at various points of use in the open and closed system DCUs under study (Appendix B). There is no legislation or policy parameters that guide the presence of *P. aeruginosa* in DUWLs, but their presence is of concern for individuals who are at risk of infection, and therefore DWULs should be monitored on a regular basis.

P. aeruginosa was measured by taking a swab sample of the inner distal surfaces of taps that supplied open system DCUs with water. The results showed that *P. aeruginosa* was present in open system DCUs O2.1 (8.85×10^4 CFU 100 ml⁻¹), O2.2 (1.06×10^5 CFU 100 ml⁻¹), O2.3 (8.98×10^4 CFU 100 ml⁻¹), and O2.4 (7.94×10^4 CFU 100 ml⁻¹) (Figure 4.11). It can be assumed that the surfaces of the taps feeding water to these units were not subject to adequate decontamination processes and this may also have influenced the water quality, because the water of these DCUs was also contaminated with *P. aeruginosa*. It was expected that, because the tap water and the inner distal surfaces of these taps exhibited *P. aeruginosa* contamination, it would result in the contamination of the lumen surfaces of the dental waterlines with *P.*

aeruginosa because of possible biofilm formation within the DUWLs, and that this might consequently influence the surfaces of the fast handpieces.

P. aeruginosa was detected on the inner surfaces of taps supplying DCUs O2.1, O2.2, O2.3, and O2.4. However, no *P. aeruginosa* was detected on any inner surfaces of the waterlines supplying the fast handpieces of open system DCUs with water.

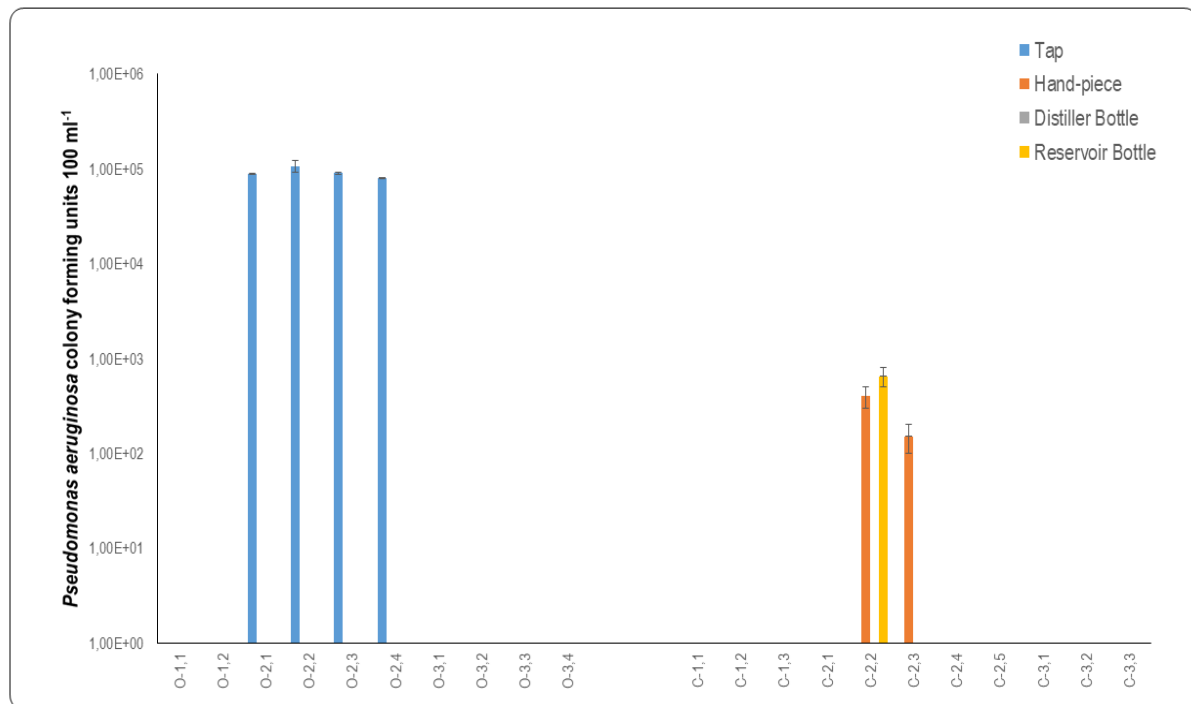


Figure 4.11: Graphic representation of data from oral healthcare facilities for biofilm-associated *Pseudomonas aeruginosa* counts for swab samples of open and closed system dental units. Water samples were taken from taps and fast handpieces in open system dental chair units (O1.1 to O3.4) and from taps, distiller bottles, reservoir bottles, and fast handpieces of closed system dental chair units (C1.1 to C3.3).

The couplings of fast handpieces were removed to expose the waterline for a swab sample, and the results showed that no *P. aeruginosa* was present on the inner surfaces of the waterlines of fast handpieces (Figure 4.11). The absence of *P. aeruginosa* on the inner surfaces of the fast handpieces could indicate that flushing of waterlines and/or the use of decontaminating agents may have resulted in the absence of *P. aeruginosa* in this instance. An earlier study investigated biofilm production on high-speed handpiece surfaces by cutting aluminium fragments from them and testing these fragments. The number of adhered cells for *P. aeruginosa* on the surfaces of the aluminium pieces was 9×10^6 CFU cm⁻² (Freitas, Sand and Simonetti, 2010).

In the current study, *P. aeruginosa* was measured in closed system DCUs by taking a swab sample of the inner surfaces of taps that supplied water to the DCUs. The results indicated that no *P. aeruginosa* was present on the inner surfaces of the taps that were used to fill distiller bottles (Figure 4.11 and Figure 4.12). *P. aeruginosa* was also not found on the inner surfaces of distiller bottles (Figure 4.11 and Figure 4.12). However, *P. aeruginosa* was detected in reservoir bottles of closed system DCUs, at a mean count of 5.91×10^1 CFU 100 ml⁻¹. *P. aeruginosa* was also detected on the inner surface of the reservoir bottle attached to DCU C2.2 (6.50×10^2 CFU 100 ml⁻¹). This reservoir bottle may have become contaminated due to ineffective decontamination practices or by improper handling. The water in this reservoir bottle was also contaminated with *P. aeruginosa* and may have colonised the reservoir bottle surface.

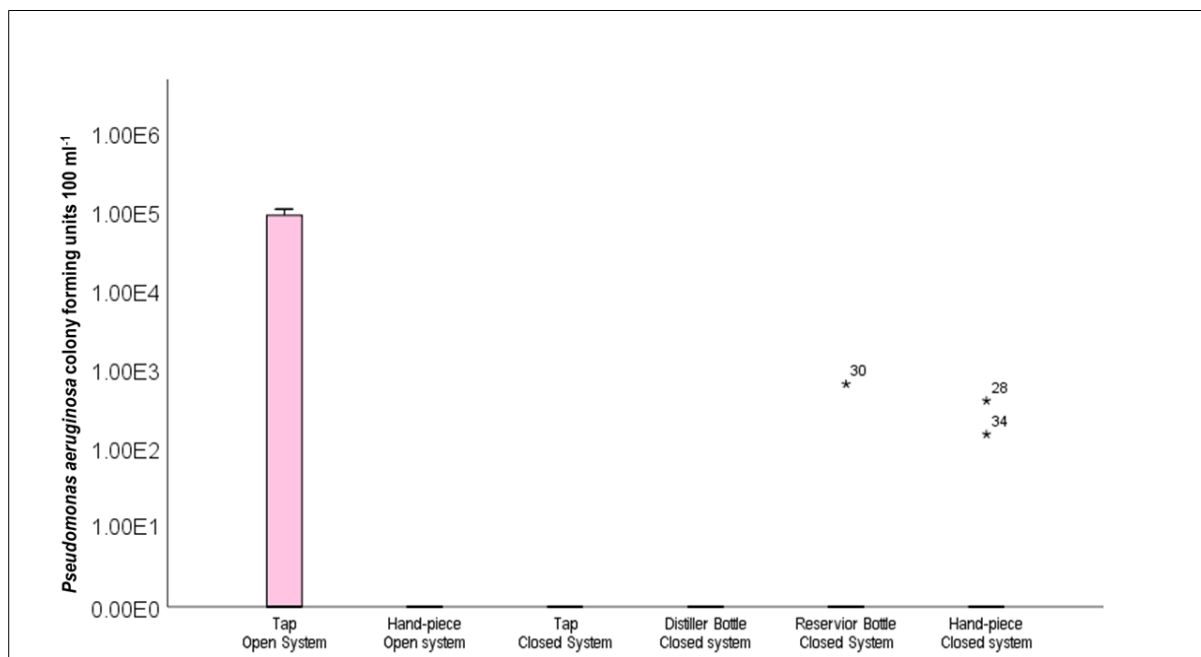


Figure 4.12: 'Box and whisker' plots and descriptive statistics of *Pseudomonas aeruginosa* counts of swab samples for open and closed systems. The bottom and top of each box indicate the 25th and 75th percentiles, with the height of the box therefore indicating the interquartile range. The line dissecting the box is the median value. The 'whiskers' indicate the range of values (minimum and maximum). The markers for outliers are indicated by a ° out values and * extreme out values. The red dotted line in the figure corresponds to the SANS 241:1(SANS, 2015) limitation for total coliform bacteria per 100 millilitres.

The couplings connected to the waterline of the fast handpieces were disconnected and a swab sample was taken of the inner surfaces of the fast handpiece waterlines. It was expected that the surface of the fast handpiece in DCU C2.2 would reveal *P. aeruginosa* because it was present on the inner surface of the reservoir bottle and in the water that filled the reservoir bottle of this DCU (Figure 4.11). *P. aeruginosa* was indeed present on the inner surface of the fast handpiece waterline of this unit at a count of 4.00×10^2 CFU ml⁻¹ (Figure 4.11). *P. aeruginosa* was also present on the inner surface of the fast handpiece of another closed system DCU, namely C2.3 at 1.50×10^2 CFU ml⁻¹ (Figure 4.11).

4.5 Conclusion

Biofilms are known to be widely distributed in aquatic environments and are formed by the attachment of microorganisms to surfaces and the production of extracellular polysaccharides. Biofilms are reportedly present on and in a range of healthcare devices, including DUWLs. They pose a serious risk to public health because of the increased resistance of biofilm-associated organisms to antimicrobial agents as well as their ability to cause infections in immunocompromised individuals (Revdiwala, Rajdev and Mulla, 2012). The swab samples taken from open and closed system DCUs in this study indicated the presence of biofilm-associated organisms and possible biofilm growth within the DUWLs.

Tested within the limitations of the study, it was found that the mean HPC for surface swab samples at all sampling sites did not comply with the SANS 242:1 (SANS, 2015) stipulation of the acceptable maximum count of 1×10^3 CFU ml⁻¹. Total coliforms were present on the inner surfaces of taps supplying water to open system DCUs. These pipelines should be disinfected and monitored regularly. The reservoir bottles of open system DCUs also contained total coliforms, probably as a result of ineffective or lacking decontamination processes and improper handling. The surfaces of the reservoir bottle and the waterline of the fast handpiece of closed system DCU C2.2 showed the presence of *P. aeruginosa* in the form of biofilm. *P. aeruginosa* was also detected in the waterline supplying the fast handpiece of closed system DCU O2.3. This finding is noteworthy as *P. aeruginosa* can be an important indicator of biofilm growth in DUWLs (Lizzadro et al., 2019). The fact that *P. aeruginosa* was present on

the inner distal surfaces of taps supplying water to open and closed system DCUs as well as in the waterlines of fast handpieces when oral healthcare procedures were performed is concerning, as its presence poses a real threat to immunocompromised patients as well as OHCPs and their personnel. *P. aeruginosa* was proven to be responsible for infections in immunocompromised patients who were treated using DCUs that harboured these organisms. Moreover, exposure to fragments of biofilm dispersed by aerosols may also constitute a serious threat to the pulmonary system of patients that inhale contaminated aerosols.

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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


 Central University of
Technology, Free State
 
 Department of Life Sciences
Faculty of Health and Environmental Sciences
 
 10
YEARS
AS A UNIVERSITY
OF TECHNOLOGY

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 (akasiakh@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 Kader

Contact Details for communication

Name of the Facility:				
Dentist in charge:				
Physical Address:				
Postal Address:				
Tel no:	()		Cell no:	
Fax 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 unit waterlines

		Tap		Handpiece		Reservoir Bottle	Distiller Bottle
Organism (unit)	Statistic	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 Coliforms (CFU 100 ml⁻¹)	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
	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	27.27%	10%	45.45%	20%	9.09%	90.90%
Compliance							
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	100%	70%	100%	100%	27.27%	100%
Compliance							
<i>Pseudomonas</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%