A quantitative and qualitative assessment of dental aerosols within a dental clinical unit: an avenue for the transmission of resistant nosocomial infection.

By

DR. SONIA THERESA BREDENKAMP

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Supervisor

Professor Charlene WJ Africa

KEY WORDS

Dental aerosols

Scaling and polishing

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Blood agar

Gram stain

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Microbial analysis

VITEK®

ABSTRACT

Introduction: Nosocomial infections are infections that occur 48 hours after receiving care for an unrelated condition in a clinic or a hospital environment, many of which are resistant to at least one of the drugs most commonly used to treat them. The dental clinical settings are reservoirs for the transmission of microbes through aerosols produced by routine dental procedures.

Aim:

The aim of the present study was to qualitatively and quantitatively assess microbial dental aerosol in order to determine the risk for nosocomial transmission of resistant bacteria within a dental clinical setting.

Methods and materials:

Passive sampling was employed to collect dental aerosol samples within a 6.96m^2 dental cubicle during basic conservative dental procedures performed on 40 patients in a university dental training clinic. Sterile Blood Agar plates were placed at predesignated sites (counter and tray), 40 cm from the operatory site for a duration of 2 hours. After overnight incubation at 37 ºC, Colony Forming Units (CFU) on the blood agar plates were quantified and their identification and antimicrobial susceptibility assessed using the VITEK®2 system. The microbial index of contamination was compared for different dental procedures as well as for the presence and absence of dental students within the clinic.

Results:

The CFUs were highest for scaling and polishing procedures and when students were present in the undergraduate clinic. They were equally distributed for the counter and tray placements. A total of 119 pure isolates were identified of which the predominant isolate was *Micrococcus luteus* (31%) followed by *Staphylococcus hominis* ssp *hominis (*8.4%) and *Kocuria rosea* (7.6%). Of these, *Staphylococcus ssp hominis* met the criteria for VITEK® susceptibility testing. All ten strains of *Staphylococcus hominis ssp hominis* showed susceptibility to Gentamycin, Ciprofloxicin, Clindamycin, Linezolid, Teicoplanin, Vancomycin, Tigecycline, Rifampicin and Trimethoprim/Sulfamethoxazole. Resistance was shown by five strains to Fosfomycin and three strains to Erythromycin. One strain showed resistance to Oxacillin, Daptomycin, Tetracycline and Fusidic acid.

Conclusion:

The microbial load in dental aerosol constitutes a complex heterogeneous mixture of isolates, with Gram-positive cocci predominating. The isolation of resistant species poses a health challenge, especially for nosocomial infection in dentistry where it is not being monitored and no surveillance thereof pursued.

DECLARATION

I, Sonia Bredenkamp, certify that "The quantitative and qualitative assessment of dental aerosols within the dental clinical unit at the University of the Western Cape: An avenue for the transmission of resistant nosocomial infection" is my own work and has not been submitted to another institution, and that all the information sources that have been used or quoted have been acknowledged with complete citations.

This research was conducted in the Faculties of Dentistry and Natural Sciences, at the University of the Western Cape, Cape Town, South Africa, under the supervision of Professor Charlene WJ Africa

Dr.Sonia Theresa Bredenkamp

Signed:…… Date: 30 November 2020

DEDICATION

I dedicate this thesis to my Supervisor, Professor CW Africa for believing in me and providing the support, encouragement and inspiration to complete this overwhelming task during these challenging times of COVID 19. My interest in research was ignited by her passion for research and her pursuit in helping others achieve their goals.

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AST- Antimicrobial susceptibility testing

BA- Blood Agar

CFU/m^{3-} Colony forming units per cubic meter

DCU's -Dental chair units

DUWL's- Dental unit water lines

ID- Species identification

GN - Gram-negative fermenting and non-fermenting bacilli

GP - Gram-positive cocci and non-spore-forming bacilli

MRSA-Methicillin-resistant *Staphylococcus aureus*

NaCL-Sodium Chloride

TSA- Trypticase Soya Agar

V2C- VITEK® 2 compact

CDC- Centre for Disease Control

IMA-Index of microbial air contamination

CHAPTER 1: INTRODUCTION

Nosocomial infections are infections in hospital inpatients that were neither present nor incubating at the time of the patient's admission (Jenkins, 2017; Khan *et al*., 2017). Among the general risk factors identified for nosocomial infections are the environment and the susceptibility and the condition of the patient (e.g. immunosuppression and prolonged antibiotic use) as well as the lack of awareness of infection control policies amongst healthcare workers. A lack of infection control results in poor hygienic practices and inadequate waste disposal within health facilities, along with the inappropriate use of invasive devices (Khan *et al*., 2017).

Within the dental environment, the pathways of contamination can be bidirectional i.e. an infectious microorganism can be transferred from the patient to the dental staff and vice versa through the patient's saliva and blood and accidental punctures by contaminated needles (Laheij et al., 2012). Transmission may also occur as a result of a contaminated surface, inadequately sterilised dental instruments, dental aerosols/splatter and dental unit waterlines (Harrel and Molinari, 2004; Laheij et al., 2012). The distribution of dental splatter is towards the dentist's face and patient's chest whereas aerosols may be spread throughout the dental clinic (Leggat and Kedjarune, 2001).

The topic of dental aerosol has had a timeline spanning from the 1960's when dental aerosol was first defined (Micik *et al*., 1969). Since the world health organization (WHO) declared the outbreak of the Severe Acute Respiratory Syndrome coronavirus 2 (SARS- CoV-2), also known as Coronavirus disease 2019 or Covid -19) to be a pandemic toward the end of December 2019, the spread of viruses and other microbes in aerosol has become the focal point within the global health community. The dental profession is categorised as the profession at

highest risk for contracting and transmission of disease by virtue of the nature of the work, which involves the generation of bioaerosol (Monaghan, 2016; Tellier *et al*., 2019).

Despite the well-documented risks and case reports associated with dental aerosols, only a few studies report on the monitoring of the microbial condition of air and removal of microbiological contaminated air from dental rooms in the private and public sector (Kimmerle et al.,2012; Pasquarella et al., 2012; Manarte-Monteiro et al., 2013; Polednik, 2014; Singh et al., 2016). This information is of utmost importance for the implementation of effective infection control measures (Szymanska and Dutkiewicz, 2008).

The aim of the present study was to employ passive air sampling to qualitatively and quantitatively assess microbial dental aerosol in order to determine the risk for nosocomial transmission of resistant bacteria within a dental clinical setting.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction: The definition and scope of nosocomial infection

Nosocomial infections are classified into 13 types involving 50 infection sites based on biological and clinical criteria (Khan *et al.*, 2015). For every 100 hospitalized patients, seven in developed and ten in developing countries can acquire one of the healthcare associated infections (Khan *et al*., 2017). A point prevalence survey of 231,459 patients from 947 acute care hospitals across 30 European countries in 2011/12 revealed that 5.7% suffered from a nosocomial infection (Jenkins, 2017). Nosocomial infections can be fatal or cause delayed recovery, functional impairment or aesthetic damage (Jenkins, 2017). Nosocomial infections are responsible for 4-56% of neonatal deaths with the frequency of infections in low-income countries reported to be three times higher than in high-income countries (Khan *et al*., 2017).

Routes of nosocomial infections include translocation, transmission, healthcare workers and the environment. Translocation involves an infection caused by the affected patient's own microbiota moving from its natural habitat to the site of subsequent infection. Transmission may be direct, via person to person contact (e.g. between health worker and patient), or indirect, such as an environmental setting (contaminated water and food, or via shared items and surfaces such as lamps, chairs etc). Healthcare workers may be a source of infection if infected with blood borne viruses (e.g. HIV, hepatitis B and C) respiratory infections (influenza, pertussis and tuberculosis) or skin infections such as herpetic whitlow. The inhalation of airborne spores of environmental fungi such as *Aspergillus* species poses a risk to immunocompromised patients (Jenkins, 2017).

The most frequent types of nosocomial infections include central line associated bloodstream infections (death rate between 12-25%), followed by catheter-associated urinary tract infections (>12% of reported infections), surgical site infections (2-5% of surgical patients) and ventilator associated pneumonia (9-27% of patients), with catheter-associated urinary tract infections being the most common nosocomial infection reported globally (Khan *et al*., 2017).

Pathogens responsible for nosocomial infections are bacteria, viruses and fungi, of which bacteria are the most commonly found. These micro-organisms vary depending on different patient populations, medical facilities and even differences in the environment (Khan et al., 2017). Opportunistic pathogens form part of the normal human microbiota and occur naturally on the epithelial cells of the skin, oropharynx, gastrointestinal tract and the genitourinary tract (Jenkins, 2017). Among the microbial agents implicated in nosocomial infections are *Streptococcus* spp., *Acinetobacter* spp., enterococci, *Pseudomonas aeruginosa*, coagulase-negative staphylococci, *Staphylococcus aureus*, *Bacillus cereus, Legionella, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli,* and *Serratia marcescens* (Zemouri *et al,* 2017). Also implicated are *Stenotrophomonas maltophilia* and *Aspergillus* species (Jenkins, 2017). *Acinetobacter* is the most frequently reported genus responsible for infections occurring in ICU's with *Bacteriodes fragilis, Clostridioides difficile* and *Enterobacteriaceae* such as *Klebsiella and Escherichia coli* causing co-infections, inflammation of the colon and transmission from infected patients to others through inadequate hand hygiene respectively (Khan *et al*., 2017). *Staphylococcus aureus* is a normal commensal habitant of the oropharynx and skin and may cause skin infections, septicaemia, endocarditis, osteomyelitis, pneumonia and toxic shock syndrome. (Laheij, *et al*., 2012).

Viral nosocomial infections are transmitted via hand and mouth, the respiratory route and faecal oral route and include hepatitis, influenza, HIV, rotavirus, herpes simplex and the SARS coronavirus (Khan *et al*., 2017, Peng, 2020).

Fungal parasites such as *Aspergillus* spp *Candida albicans* and *Crypotococcus neoformans* are opportunistic pathogens causing nosocomial infections in immune-compromised individuals. Candidiasis is the most common fungal infection (Damasceno *et al*., 2017) and results from the patient's endogenous microflora, while *Aspergillus* infections result from the inhalation of fungal spores as a result of contaminated air that arises from the construction/renovation in a healthcare facility (Damasceno *et al*., 2017, Khan *et al*., 2017).

The Centre for Disease Control (CDC, 2001) reports that > 70% of the bacteria causing nosocomial infections are resistant to at least one of the drugs most commonly used to treat them. Thus, the need to identify and control the transmission of such infections is paramount in the prevention of patient morbidity. However, stringent infection control in most healthcare facilities in most countries remains limited (CDC 2001b).

2.2 Antimicrobial resistance

Antimicrobial resistance occurs when microbes develop the ability to resist the effects of drugs (Khan *et al*., 2017). Defined terms are used to describe the extent of resistance. Multidrug resistant (MDR) bacteria are resistant to at least one agent in three or more antimicrobial categories. Extensive drug resistance (XDR) is resistant to at least one agent in all but two or fewer antimicrobial categories. Pan drug resistance (PDR) is resistance to all agents in all antimicrobial categories (Jenkins, 2017).

Multidrug resistant bacteria pose a major health risk worldwide. These bacteria are mainly transmitted by either direct or indirect contact as a result of contaminated surfaces (Laheij, *et al*., 2012). Multidrug resistant bacteria that cause nosocomial infections are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin –resistant enterococci (VRE) (Umar *et al*., 2015) and MDR Gram-negative bacilli, particularly *Escherichia coli* and *Klebsiella* species (Jenkins, 2017).

The extended–spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* and carbapenemase-producing Gram-negative bacteria are the most problematic multi-resistant organisms (McCormack *et al.*, 2015), demonstrating resistance to penicillin, second and third generation cephalosporins and monobactams (Laheij, *et al*., 2012). MRSA are resistant to all β-lactam antibiotics including methicillin, dicloxacillin, cephalosporins and carbarpenems (Laheij *et al*., 2012). For hospitalised patients receiving treatment, those with oral cancer showed an increased risk for MRSA colonization (Laheij *et al*., 2012).

The CDC and Prevention reports that each year 100 million courses of antibiotics are prescribed by office-based physicians, 50% of which are unnecessary (CDC, 2016). This defeats the aim of anti-microbial therapy which is, to effectively act against the pathogen with no resistance and adverse effects (Khan *et al*., 2017). Self-medication with antibiotics, incorrect dosage, prolonged use and a lack of standards for healthcare workers are the main factors for the increase in antimicrobial resistance, which threatens the effective control against bacteria (Khan *et al*., 2017).

In South East Asia, a high resistance of *E.coli* and *K. pneumoniae* to third generation cephalosporin has been reported, with more than a quarter of *S. aureus* infections being methicillin resistant (Khan *et al*., 2017).

Prevention is the best approach to reduce the incidence of nosocomial infections. It can be addressed by taking into consideration the interaction between pathogens and patients within the clinical practice where health care is delivered (Jenkins, 2017).

2.3 The risk for nosocomial infections in dentistry

The oral environment is a complex dynamic system with diverse conditions comprising of specialized surfaces of soft and hard tissues (Zawadski *et al.*, 2017) collectively harbouring up

to 700 different species of bacteria, fungi, protozoa and viruses (Laheij *et al*., 2012; Lasserre *et al*, 2018). Routine dental procedures are invasive in nature, increasing the risk of exposure to these micro-organisms (Laheij *et al*., 2012).

The study of airborne particles in the dental office has been designated "Dental aerobiology" by Micik *et al*., (1969), the pioneers of this research niche in the field of dentistry. More importantly, the relationship of these airborne particles and the health of dental personnel and patients have been of particular research interest for many decades. This remains as important today within the dental environment, as it was then. Airborne materials are produced during dental procedures which could be in the form of aerosols and splatter generated by dental procedures (Bennet *et al*., 2000; Szymanski and Dutkiewicz, 2008).

Aerosols are defined as particles less than 50 micrometres in diameter which remain airborne for a period of time before either settling on environmental surfaces or settling within the respiratory system (Harrel and Molinari, 2004). The term splatter has been defined as particles larger than 50 micrometres in diameter and behave in a ballistic manner, which means that these particles are ejected forcibly from the operating site (Harrel and Molinari, 2004).

The particle size influences the site of deposition in the respiratory tract and the survival of microbes in the aerosol (Gowtham and Deepthi, 2014). Smaller particles such as bacteria (0.25µm – 4.0 µm) and viruses (0.02µm – 0.4µm) deposit within the lungs. The larger particles (>10 µm) such as fungi deposit in the upper respiratory tract (Gowtham and Deepthi, 2014).

A recent, comprehensive, scoping review on bio-aerosol in the dental environment, concluded that the risk for health care workers, especially dentists, working in an environment where there is chronic exposure to bio-aerosols, remains unclear (Zemouri *et al*, 2017). This review highlighted that the methods used in these studies did not investigate the presence of parasites

and viruses in the aerosols generated and concluded that bio-aerosols may be hazardous to certain populations who are chronically exposed to these bio-aerosols and who are immunocompromised (Zemouri *et al,* 2017).

2.4 Sources of microbial aerosols in the dental clinic

A combination of cutaneous, oral, respiratory and environmental bacteria may be isolated from the microbial aerosol (Decreane *et al*., 2008). Microbes indigenous to the oral cavity such as viridans streptococci, *Actinomyces* spp, *Haemophilus* spp, *Neisserria* spp *and Lactobacillus* spp have been reported (Decreane *et al*., 2008). Researchers studying the microbiological condition of air within dental surgeries believe that streptococcal bacteria, which make up 85-95% of the dental aerosol, are believed to be amongst the most dangerous contamination carriers as these bacteria can cause an array of disease including dental caries and bacterial endocarditis (Szymmanska, 2007).

Three potential sources of microbial aerosols have been identified, namely, salivary/respiratory, dental instrumentation and the operative site (Leggat and Kedjarune, 2001; Harrel and Molinari, 2004; Szymanska and Dutkiewicz, 2008; Kimmerle *et al.,* 2012; Swaminathan and Thomas, 2013; Gowtham and Deepthi, 2014; Polednik, 2014; Sharashchandra and Ambika, 2014; Umar *et al*, 2015).

2.4.1 Salivary/respiratory source

Saliva contains many free-floating bacteria per millimetre that progressively deposit and adhere to the intra-oral sites (Lasserre *et al,* 2018). The initial colonizers of early dental plaque are Gram-positive bacteria, mostly cocci, then later the composition of the dental plaque comprises of Gram-negative bacteria (Lasserre *et al,* 2018).

The saliva as well as the nasopharyngeal secretions may harbour pathogenic organisms including Herpes Simplex virus (HSV), Varicella-Zoster virus (VZV), Human immunodeficiency virus (HIV) and Hepatitis B, C and D viruses (Laheij et al., 2012; Gowtham and Deepthi, 2014), the common cold and influenza viruses, streptococci and staphylococci (Harrel and Molinari, 2004; Szymanska and Dutkiewicz, 2008). When conditions such as inflammation and bleeding are present, saliva may be contaminated with blood and aerolized, thus posing a risk for a hepatitis infection (Sharashchandra and Ambika, 2014).

2.4.2 Dental instrumentation

The composition of the air shared between the patient and dental personnel while using dental hand pieces during treatment is largely ignored in microbiological analyses (Szymanska, 2007). Within the dental practice, the use of high-speed drills and ultrasonic scalers in the presence of salivary and blood secretions, generate aerosols containing blood and oral micro-organisms (Bennet *et al*., 2000). Dental handpieces, ultrasonic scalers, air polishers and air abrasion units have been identified to produce the most aerosols (Harrel and Molinari, 2004; Polednik, 2014) and are significant sources of submicrometer particles in the dental office (Polednik, 2014). The ultrasonic scaler is reported to produce the most airborne contamination (Bennet *et al*., 2000; Harrel and Molinari, 2004; Swaminthan and Thomas, 2013) with cavity preparation producing a significant increase in the level of bioaerosols (Hallier *et al., 2*010).

High bacterial counts have been associated with the use of ultrasonic scaling compared to high speed drilling (Bennet *et al*., 2000; Grenier, 1995). For ultra-sonic scaling, the number of colony forming units were reported to increase from 12 ± 4CFU/m^3 to 216 ± 75CFU/m^3 with a drop of 80% to 44 ± 14 CFU/m^3 after treatment, while for high speed drilling with rubberdam use, the level of air contamination increased to 75 ± 22 CFU/m^3 with the microbes remaining

airborne for 7 hours in the multi-clinic site and 2 hours in the closed operatory site (Grenier, 1995).

2.4.3 Operative site

The European Union Medical Devices Directive classifies dental chair units (DCU's) as medical devices (Rodrigues, *et al*., 2017). Each DCU is equipped with narrow bore flexible plastic tubing called DUWLs, the texture and composition of which harbours the growth of microbial biofilms (Rodrigues, *et al*., 2017).

Water-borne microorganisms may cause infection in 4 ways within the dental environment e.g. patients undergoing dental treatment, haematogenous spread during surgical procedures, local mucosal (oral or conjunctival) contact, ingestion and inhalation (Rodrigues *et al*, 2005).

Conjunctival infection risks include *Pseudomonas aeruginosa* and amoebic keratitis contamination caused by microorganisms isolated from the dental unit water lines (DUWL) (Swaninathan and Thomas, 2013).

The monitoring of the quality of water used in the dental units is imperative as patients and oral healthcare workers are exposed routinely to the water and dental aerosols produced during treatment (Damasceno *et al*, 2017; Rodrigues, *et al*., 2017). The water that circulates within the system is impure as a result of an increase in microorganism density with increased resident time of the water (Damasceno *et al*, 2017). The microorganisms found in dental unit waterlines are either planktonic (free floating) or sessile i.e. attached to the inside wall of the waterline forming a biofilm (Rodrigues, *et al*, 2005). Stagnant water, biofilm formation and lack of disinfection measures help microorganisms to proliferate within the dental unit water systems (Damasceno *et al*, 2017).

Besides water stagnation and the presence of low concentrations of bacteria with adherent properties that serve to pioneer biofilm formation, other factors contribute to the formation of

the DUWL biofilm. These include waterline openings, retraction through the handpiece or water air syringe, nutrients provided by incoming bacteria, increased biofilm attachment to the tubing, along with the water flow to the dental equipment (Rodrigues, et *al.*, 2005; Szymanski and Dutkiewicz, 2008; Swaminathan and Thomas, 2013; Gungor, *et al.*, 2014; Gowtham and Deepthi, 2014; Fotedar and Ganju 2015; Paramashivaiah *et al.*, 2016; Volgenant and Persoon, 2018).

The American Dental Association (ADA) recommends that the water used for non-surgical dental procedures should contain no more than 200CFU/ml compared to the Centre for Disease Control (CDC) recommendation of 500 CFU/ml for drinking water (Pankhurst and Coulter, 2007; Gungor, *et al.*, 2014; Fotedar and Ganju, 2015 Rodrigues S *et al*.,2017).

Most of the micro-organisms within the dental unit waterlines are opportunistic pathogens (Rodrigues, *et al*, 2005: Gungor, *et al.*, 2014; Fotedar and Ganju, 2015). The micro-organisms with pathogenic potential commonly present within the dental unit waterlines include *Legionella pneumophilia*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus* spp and *Stenotrophomonas* (Rodrigues, *et al*, 2005; Pankhurst and Coulter, 2007; Gungor, *et al.*, 2014; Fotedar and Ganju, 2015 and Paramashivaiah *et al.*, 2016). Gram-negative bacteria constitute the most common species (Paramashivaiah *et al.*, 2016) with *Pseudomonas aeruginosa* isolated from 15 to 24% of samples with water concentration of 2 x 10^5 CFU/mL (Rodrigues, *et al*, 2005) and *Legionella* sp having a water concentration of 10^2 to 10^4 CFU/mL (Rodrigues, *et al*, 2005). Nontuberculous mycobacteria (*Mycobacterium gordonae* and *Mycobacterium chelonae*) have dental unit waterline concentrations 400 times greater than tap water (Rodrigues, *et al*, 2005).

In order to reduce the formation of biofilms within the dental unit waterlines, the employment of anti-retraction valves to reduce suck back from the oral cavity is recommended, as well as

flushing the waterlines for 2 minutes in the morning and 20-30 seconds between patients, along with the use of filtered independent bottled water systems (Rodrigues, *et al*, 2005 and Paramashivaiah *et al.,* 2016).

Although the placement of portable microbial filters will prevent suspended bacteria from entering the handpiece, this, however, does not remove the biofilm (Rodrigues, *et al*, 2005). In addition, some filters are impregnated with iodine, which could adversely affect patients with an allergic reaction to this anti- bacterial product (Rodrigues, *et al*, 2005). Disinfectant use with the following active agents such as hydrogen peroxide, citric acid, iodine, chlorohexidine 1:10 household bleach, ozone and electrochemically activated water may also pose a health risk, as there are no official recommendations for the use of this water within the mouths of dental patients (Rodrigues, et *al*, 2005; Paramashivaiah *et al.,* 2016).

The independent bottled system has the advantage of bypassing the main water system, however, it is the tubing that becomes contaminated with the biofilm infecting the distilled/sterile water (Rodrigues, *et al*, 2005). Ideally the bottles should be disinfected at the end of the day, stored dry and inverted (Rodrigues, *et al*, 2005).

Nanotechnology has been used extensively in dentistry and this application has recently also been introduced as an alternative to the conventional methods for water decontamination to reduce the formation of biofilms within the dental unit waterlines (Paramashivaiah *et al.*, 2016). A lack of student compliance in maintaining appropriate water quality in a Dutch dental school, revealed a need for routine water checks in conjunction with the preventive recommendations to reduce the formation of biofilms (Volgenant and Persoon, 2018)

Known risks to dental personnel and patients as a result of dental aerosols have been documented to be pneumonic plague, tuberculosis, influenza, legionnaires' disease, severe

acute respiratory syndrome (SARS) and herpesviruses (Harrel and Molinari, 2004; Gowtham and Deepthi, 2014).

Decreane *et al* (2008) found the predominant airborne microbes to be *Propionibacterium acnes, Micrococcus luteus* and *Staphyloccocus epidermidis i*n periods of high clinical activity. These microbes are normally found on the skin, but may reside in the oral cavity as well and may cause contamination of the dental surfaces through the air-borne route (Decreane *et al.*, 2008; Kimmerle *et al*., 2012).

2.5 Preventive measures

Within the dental environment, microbial aerosols are considered to pose a serious health risk to dental personnel and patients especially if patients harbour blood-borne and respiratory viruses (Bennet *et al.,* 2000).

To protect the dental team and patients alike, the following strategies are still being advocated to reduce the risk of respiratory infection viz; barrier protection, pre-procedural mouth rinses, high volume evacuation and the use of air filters and air sanitizers. An outbreak of severe acute respiratory syndrome in Hong Kong (2002-2004) and the recent COVID-19 pandemic, highlight the need for implementing these strategies strictly by all dental personnel (Harrel and Molinari, 2004 and Peng, 2020).

The screening of patients is the first preventive measure before any dental treatment is commenced (Swaminathan and Thomas, 2013). The use of gloves, masks, safety glasses, the routine use of a pre-procedural antiseptic mouthwash, rubberdam placement and the use of a high volume evacuator are the next line of preventive measures (Leggat and Kedjarune, 2001; Harrel and Molinari, 2004; Gowtham and Padma, 2014). The use of a pre-procedural mouth rinse and suction should be made mandatory before any invasive restorative procedure (Sawhney *et al*, 2015).

Immunization against Hepatitis A and B, influenza, mumps, measles, tetanus, rubella, tuberculosis and whooping cough should be adhered to and emphasis put on the personal and hand hygiene of the dental personnel (Swaminathan and Thomas, 2013).

As mentioned above, flushing of the dental waterlines needs to commence at the start of each clinical day and for a duration of 30 sec to 1 minute between patients. This eliminates microbial accumulation due to overnight waterline stagnation. At the end of the day, the suction lines must be cleaned with ammonia or an enzymatic detergent (Swaminathan and Thomas, 2013).

Surface contamination barriers such as thin plastic bags, wraps or aluminium foils should be used on dental unit light handles, electrical and mechanical controls, the head and arm rests, dental unit controls, high and low speed handpieces, ultra-sonic scaler, air/water syringe, ejector and high vacuum evacuator (Swaminathan and Thomas, 2013).

In addition, regular cleaning and servicing of ventilating systems and microbiological monitoring should be implemented to detect risk factors and introduce infection control measures (Swaminathan and Thomas, 2013).

2.6 Microbial sampling of bacterial aerosols within the dental environment

The identification and antimicrobial assessment of environment-associated opportunistic pathogens may be achieved using passive or active air sampling. Passive sampling (agar plates) may be used to quantify airborne bacteria (Manarte-Monteiro, *et al.*, 2013) and allow for cumulative measurement of contamination, while active air samplers measure particles suspended in the air during a specific time period as well as the level of microbial air contamination. The number of colony forming units is measured in $1cm^3$ of air over a period of 15 minutes (Decraene *et al*., 2008).

The method used to collect a sample should be standardized as well as the clinical environment. This may impact on the results obtained since microbiological particles may differ in size and

density and may be distributed differently when airborne (Kimmerle *et al.*, 2012). Zemouri, et al., (2017) listed both air samplers and settling plates as examples of air sampling methods given in studies that met the stringent criteria for a scoping review with respect to dental bio-aerosols.

The advantage of air samplers is that they form the basis for official standards in air control and are therefore considered the appropriate sampling method (Decraene *et al*., 2008). The disadvantages include difficulty in sterilization, cost, the need for continuous calibration and the existence of many types of air samplers. Passive sampling on the other hand has the advantage of costing less.

The microbial identification of Gram-negative and Gram-positive bacteria is the first step for the interpretation of antimicrobial susceptibility tests (Levesque *et al*., 2015). The more traditional methods of microbial identification which include phenotypic methods, growth on selective and non-selective media, and morphology of colonies, Gram stain, microscopic morphology and biochemical reactions are time consuming and laborious (Levesque *et al.*, 2015) and are rapidly being replaced by the use of new technology systems such as VITEK® and the Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Levesque *et al.*, 2015). Both systems provide a high level of accurate identification for a wide range microorganisms (Levesque *et al*., 2015).

There are a series of fully automated VITEK® systems (bioMerieux, MarcyI'Etoile,France) providing species identification (ID) and antimicrobial susceptibilty testing (AST) of clinical isolates (Nakasone *et al*, 2007). Extensive upgrades to this system have been done in the past three decades which included the re-introduction of colorimetric reading in lieu of fluorescence technology, and the addition of several biochemical substrates and taxa (Nakasone *et al*, 2007).

The quality of microbial identification is determined by the age of the culture (8-24 hour cultures) and the inoculum load (Mcfarland standard of 0.5) (Funke *et al.*, 1998).

The API test strips used for microbial identification have long been considered as the gold standard in this niche of microbial identification with 97.5% accuracy (Nakasone *et al.*, 2007). However the 98.3 % accuracy level of the VITEK-2 system makes it a reliable and highly acceptable method for the accurate identification of clinical isolates (Bourbeau and Heiter, 1998, Wallet, *et al*., 2005; Nakasone *et al*., 2007).

2.7 Aims and objectives of the study

The hypothesis was formulated based on the research question: Are dental aerosols reservoirs for antimicrobial resistant bacterial species implicated in nosocomial infections?

H_0: Dental aerosols do not contain antimicrobial-resistant bacterial species frequently implicated in nosocomial infections.

H_1: Dental aerosols contain antimicrobial-resistant bacterial species frequently implicated in nosocomial infections.

The hypothesis was tested by qualitatively and quantitatively assessing microbial dental aerosol in order to determine the risk for nosocomial transmission of resistant bacteria within a dental clinical setting by meeting the following objectives:

1. To determine the quantitative and qualitative assessment of dental aerosols within a university dental clinical environment when routine dental procedures (fillings and scaling) are performed.
2. To compare the bacterial concentration of dental aerosols between ultra-sonic scaling and the use of the high speed drill in cavity preparation.

3. To compare the bacterial concentration at different locations within the dental operatory site when performing routine dental procedures.
4. To compare the bacterial concentration with and without student clinical activity when performing routine dental restorative procedures
5. To identify bacterial species within the bacterial concentrate using VITEK® Technology
6. To determine the antibiotic susceptibility of the bacterial isolates using VITEK® technology

CHAPTER 3: Materials and Methods

3.1 Study design and area

This is a clinico-microbial study.

Microbial monitoring of the dental clinical environment was undertaken at the Tygerberg Dental Faculty, University of the Western Cape within the restorative undergraduate clinic. The clinical area is divided into eight dental cubicles. Each cubicle measured 696$m^{2,}$ with a length of 2 960 m, breadth of 2 440m and a height of 15 330m.

Microbial identification and characterization was performed at the Faculty of Natural Sciences in the department of Medical Biosciences in collaboration with Cape Peninsula University of Technology (CPUT).

3.2 Ethical clearance

Ethical clearance was obtained from the Biomedical Science Research Ethics Committee of the University of the Western Cape (UWC) (Ethics Reference Number**:** BM18/3/18). The patients were informed of the nature of the study and given the option to withdraw without consequences to future treatment requirements. Routine dental treatment was carried out as per the normal clinical services rendered protocol within the Conservative Dentistry Department. Patients were informed that the data collected from the exposed sample plates would be used for research purposes only and kept confidential.

3.3 Sample size estimation

Consultation with a statistician was done before commencement of the study and it was estimated that 40 patients would be needed to generate the number of samples required to obtain a statistically viable result for testing the hypothesis. An ANOVA power analysis was performed to determine the sample size for this study (see appendix 2).

3.4 Clinical protocol

Aerosol collection within the dental clinic was performed using the passive sampling method. Patients were booked as per the normal booking protocol for the undergraduate dental clinic at the faculty of Dentistry. The normal booking protocol at the faculty of dentistry involves screening of patients by services rendering dentists who then refer them to the relevant department depending on their treatment needs. The screening form includes the medical history, patient's main complaint and demographics. For the present study, all patients needing restorations and a scale and polish according to the faculty's screening process were referred to the primary investigator. A total number of 40 patients were treated on the clinical platform.

Microbial monitoring of aerosols occurred during basic restorative work on two to three patients per day over a three week period. Within this 3-week period, microbial monitoring included student activity in the clinics as students also performed treatment for one and a half weeks of the study period.

The basic restorative work comprised of cavity preparation for anterior and posterior restorations using a high-speed hand-piece (Alegra TE98, W& H) and for caries removal, a low-speed hand-piece (MKdent/Ecoline/ LE11, MK-dent). For the scaling and polishing treatment option, the ultrasonic scaler (SONICflex 2000N /M161707, Kavodental) was used.

3.5 Assessment of the index of Microbial Air Contamination

3.5.1 Dental cubicle preparation

Before each appointment, the counter top, dental chair, overhead light, dental stool and dental unit waterline were disinfected using 80% isopropyl alcohol (Batch number 19/033, AR agencies, Athlone Industria)

Protective clothing such as a clean white coat, protective eyewear, masks and gloves were worn by the researcher/dentist and dental assistant.

A pre-procedural mouth rinse was not done as this did not form part of the clinical protocol.

In order to limit the amount of air turbulence, only three people were present in the cubicle, viz., the researcher, dental assistant and patient. The ventilation system remained unaltered for the duration of the sampling.

3.5.2 Aerosol collection

For this study, 90 mm Blood Agar (BA) plates were prepared by dissolving (39g) of Columbia agar base (Thermo Scientific, CM0331) in one litre of distilled water. The media was boiled to dissolve the powder completely and autoclaved at 121°C for 15 minutes. The media was cooled down to 50°C and 5% sterile defibrinated horse blood (MRC horse farm) was aseptically added, mixed and thereafter poured into sterile 9mm petri dishes. The plates were left to solidify at room temperature then stored at 4°C until use (Figure 2).

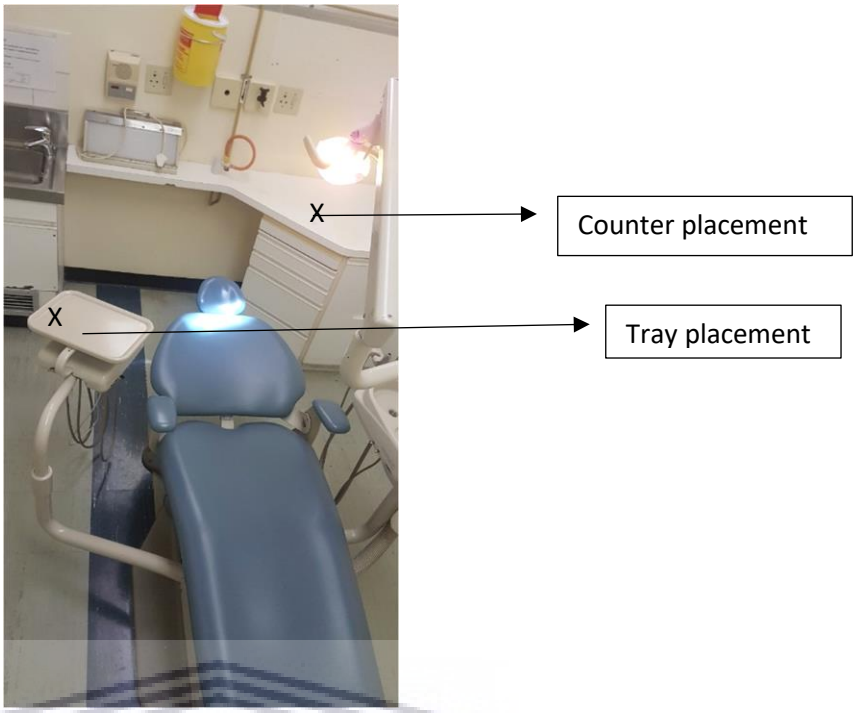

Figure 1. Location of the blood agar plates within the dental cubicle, i.e. counter and tray placements. A control plate was placed in the cubicle 1 hour prior to treatment, 1 meter from the floor and 1 meter from the cubicle wall.

A total of 99 BA plates and 19 control plates were used in line with the power calculation (Appendix 2).

For the control, the prepared blood agar (BA) plate was placed in the clinic one hour before commencement of passive sampling on each day of sampling. The Index of Microbial Air Contamination (IMA) was determined by exposure of opened petri dishes to air for the duration of 1 hour, placed 1m from the floor and 1m away from the walls (Pasquarella *et al*, 2000).

The operational BA plates were placed at two designated sites within the dental cubicle namely, the counter and the tray. The distance from the location of the sample plate placed on the tray to the operatory site was 40 cm and from the counter to the tray was 40 cm.

Before placement, the BA plates were labelled with the date, patient number and designated site (counter/tray). After completion of the operational sampling, the agar plates were collected

and incubated for 48 hours at 37ºC for quantitative and qualitative analysis in the microbiology culture laboratory within the department of Medical Biosciences.

3.6 Microbiological examination

3.6.1 Examination of blood agar plates for colony forming units (CFU)

Post incubation, the blood agar plates were inspected for bacterial growth. The colony morphology and the colony counts were recorded.

Colony counts were performed using the Gallenkamp20//CX-300 colony counter (Gallenkamp Co.Ltd.,UK).by placement of the agar plate on the lighted surface under the magnifying glass of the colony counter. A black felt tip pen was used to mark each colony as it was counted, to ensure that it was counted once only.

The morphology of each colony on the blood agar plate was recorded by describing its size, shape, border and texture. Colonies were subcultured onto Trypticase Soya agar plates (RMR0004, Merck Life Sciences GmbH, The Biovac Institute) in order to obtain pure cultures of single colonies for use in the identification and characterisation of the bacterial isolates.

3.6.2 Identification and characterisation of bacterial isolates

For purity of culture, Trypticase Soya (TSA) plates were used for subculturing from the BA plates. These TSA plates were labelled according to the date, batch number and isolate number and then incubated for 24-48 hours at 37°C. Post incubation, plates were inspected for purity and each isolate was subjected to Gram staining, catalase testing, VITEK® identification and susceptibility testing.

3.6.2.1 Microscopy

Each slide was labelled according to the corresponding isolate number as represented on the TSA agar plate. An inoculation loop was flamed, cooled and a drop of saline transferred to a labelled clean glass slide.

The inoculation loop was flamed again, cooled and a small amount of the isolate picked and mixed with the saline to produce an even smear on the slide. Smears were allowed to dry at a slant, then heat-fixed and allowed to cool for Gram staining.

The following standard method was used for the Gram staining.

1. The fixed smear was flooded with Gram's Crystal Violet Solution and left for 60 seconds.
2. Excess stain was gently removed by washing with a slow stream of tap water.
3. The iodine solution was applied for 60 seconds to bind the stain.
4. This was followed by rinsing gently with slow streaming tap water and alcohol.
5. Smears were counterstained with Safranin solution for 60 seconds before rinsing with a gentle stream of tap water.
6. The excess water was blotted with tissue paper and smears were allowed to air dry.

Smears were examined under 100X magnification using a (Zeiss standard 20 binocular microscope ,450807-9901,230V, 50 to 60Hz,45 Va Type B) and cell morphology recorded as being either Gram-positive or Gram-negative cocci/rods.

3.6.2.2 Catalase Test

The catalase test was performed to distinguish between streptococci and staphylococci by placing a saline suspension of the sub-cultured isolate on a clean glass slide. One drop of hydrogen peroxide was added and the results recorded as either positive or negative depending on the reaction of the hydrogen peroxide with the isolate suspension.

3.6.2.3 VITEK® identification and susceptibility testing

Strict laboratory disinfection protocol was followed by spraying and wiping the surfaces with 80% isopropyl alcohol. A 24-hour pure culture of each isolate on TSA was prepared as per the standard protocol for VITEK® Identification and susceptibility testing.

The VITEK® suspensions were prepared in a Biohazard safety cabinet (Class II Type A2). For each isolate, 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCL, pH 4.5 to 7.0) was dispensed into a 12x75mm clear plastic (polystyrene) test tube. A sterile swab or applicator stick was used to transfer a colony from the pure growth on the TSA plates into the saline-containing polystyrene tube, mixed and the turbidity adjusted using the DensiCHECK™ instrument (Figure 3). This is an instrument used in conjunction with the VITEK® 2, which measures the optical density of the bacterial suspension.

Figure 2. DensiCHECK™ instrument used in conjunction with the VITEK® 2 which measures the optical density of the bacterial suspension.

If the suspension indicated a higher DensiCHECK™ reading compared to the Mcfarland standard (Table 1), more saline was added. If the DensiCHECK™ reading was too low, more

of the isolate was added to the microbial suspension until the desired Mcfarland range was obtained (see Table 1).

Gram stain morphology dictated the use of the appropriate VITEK® card. There are currently four reagent cards available for the identification of different organism classes namely,

GN - Gram-negative fermenting and non-fermenting bacilli

GP - Gram-positive cocci and non-spore-forming bacilli

YST - yeasts and yeast-like organisms

BCL - Gram-positive spore-forming bacilli

Table 1. The turbidity range of suspensions used for the VITEK ® card inoculation

Product	McFarland Turbidity Range
Gram-negative fermenting and non-fermenting bacilli	0.50-0.63
Gram-positive cocci and non-spore-forming Bacilli	0.50-0.63
Yeasts and yeast-like organisms	1.80-2.20
Gram-positive spore-forming bacilli	1.80-2.20

Once the suspensions were completed for all the isolates with acceptable McFarland turbidity values, the VITEK® cards were inserted into the VITEK® cassette rack with the tube end immersed in the bacterial suspension. The cassette rack with the individual suspensions were placed into the VITEK® compact 2 unit and the cards filled according to standard protocol by depressing the "start fill" button on the interface. It took approximately 70 seconds for the cards to be filled which was indicated by the VITEK® compact 2 unit beeping when the filling cycle was completed. Once the cards were filled, the Load Door automatically unlocked and the cassette was placed in the Load Door. Care was taken to place the cassette inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected. The cards were sealed, straws were cut and the cards were loaded automatically into the carousel. The V2C l generated a beeping sound once all cards were loaded into the cassette and the

VITEK® compact 2unit automatically processed the cards. The cassette was removed and the tubes and straws were disposed of in a biohazard container.

For the identification and susceptibility of the isolates, VITEK®ID and VITEK® AST cards were used respectively.

There were 3 outcomes with respect to the analysis of the susceptibility testing, namely, susceptible (S), resistant (R) or intermediate (I). According to the European Society of Microbiology and Infectious Diseases, a microorganism is categorised as intermediate when there is a high likelihood of therapeutic success because of increased exposure to the agent by adjusting the dosing regimen or by its concentration at the site of infection (Gunnar, 2017).

3.7 Statistical analysis

IBM SPSS version 26 was used to analyze the data. A p value <0.05 indicated statistical significance.

Colony forming units were summarized using median and interquartile range due to their distribution being significantly non normal. Distributions of colony forming units were compared according to location, dental treatment, and student activity using non parametric Mann-Whitney test in the case of two independent samples (Mann & Whitney, 1947) and Kruskal-Wallis tests in the case of more than two independent samples (Kruskal & Wallace, 1952). Box and whisker plots were used to visualize the distributions by groups.

CHAPTER 4: RESULTS

The results of this study were both clinical and microbiological. The clinical aspect comprised of a qualitative and quantitative assessment with respect to colony counts in a dental setting during the basic dental treatment of 40 patients.

The microbiological aspect comprised of a qualitative and exploratory component with respect to the identification of the isolates and susceptibility testing. In total, 119 isolates were identified comprising 23 species, all of which underwent susceptibility testing using the VITEK® 2 compact system.

4.1 Calculation of the Index of Microbial Air Contamination

The number of plates for each location is demonstrated in Figure 3, with an equal number of plates placed on the counter and tray.

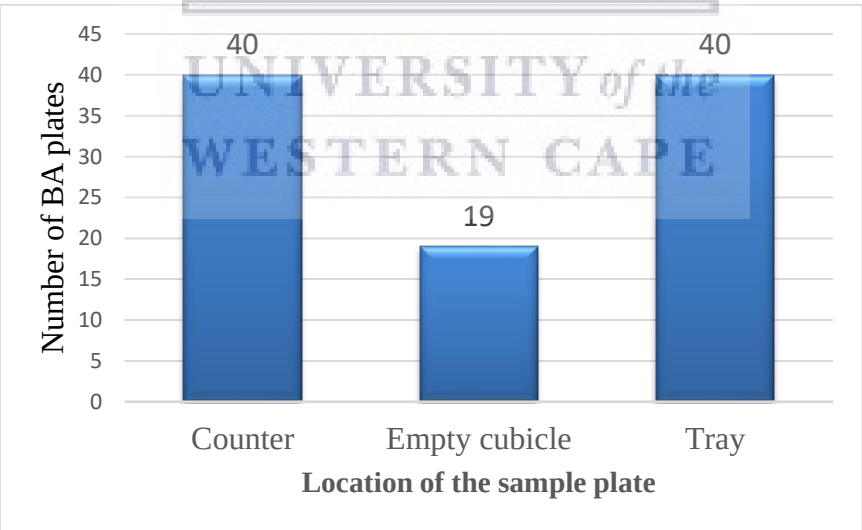

Figure 3. The number of BA plates and the location of the sample plates within the dental cubicle.

There was a median value of 5 in each of the locations (Table 2) and therefore no significant difference was observed between the distribution of colony counts when the control was compared with each of the counter and tray locations (p=0.881)

Table 2: A comparison between the baseline colony counts for the control, tray and counter placements.

		Colony counts on the sample plate		
		Median	Percentile 25	Percentile 75
Location of sample plate	Control n=19	5	3	8
	Counter n=40	5	3	8
	Tray n=40	5	3	9

Statistical significance was defined as $p < 0.05$.

Table 3 summarises the descriptive statistics for the location and number of test plates, the dental treatment administered at the time of plate exposure and their relative colony counts. The mean CFU/plate was higher during posterior restorations than during anterior restorations on the Tray plates.

The Kruskal-Wallis test compared the colony counts on plates exposed during different dental treatments and no significant differences were observed between the counter (p value = 0.360) and tray (p value = 0.475) placements (Table 3).

Table 3. A summary of the descriptive statistics for location, number of plates, dental treatment and colony counts.

Location	Number of plates	Dental treatment	CFU/plate count			
			Mean	Standard deviation	Median	P value (Kruskal- Wallis test) for comparison between dental treatments
Control	19	No treatment	5.6	3.7	5.0	N/A*
Counter	40					0.360
	4	Anterior restoration	4.3	3.3	4.0	
	9	Posterior restoration	4.4	4.0	4.0	
	27	Scale and polish	7.5	7.7	5.0	
	13	All restorations	4.4	3.6	4.0	
Tray	40					0.475
	4	Anterior restoration	4.3	1.0	4.5	
	9	Posterior restoration	5.0	3.5	5.0	
	27	Scale and polish	8.2	7.7	6.0	
	13	All restoration	4.8	2.9	5.0	

Statistical significance was defined as $p < 0.05$.

* N/A since no comparison groups were tested

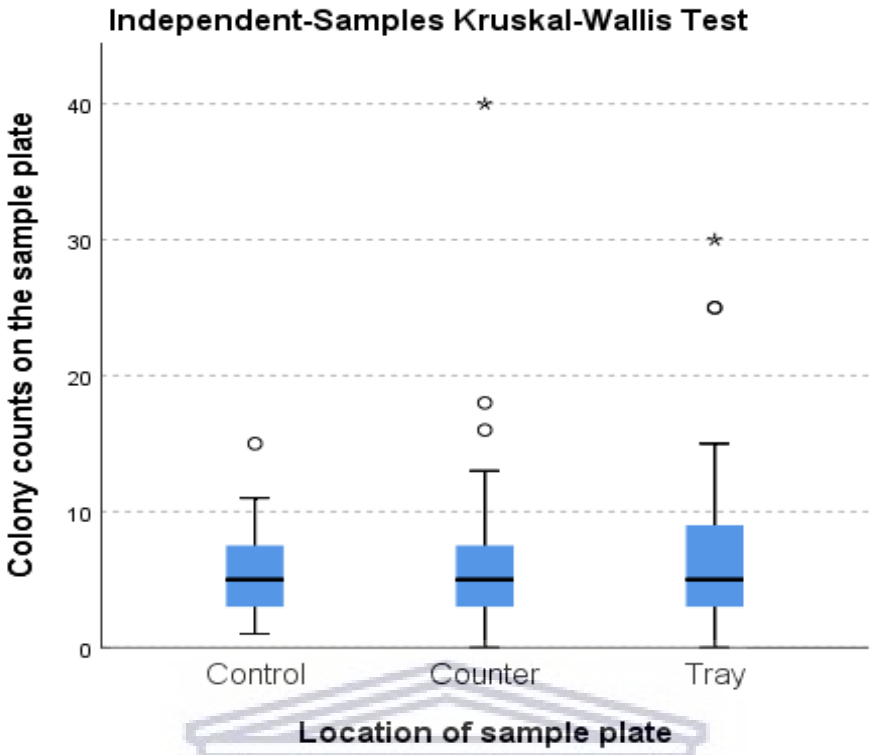

Figure 4. Box and Whisker plot of the distribution of colony counts by dental location of the sample plate. The boxes represent the interquartile range of the data while the line in the middle of the box is the median. The "whiskers" represent the range of the data while circles are outliers and asterisks are extreme points.

The distribution of the colonies on the tray were higher than the control and counter placements which looked similar (Figure 4). The Kruskal-Wallis test showed that this was not statistically significant (P=0.881)

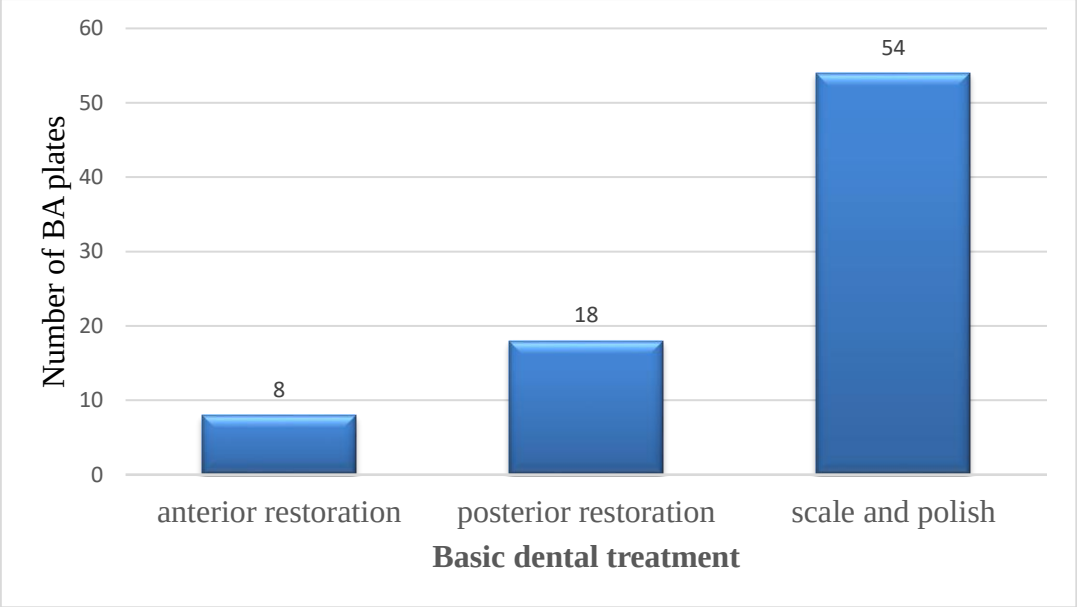

Figure 5. The total number of BA plates exposed (both counter and tray locations) during the different basic dental treatments

Figure 5 shows the total number of plates exposed during the different dental treatments. Scale and polish was the predominant treatment procedure and therefore had the highest plate count (54 CFU/plate), followed by posterior restorations and anterior restorations (26 CFU/plate).

The distribution of colony counts (CFU/ml) for the different plate placements during the different dental treatments was found to be the same for both the counter (Figure 6) and tray (Figure 7) BA placements, with no statistical significance observed between the colony counts of the counter ($p=0.360$) and tray ($p=0.475$) placements when exposure during different dental treatments were compared.

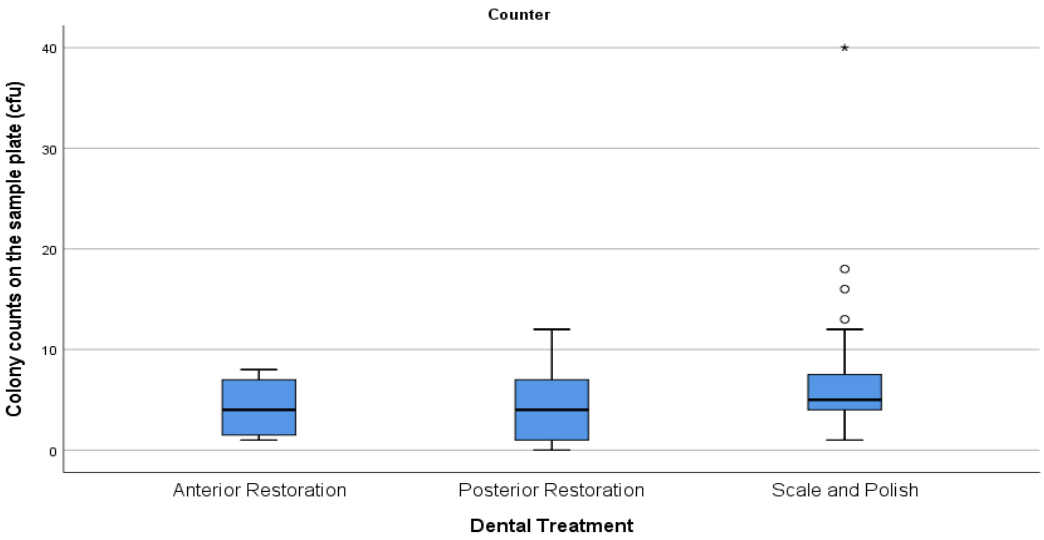

Figure 6. Box and Whisker plot of the distribution of colony counts by dental treatment in counter placements. The boxes represent the interquartile range of the data while the line in the middle of the box is the median. The "whiskers" represent the range of the data while circles are outliers and asterisks are extreme points.

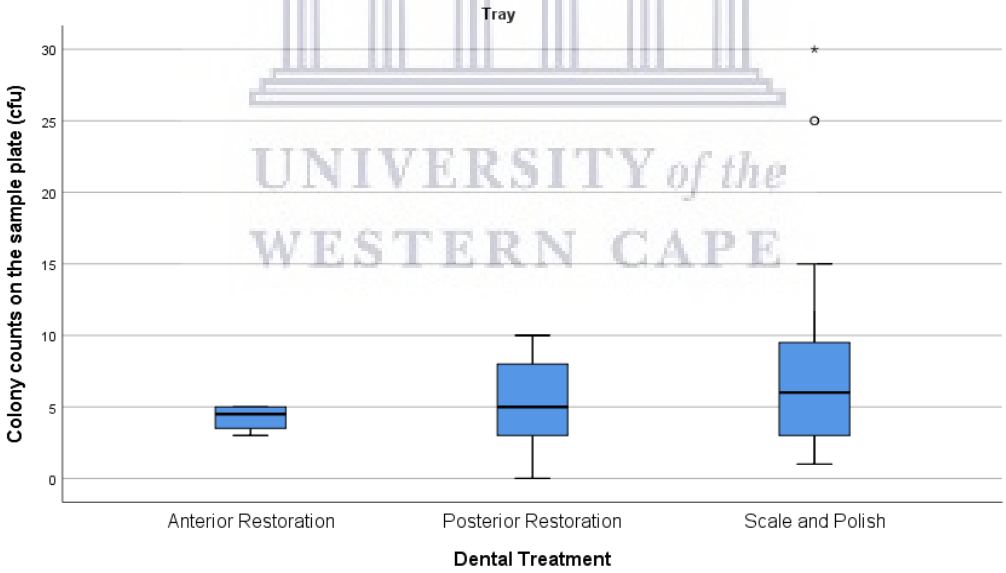

Figure 7. Box and Whisker plot of the distribution of colony counts by dental treatment in tray placements. The boxes represent the interquartile range of the data while the line in the middle of the box is the median. The "whiskers" represent the range of the data while circles are outliers and asterisks are extreme points.

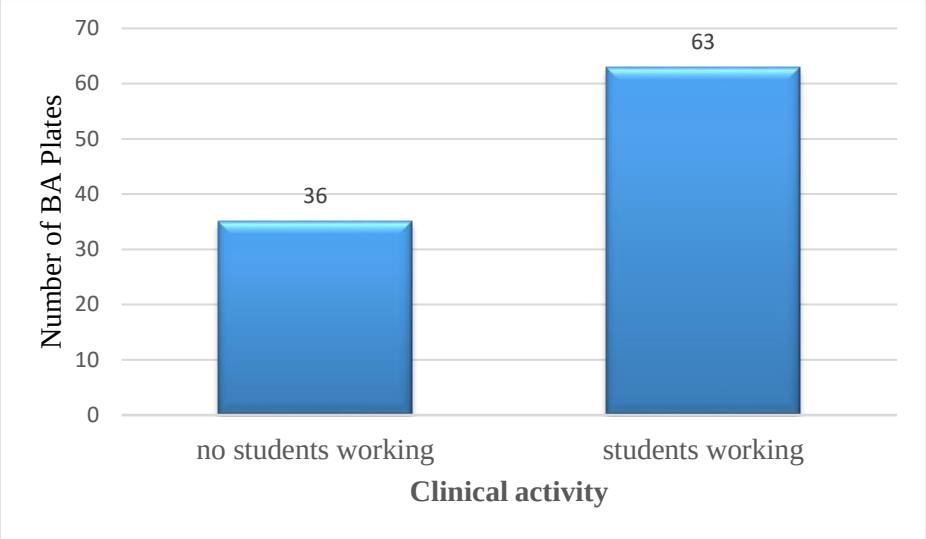

Figure 8. The number of BA plates and related student activity in the clinic during sampling

Since dentistry students receive their training in the clinic where the air contamination was being monitored, the number of plates exposed during student activity almost doubled during the period of sampling (Figure 8) resulting in a significant increase (p=0.002) in colony counts (Figure 9) as shown by the Mann-Whitney U-test.

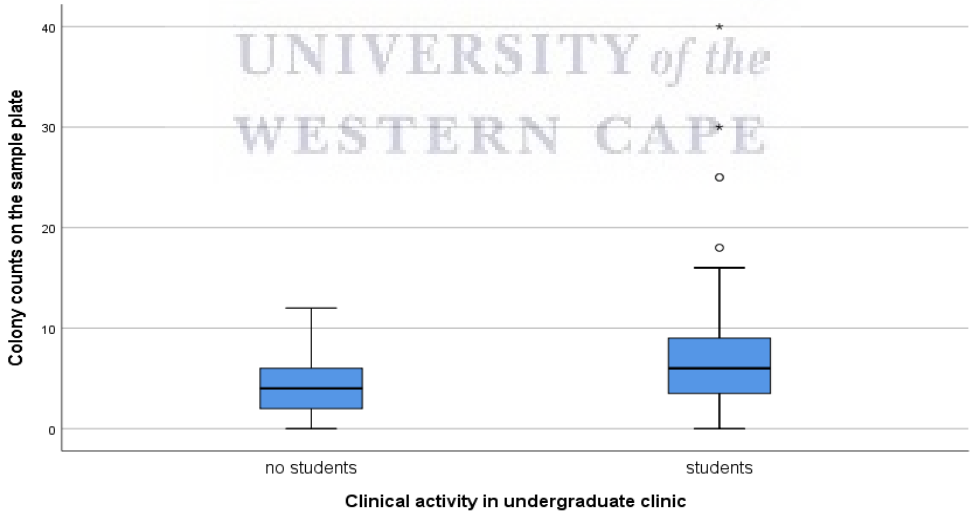

Figure 9. Box and Whisker plot of the distribution of colony counts and the student's clinical activity. The boxes represent the interquartile range of the data while the line in the middle of the box is the median. The "whiskers" represent the range of the data while circles are outliers and asterisks are extreme points.

4.2 Isolation and identification of colonies

For the identification of bacterial species, pure cultures were obtained, their colonial morphology and Gram stain reactions were recorded and species definition obtained using the VITEK® system. A total number of 119 pure isolates were obtained from the BA sample plates.

VITEK® identification cards were selected according to the Gram stain morphology of the isolates. Gram-positive cocci constituted 104 (87%) of the total number of isolates (n=119) of which 11(9.2 %) were Gram-positive bacilli, 1(0.8 %) was a Gram-negative bacillus and 3 (2.5 %) were Gram-negative cocci (Figure 10).

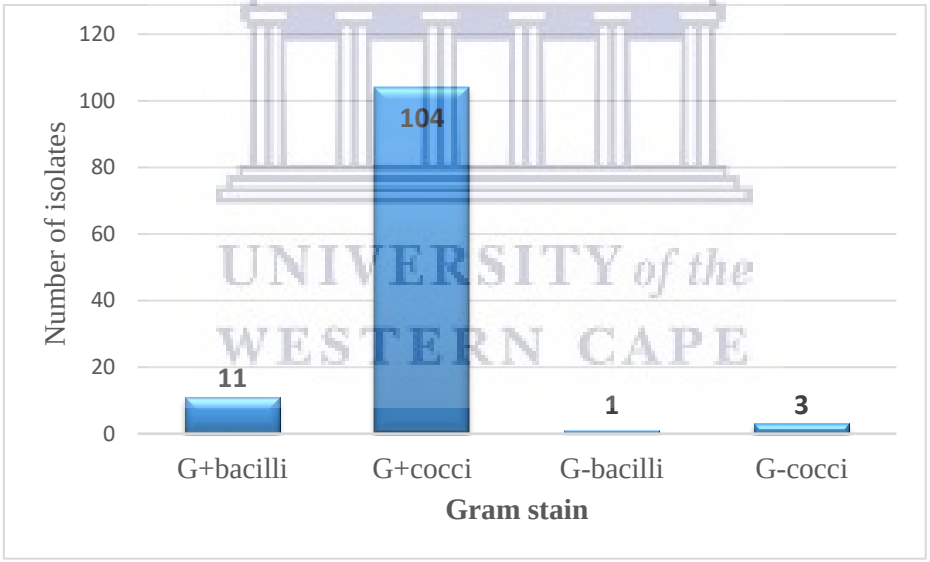

Figure 10. Distribution of Gram-positive and Gram-negative morphotypes isolated

Table 4. Frequency and percentage distribution of bacterial morphotypes according to dental procedures.

Gram stain	Dental treatment			
	Control n (%)	Restorations n (%)	Scale and Polish n (%)	Total n (%)
G-bacilli	0 (0.0)	0 (0.0%)	1 (1.5)	1 (0.8)
G-cocci	1 (4.17)	1 (3.4)	1 (1.5)	3 (2.5)
G+bacilli	4 (16.67)	0 (0.0)	7 (10.6)	11 (9.2)
G+cocci	19 (79.17)	28 (96.5)	57 (86.3)	104 (87.3)
Total	24 (100)	29(100)	66 (100)	119 (100)

Gram-positive cocci were the predominant isolates (Table 4) followed by Gram-positive bacilli, with the highest colony counts observed on plates following scaling and polishing.

Table 5. Frequency and percentage distribution of bacterial morphotypes according to the location of BA plate.

Gram stain	Location of the BA plate			
	Control n (%)	Counter n (%)	Tray n (%)	Total n (%)
G-bacilli	0 (0.0)	0 (0.0)	1 (1.8)	1 (0.85)
G-cocci	1 (4.2)	1 (2.5)	1 (1.8)	3 (2.5)
G+bacilli	4 (16.7)	0 (0.0)	7 (12.7)	11 (9.2)
G+cocci	19 (79.2)	39 (97.5)	46 (83.6)	104 (87.4)
Total	24 (100)	40 (100)	55 (100)	119 (100)

With regard to the location of sampling plates, Gram-positive cocci were the predominant isolates (Table 5) with the highest colony counts observed on the plates placed on the tray.

4.3 VITEK® identification of isolates

A VITEK® report as demonstrated in Figure 11 was generated for each isolate. The lab report showed different identification levels. These identification levels could be categorised as either excellent, very good, good, acceptable, low discrimination or unidentified organism.

bioMérieux Customer:
System #:

Laboratory Report

Printed Mar 28, 2019 11:49 CAT
Printed by: Rose

Isolate: BN45.2-1 (Approved)

Card Type: GP Bar Code: 2420801103459412 Testing Instrument: 000017227F7D (13707)
Setup Technologist: Rose Gwanpu(Rose)

Bionumber: 040032310000000
Organism Quantity:

Selected Organism: Micrococcus luteus

Comments:	

Identification Information	**Card:**	GP	**Lot Number:** 2420801103	**Expires:**	Feb 5, 2020 12:00 CAT
	Completed:	Mar 27, 2019 19:40 CAT	**Status:** Final	**Analysis Time:**	5.82 hours
Organism Origin	VITEK 2				
Selected Organism	98% Probability	**Micrococcus luteus**			
	Bionumber:	040032310000000		**Confidence:**	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	APPA	(-)	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	+	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-

Figure 11. An example of a VITEK® Lab report showing *Micrococcus luteus.*

The results showed that 90% of the isolates were successfully identified, 4.2 % of the isolates could not be identified and 5% had no or low reactive biopatterns. The number and distribution of species are listed in Table 6.

The predominant isolate was *Micrococcus luteus* (31%) followed by *Staphylococcus hominis* ssp *hominis (*8.4%) and *Kocuria rosea* (7.6%). Other Gram-positive cocci included the genera *Aerococcus, Alloiococcus, Dermacoccus, Gamella, Globicatella, Granulicatella, Lactococcus and Leuconostoc* (Table 6), while *Erysipelothrix and Rothia* were representative of the Gram-positive bacilli. The Gram-negative cocci and bacilli could not be identified by VITEK ®.

Each bacterial species could be traced to its original BA plate within the clinical location, the dental treatment performed as well as student clinical activity.

Micrococcus luteus remained the predominant isolate regardless of the location (30% for the counter and 30.9% for the tray), student activity (24.1% versus 36.9% for no student activity), dental treatment procedure (37.9% for scale and polish and 27.3% for restorations). A similar trend was observed for each identified isolate within Table 6.

Table 6: VITEK® identification and distribution of isolates

Bacterial species	N (%)	BA placements n (%)			Clinical activity n (%)		Dental treatment n (%)		
		Counter	Control	Tray	Students	No students	No	S/P	Res
Aerococcus viridans	2 (1.7)	2 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.1)	0 (0.0)	0 (0.0)	2 (3.0)
Alloiococcus otitis	3 (2.5)	0 (0.0)	1(4.2)	2 (3.6)	3 (5.6)	0 (0.0)	1 (4.2)	0 (0.0)	2 (3.0)
Dermacoccus nishinnomiyaensis	5 (4.2)	1 (2.5)	0 (0.0)	4 (7.2)	5 (9.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (7.6)
Erysipelothrix rhusiopathiae	1 (0.8)	0 (0.0)	0 (0.0)	1(1.8)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)
Gamella bergeri	1 (0.8)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	1 (1.5)	1 (4.2)	0 (0.0)	0 (0.0)
Globicatella sanguis	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (1.5)
Granulicatella adiacens	2 (1.7)	1 (2.5)	1 (4.2)	0 (0.0)	1 (1.9)	1 (1.5)	1 (4.2)	0 (0.0)	1 (1.5)
Granulicatella elegans	4 (3.4)	3 (7.5)	0 (0.0)	1 (1.8)	1 (1.9)	3 (4.6)	0 (0.0)	2 (6.9)	2 (3.0)
Kocura varians	1 (0.8)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	1 (3.5)	0 (0.0)
Kocuria kristinae	2 (1.7)	1 (2.5)	1 (4.2)	0 (0.0)	1 (1.9)	1 (1.5)	1 (4.2)	0 (0.0)	1 (1.5)
Kocuria palustris	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)
Kocuria rhizophila	6 (5.0)	1 (2.5)	1 (4.2)	4 (7.2)	3 (5.6)	3 (4.6)	1 (4.2)	0 (0.0)	5 (7.6)
Kocuria rosea	9 (7.6)	4 (10.0)	1 (4.2)	4 (7.2)	7 (13.0)	2 (3.1)	1 (4.2)	2 (6.9)	6 (9.1)
Lactococcus lactis	1 (0.8)	1 (2.5)	0 (0.0)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)
Leuconostoc mesenteroides ssp cremoris	3 (2.5)	1 (2.5)	0 (0.0)	2 (3.6)	0 (0.0)	3 (4.6)	0 (0.0)	3 (10.3)	0 (0.0)
Leuconostoc pseudomesenteroides	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	1 (3.5)	0 (0.0)
Low reactive biiopattern	3 (2.5)	1 (2.5)	1 (4.2	1 (1.8)	2 (3.7)	1 (1.5)	1 (4.2)	1 (3.5)	1 (1.5)
Micrococccus luteus	37 (31.1)	12 (30.0)	8 (33.3)	17 (30.9)	13 (24.1)	24 (36.9)	8 (33.3)	11 (37.9)	18 (27.3)
Micrococcus lylae	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (1.5)

Non or Low reactive biiopattern	3 (2.5)	0 (0.0)	1 (4.2)	2 (3.6)	3 (5.6)	0 (0.0)	1 (4.2)	0 (0.0)	2 (3.0)
Rothia dentocariosa	4 (3.4)	2 (5.0)	0 (0.0)	2 (3.6)	2 (3.7)	2 (3.1)	0 (0.0)	0 (0.0)	4 (6.1)
Staphylococccus cohnii ssp cohnii	4 (3.4)	1 (2.5)	2 (8.3)	1 (1.8)	4 (7.4)	0 (0.0)	2 (8.3)	0 (0.0)	2 (3.0)
Staphylococcus auricularis	1 (0.8)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	1 (3.5)	0 (0.0)
Staphylococcus capitis	3 (2.5)	3 (7.5)	0 (0.0)	0 (0.0)	2 (3.7)	1 (1.5)	0 (0.0)	1 (3.5)	2 (3.0)
Staphylococcus cohnii ssp urealyticus	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (1.5)
Staphylococcus epidermidis	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0(0.0)	0 (0.0)	1 (1.5)
Staphylococcus hominis ssp hominis	10 (8.4)	3 (7.5)	3 (12.5)	4 (7.2)	3 (5.6)	7 (10.8)	3 (12.5)	5 (17.2)	2 (3.0)
Staphylococcus saprophyticus	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	1 (3.5)	0 (0.0)
Streptococcus mitis/oralis	1 (0.8)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (1.5)
Streptococcus salivarius ssp salivarius	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (1.5)
Unidentified organism	5(4.2)	0 (0.0)	3 (12.5)	2 (3.6)	1 (1.9)	4 (6.2)	3 (12.5)	0 (0.0)	2 (3.0)
Total	119 (100)	40 (100)	24 (100)	55 (100)	54 (100)	65 (100)	24 (100)	29 (100)	66 (100)

4.4 VITEK® Susceptibility testing

Susceptibility testing was conducted on isolates which met the criteria listed in the guidelines of the *bioMérieux* instructional sheet (Ref 421040) for the VITEK® AST card, namely, Enterobacteriaceae, Non-fermenters, staphylococcci, enterococci, streptococci (including *S.pnuemoniae, S.viridans* and *beta-hemolytic* streptococci) and yeasts (Appendix 1).

In Table 6, antibiotic susceptibility patterns are presented in columns indicating S (susceptibility), R (resistance) or I (intermediate). Where only a column for R is shown, it indicates that none of the species were susceptible to that particular antibiotic. Similarly, if only a column for S is shown, it can be assumed that no resistance was found to that particular antibiotic.

The two strains of *Alloiococcus otitis* showed susceptibility to all the antibiotics tested and resistance to Fosfomycin and Fusidic acid (Table 7).

Leuconostoc mesenteroides ssp cremoris showed susceptibility to all the antibiotics and resistance to Erythromycin.

All four strains of *Staphylococccus cohnii ssp cohnii* showed susceptibility to the following antibiotics Gentamycin, Ciprofloxacin, Tigecycline, Trimethoprim/Sulfamethoxazole, two strains showed resistance to Oxacillin, one strain was resistant to Erythromycin, Clindamycin, Linezolid, Daptomycin, Teicoplanin, Vancomycin, Tetracycline, Fosfomycin, Fusidic acid and Rifampicin. Three strains exhibited resistance to Fusidic acid. Only one intermediate result was shown with Rifampicin. *Staphylococcus capitis* showed susceptibility to all the antibiotics and resistance to Fosfomycin. *Staphylococcus cohnii ssp urealyticus* showed susceptibility to most of the antibiotics and resistance to Oxacillin and Fusidic acid. An intermediate result was shown for Clindamycin and Tetracycline. *Staphylococcus epidermidis* showed resistance to

Oxacillin, Erythromycin, Clindamycin, Tetracycline and Trimethoprim/Sulfamethoxazole and susceptibility to the rest of the antibiotics. All ten strains of *Staphylococcus hominis ssp hominis* showed susceptibility to Gentamycin, Ciprofloxicin, Clindamycin, Linezolid, Teicoplanin, Vancomycin, Tigecycline, Rifampicin and Trimethoprim/Sulfamethoxazole. Resistance was shown by five strains to Fosfomycin and three strains to Erythromycin. One strain showed resistance to Oxacillin, Daptomycin, Tetracycline and Fusidic acid. *Staphylococcus saprophyticus* showed resistance to all the antibiotics except for Erythromycin, Fusidic acid and Fosfomycin.

Both *Streptococcus mitis/oralis* and *Streptococcus salivarius ssp salivarius* showed susceptibility to Vancomycin, Ampicillin, Penicillin and Clindamycin.

Table 7: VITEK® susceptibility profiles of isolates

Species (n=23)	Ox		Ge	Ci	Er		Cl			Li		Da		Te		Va		Tet			Ti	Fo		FA		Rf			TS	Am	P	Cl2
	R	S	S	S	R	S	I	R	S	R	S	R	S	R	S	R	S	I	R	S	S	R	S	R	S	I	R	S	R	S	S	S
A. otitis (2)	0	2	2	2	0	2	0	0	2	0	2	0	2	0	2	0	2	0	0	2	2	2	0	2	0	0	0	2	0	2	0	0
L. mesenteroides (1)	0	1	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	0
S. cohnii (4)	2	2	4	4	1	3	0	1	3	1	3	1	3	1	3	1	3	0	1	3	4	1	3	3	1	1	1	2	0	4	0	0
S. capitis (1)	0	1	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	1	0	0	1	0	0	1	0	1	0	0
S. cohnii ssp urealyticus (1)	1	0	1	1	0	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	0	1	0	1	0	0
S. epidermidis (1)	1	0	1	1	1	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0
S. hominis (10)	1	9	10	10	3	7	0	0	10	0	1 0	1	9	0	1 0	0	1 0	0	1	9	10	5	5	1	9	0	0	10	0	10	0	0
S. saprophyticus (1)	0	1	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	1	0	1	0	0	0	1	0	1	0	0
S. mitis/oralis (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
S. salivarius ssp salivarius (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

Ox=Oxacillin, Ge=Gentamicin, Ci=Ciprofloxacin, Er=Erythromycin, Cl=Clindamycin, Li=Linezolid, Da=Daptomycin, Te=Teicoplanin, Va=Vancomycin, Tet=etracycline, Ti=Tigecycline, Fo=Fosfomycin , FA=Fusidic acid, Rf=Rifampicin, TS=Trimethoprim/Sulfamethoxazole, Am=Ampicillin, P=Penicillin, Cl2=Clindamycin2.

CHAPTER 5: DISCUSSION

Within the current climate of Covid-19, the opinion of the author, Monaghan (2016) has truly manifested. He predicted that based on past experience, a respiratory infection would emerge with the potential for rapid global spread and resultant loss of life (Monaghan, 2016). He advocated that dental surveillance was imperative for emerging viral and bacterial infections and since viral infections spread through droplets and aerosols, this places dentistry at a high risk for the transmission of these infections (Monaghan, 2016).

The rapid spread of SARS-CoV-2 and the high death toll globally, has created an awareness of infection control protocols and an upsurge of research articles (predominantly from China where the spread of the infection originated) on the topic of dental aerosol, and more so, the implications for dentistry (Alharbi *et al*., 2020; Ather *et al*., 2020; Ge *et al*., 2020; Meng *et al*., 2020; Peng *et al*., 2020 and Singh *et al*, 2020).

The passive method for measurement of IMA is a reliable and cost effective tool for quantifying and monitoring the settlement of microbes from the air onto exposed Petri dishes and its application extends from hospitals and food industries to public open spaces (Pasquarella *et al* 2000).

In the present study the baseline colony counts for the control did not significantly differ from the tray and counter placements (p value = 0.881). This shows that the use of the turbines and scaler did not significantly affect the colony counts. This finding slightly differed from the study by Manarte-Monteiro *et al*., (2013), where the IMA value in the dental clinic was significantly lower before endodontic treatment and restorations. The median values were 3.4 CFU/dm^2/h compared to clinical attendance with a baseline median value of 10.4 CFU/dm2/h. The only difference between the former and current study was that 12 settling plates were

positioned in 3 random selected dental units as the control group for the Manarte-Monteiro *et* al. (2013) study, while the present study had a total number of 19 control plates placed in one cubicle for 1 hour before treatment commenced for each patient, with much higher colony counts for the tray placements compared to the counter placements.

The results from the present study demonstrated that scaling and polishing generated higher colony counts than anterior and posterior restorations. This is in agreement with previous studies (Bennet *et al.*, 2000; Hallier *et al*., 2010 Swaminthan and Thomas, 2013; Singh, *et al*., 2016; Peng *et al*., 2019; Alharbi *et al*., 2020; Ge *et al*., 2020) which reported that scaling and polishing generate the most aerosol, followed by the use of high-speed handpieces for cavity preparation.

The mean CFU/plate was higher for the posterior restorations as compared to the anterior restorations on the tray plates. This may probably be explained by the fact that more time was needed on the posterior restoration due to the complexity of the cavity depth preparation. At the time of writing, there were no comparable studies to support or negate these findings.

Even though the potential risk of dental aerosol to dental personnel and patients has been pursued in recent studies (Raghunath *et al*., 2016; Singh *et al*., 2016; Kobza *et al*., 2018; Peng *et al*., 2019), only a few studies have reported on the settling distance of the dental aerosol from the operatory site for infection control protocol purposes (Timmerman *et al.*, 2004; Rautemaa *et al.*, 2006; Manarte-Monteiro *et al*., 2013; Veena *et al.*, 2015, Zemouri *et al*., 2020). High Colony forming units (CFU) /plate were reported by different researchers for different distances including 0.5m with a mean value of 16.6 (SD 10.4) CFU/plate compared with 2m for dental procedures with a mean value of 13.6 (SD 6.9) CFU/plate (Manarte-Monteiro *et al*., 2013), while distances of 40 cm and <1m from the operatory site were reported by Timmerman *et al*., (2004) and Rautemaa *et al*., (2006) respectively. The colony count as reported by

Timmerman *et al*., (2004) was 8.0 CFU when high volume suction was used and 17.0 CFU's when conventional suctioning was used for scaling and polishing procedures only at 1.5 m. The colony forming units at a distance of 40 cm for scaling and polishing only was 8.1 CFU for high volume suctioning and 10.3 CFU with conventional suctioning. Rautemaa *et al*., (2006) reported a mean density of 823 CFU/m^2 / h less than 1m and a mean density of 1120 CFU/m^2 / h for a distance from the operatory site of more than 1.5 m.

Although Sawhney *et al*., (2015) used 3 standardized positions for placement of the agar plates namely, the chest of the patient, the dental tray and 15.24 cm from the operatory site, nothing was reported with respect to the settling distance of the dental aerosol. Instead, the study concluded that the use of 0.2% Chlorhexidine, compared to Listerine and water, was the most effective in reducing aerosol contamination with the use of high evacuation suction. A more recent study reported that aerosol contamination from the operatory site settled in close proximity to the head of the patient (Zemouri *et al*., 2020).

In the present study, passive sampling and VITEK® technology allowed for the identification and susceptibility testing of 119 species of which 87% were Gram-positive cocci. The motivation for the use of VITEK® technology for the lab analysis was twofold, firstly, to establish a protocol for routine passive sampling of dental aerosol and identification of isolates for infection control and secondly, for collaborative strategies between the Faculty of Dentistry and the Department of Medical Biosciences in the University of the Western Cape.

VITEK® technology was reported to be a useful, rapid, reliable and credible instrument for the identification of bacterial isolates (Ligozzi *et al.*, 2002; Spanu *et al*., 2003; Wallet *et al*., 2005; Nakasone *et al*., 2007; Da Silva Paim *et al*., 2014; Levesque *et al*., 2015). The advantages of the VITEK system include the speed and decreased turnaround time of about 20 minutes for the accurate identification of 10 strains through a high degree of automation (Funke *et al*.,

1998). The one disadvantage is the limited database consisting of only ordinary routine clinical taxa and is thus not equipped to identify many of the recent taxa (Bourbeau and Heiter, 1998; Funke *et al.*, 1998; Ling *et al.*, 2003; Spanu *et al*., 2003), thus requiring supplemental tests for the identification of less common microorganisms (Funke *et al*., 1998, Levesque et al., 2015).

Other than the present study, applications of the use of VITEK for the characterisation of oral microbes included studies of the prevalence of *Candida albicans* and *Candida dubliniensis* in caries free and caries active children (Al-Ahmad *et al.,* 2016); antimicrobial susceptibility profiles of isolates from acute dento-alveolar infections (Smith , 2017) and the bacterial profile of dental plaque in children with Down syndrome (Cakolli *et al.*, (2019).

In the present study, Gram-positive cocci were the predominant isolates regardless of the placement of the Petri dishes, clinical activity or dental treatments. Gram-positive cocci were also the predominant bacteria among the isolates reported in previous studies (Manarte-Monteiro *et al.,* 2013; Zemouri *et al*., 2017 and Anjum *et al*., 2020), and as in the present study, the Gram-positive cocci included genera such as *Micrococcus, Staphylococcus, Streptococcus,* as well as Gram-positive bacilli such as *Actinomyces, Bacillus* and *Corynebacterium* (Manarte-Monteiro *et al.,* 2013; Zemouri et al., 2017; Anjum *et al*., 2020; Jain *et al*., 2020; Zemouri *et al*., 2020).

In the present study, the bacterial composition of this particular sample comprised of a heterogeneous mixture of isolates from the environment, skin and oral cavity and although in small numbers, identified several less reported species, many of which appear to be developing resistance to commonly used antibiotics.

The predominant isolate was *Micrococcus luteus* which, although of low virulence, has the potential to become pathogenic in patients with impaired resistance, colonizing the surface of heart valves (Militiadous and Elisaf, 2011). This is of significance in dentistry where bacterial

endocarditis may be a sequela to dental treatment and in this study, *Micrococcus* way outnumbered *Streptococcus* which is considered to be one of the main causes of bacterial endocarditis (Toroglu *et al.*, 2001).

Several species of coagulase-negative staphylococci were identified in this study, all of which occur very commonly as harmless commensals on human and animal skin and have been implicated in nosocomially acquired bacterial endocarditis (Chu *et al*., 2004). Other Gram-positive cocci which are rarely considered pathogenic, but which have been reported to cause endocarditis as a result of nosocomial infection include *Granulicatella,* which is considered to be under reported (Cargill *et al.*, 2012), *Lactococcus lactis,* which produces a surface glycoprotein allowing it to attach to heart tissue (Freires *et al.*, 2017) and *Gamella* species. (Ural *et al*., 2014). *Leuconostoc* nosocomial outbreaks (Bou *et al*., 2008) as well as outbreaks due to *Micrococcus luteus* (Militiadous and Elisaf, 2011) have been more frequently reported.

From previous reports (Szymanska and Dutkiewicz, 2008; Hallier *et al*., 2010; Adhikari *et al*., 2017; Kobza *et al*., 2018; Anjum *et al*., 2020; Jain *et al*., 2020; Zemouri *et al*., 2020) and also from the findings in the present study, it is evident that there is a variation in the bacterial composition within a dental setting when dental aerosol is generated.

Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella spp, Enterococcus spp, Escherichia coli and coagulase-negative staphylococci are among the most common antibiotic resistant, nosocomially acquired pathogens medically (Jenkins, 2017; MacGowan and Macnaughton, 2017) as well as dentally (Zawadzki *et a*l., 2017; Lasserre *et al*., 2018). The body of evidence suggests that these bacteria are opportunistic, especially in this era where antibiotics are either overused or misused.

There is a paucity of publications with regard to the potential risk for nosocomial infection within the dental setting (Kurita *et al.*, 2006; Laheij *et a*l., 2012; McCormack *et al*., 2015) as

compared to the medical setting (Khan *et al.,* 2017) with very few studies conducted in Africa (Bayingana *et al.,* 2017). Kurita, *et al* (2006) raised awareness that the dental operatory clinical room may be a potential reservoir for methicillin resistant *Staphylococcus aureus* (MRSA), the spread of which was believed to be from the hands and gloves of the medical staff since the anti-biograms showed the same strain present in the infected patients as that found on the surfaces of the operatory room. However, only a few documented cases of MRSA transmission in the dental setting between staff and dental students have been reported (Laheij *et al*., 2012) although dental clinics were considered to be possible reservoirs of MRSA following the observation that greater contamination of surfaces with MRSA colonies occurred after patients were treated with paper dental records showing the most contamination (Faden, 2018).

However, *Staphylococcus aureus* was not detected in the present study. *Klebsiella pneumoniae* was the other nosocomial threat in health care facilities, but little is known about nosocomial transmission in dentistry even though these can be part of the oral microflora as a result of abscesses (Laheij *et al*., 2012).

Of all the Gram-positive cocci, the coagulase negative staphylococci demonstrated the greatest antimicrobial resistance with several species resistant to oxacillin, erythromycin, tetracycline, clindamycin, vancomycin, fucidin and fosfomycin. Such antimicrobial resistance has been reported previously (Chabi and Momtaz, 2019).

Adequate infection control procedures and protocols within dental facilities (private practice, private dental hospital clinics, government dental clinics and dental schools) are important as contaminated air consisting of particles from saliva, blood, dental plaque, calculus, tooth debris and restorative materials may be an important potential source of infection (Jain *et al*., 2020) and pose an occupational hazard for dental health care workers as well as their patients (Jain *et al*., 2020).

In the dental setting, infection control measures such as hand hygiene, disinfection of waterlines and surfaces, rubber dam usage, pre-procedural mouth rinses, the wearing of medical masks, eye protection and clinical coats/scrubs have always been standardised protocol to reduce the spread of bacterial, fungal and viral infections. This is of particular importance when aerosol generating procedures (AGP) are performed such as restorative procedures involving the high-speed handpieces and scaling and polishing, involving the usage of the ultrasonic scaler. The global pandemic has brought to light the urgency and importance of the review of, and compliance with, preventive measures to reduce the risk of the spread of all micro-organisms (CDC, 2020), not just viruses, in dental aerosols. The appropriate personal protective equipment (PPE) for aerosol generating procedures such as the N95 masks, eye-ware and aprons have been recommended (Abramovitz *et al*., 2020; Diegritz *et al*., 2020; Gupta *et al*., 2020). The Oral health Centre, Faculty of Dentistry at the University of the Western Cape, has also compiled a clinical protocol adapted from the infection control measures from the Western Cape Government, Department of Health. (Covid19 facility protocol, 2020)

CHAPTER 6: CONCLUSION

The results of the present study demonstrate that regular microbiological testing is plausible through collaboration between the faculties of dentistry and health sciences. It also demonstrates that VITEK® technology can be used as a reliable instrument in the identification of bacteria. This study is one of very few to use VITEK® for the laboratory analysis in an IMA assay in the dental clinic and the use of this automated technology within dentistry could generate useful data for research as well as pave the way for regular microbial reporting and adaptation of infection clinical protocols unique to the dental setting.

Many viruses, bacteria and fungi may be transmitted via aerosols, however, only a few studies have reported on bacteria within the dental setting, (Laheij *et al*, 2012; Manarte-Monteiro *et al*., 2013; Coelho *et al*., 2016; Singh *et al*., 2016; Zemouri *et al*., 2017; Barba *et al*., 2019) especially in dental schools (Coelho *et al*, 2016). This thesis is therefore one of few studies to report on the clinical and microbiological findings with respect to bacterial air contamination within a high risk area such as a training university dental unit/cubicle setting.

A scoping review of the literature (up until March 2016) published by Zemouri *et al.*, (2017) reported only 19 bacterial species within the dental setting, (three of which were Gram-negative and 16 Gram-positive). Other than that, not much has been reported with respect to the association between the qualitative analysis of Gram-positive and Gram-negative bacteria and dental activity within a dental university clinic and /nor the comparison of dental procedures such as scale and polishing and cavity preparation of restorations. This is therefore a strength of the present study.

Haque *et al.*, (2019*)* has reported on the microbial resistance from microbes isolated from the oral cavity yet little is known about microbial resistance from microbes originating from dental aerosol. This is another strength of the present study.

All of the isolates from the settle plates constituted commensal bacteria from the oral cavity and skin, and although some species were reported in other studies to have been associated with nosocomial bacterial endocarditis, these species were very few in number in the present study. Furthermore, no overt pathogens associated with antibiotic resistant nosocomial infection were isolated.

The use of settle plates for the passive assessment of the IMA provided valuable information regarding the variables which can affect the range of microbial air contamination. Passive sampling has an advantage over active air sampling in that while active sampling measures the concentrations of microorganisms present, passive sampling measures the airborne risk of aerosols and droplets on critical surfaces which may serve as reservoirs of cross infection.

In addition, this study demonstrated a favourable IMA standard in the UWC dental clinic which is an environment at risk for airborne infection/contamination due to aerosols. This indicates adequate infection control practices with a reduced risk of nosocomial infection. According to the established levels of IMA contamination proposed, the IMA could be classified as very good (0-5 CFU/plate), good (6-25 CFU/plate), fair (26-50 CFU/plate), poor (51-75 CFU/plate) and very poor (>76 CFU/plate) with maximum acceptable values for very high, high and medium risk areas designated at 5, 25 and 50 CFU/plate respectively (Pasquarella *et al*, 2000). The results from the present study averaged between very good (0-5 CFU/plate) and good (6-25 CFU/plate) thereby showing that the risk of contamination within the dental clinics at the University of the Western Cape is adequately controlled.

This study is further strengthened by the fact that not much has been published in the literature concerning the number of people (dentist, dental assistant and patient) within a dental training hospital setting within a specific time of IMA sampling and thus there are no comparable studies which include all of these variables.

Patients who were treated during the collection of these samples to determine whether or not they may have acquired a nosocomial infection needs further research as this was not one of the objectives of this study.

A limitation of the study was the failure of the VITEK system to characterise all of the isolates. The predominating species were however identified and their antimicrobial profiles determined.

The Null hypothesis states that dental aerosols do not contain antimicrobial-resistant bacterial species implicated in nosocomial infections. This study negates the Null hypothesis and supports the alternative hypothesis in that it demonstrates that aerosols within a dental clinical setting do indeed harbour antimicrobial-resistant bacteria previously implicated in nosocomial infections and could therefore be a likely source of transmission. Dental clinics should therefore be monitored and regular routine microbial surveillance should be undertaken to protect all dental personnel and patients.

REFERENCES

Abramovitz, I., Palmon, A., Levy, D., Karabucak, B., Kot-Limon, N., Shay, B., Kolokythas, A and Almoznino, G. 2020. Dental care during the coronavirus disease 2019 (COVID-19) outbreak: operatory considerations and clinical aspects, *Quintesssence International Journal*, 51(5), 418-429.

Adhikari, A., Kurellab, S., Banerjeec, P and Mitrad, A., 2017. Aerolized bacteria and microbial activity in dental clinics during cleaning procedures. *Journal of Aerosol Science*, 114, 209-218.

Al-Ahmad, A., Auschill, T.M., Dakhel, R., Wittmer, A., Pelz, K., Heumann, C., Hellwig, E and Arweiler, N.B. 2016. Prevalence of Candida albicans and Candida dubliniensis in caries-free and caries-active children in relation to the oralmicrobiota—a clinical study, *Journal of Clinical Oral Investigaion*, 20, 1963-1971.

Alharbi, A., Alharbi, S and Alqaidi, S. 2020. Guidelines for dental care provision during the COVID-19 pandemic. *Saudi Dental Journal*, 32(4), 181-186.

Anjum, A., Hosein, M., Butt, S.A., Shafiq, F and Shafiq, Y. 2020. Qualitative Analysis of Bacterial Aerosols Generated during Ultrasonic Dental Scaling. *Journal of Advances in Medicine and Medical Research*, 31(11), 1-12.

Ather, A., Patel, B., Ruparel, N.B., Diogenes, A., and Hargreaves, K.M. 2020. Coronavirus Disease 19 (Covid-19): Implications for Clinical Dental Care, *Journal of Endodontics.*46, 584-595.

Barba, M.B., García, J.M., De los Ángeles Martínez Cárdenas, M., Umegido, G. C.,

Nava, E.G., Galván, M.J.H., Márquez, J.L.P and Mendoza, D.S. 2019. Bacteriological analysis of high-speed hand pieces used in clinical practice. *Journal of* Revista *de la Asociacion Dental Mexiacana*, 76 (5), 261-266.

Bayingana, C., Gahutu, J.B., Sendegeya, A, Habarugira, F., Mukumpunga, C., Lyumugabes, F and Ndoli, J. 2017. Prevalence of bacterial species involved in nosocomial infections in paediatrics unit at Butare university teaching hospital (Chub). *International journal of current microbiology and applied sciences*, 6 (6), 3227-3232.

Bennet, A.M., Fulford, M.R., Walker, J.T., Bradshaw, D.J., Martin, M.V and Marsh, P.D. 2000. Microbial aerosols in general dental practice. *British dental journal*, 189 (12), 664-667.

Bou, G., Saleta, J.L., Sáez Nieto, J.A., Tomás, M., Valdezate, S., Sousa, D., Lueiro,F., Villanueva, R., Pereira, M.J., and Llinares, P. 2008. Nosocomial Outbreaks Caused by Leuconostoc Mesenteroides subsp. Mesenteroides. *Journal of Emerging Infectious Diseases*, 4(6), 968-971.

Bourbeau, PP and Heiter BJ. 1998. Comparison of Vitek GNI and GNI + Cards for identification of Gram –Negative Bacteria. *Journal of Clinical Microbiology*, 36(9), 2775-2777.

Cakolli, V.H., Mucaz, S., Benedetti, A and Hoxa, V.H. 2019. Detection of Bacteria in the Dental Plaque in Children with Down syndrome. *Journal of International Dental and Medical Research*, 12(4), 1474-1480.

Cargill, J.S., Scott, K.S., Gascoyne-Binzi, D and Sandoe, J.A.T. 2012. Granulicatella infection: diagnosis and management, *Journal of Medical Microbiology*, 61(6) 755–761.

Centres for Disease Control and Prevention. Campaign to Prevent Antimicrobial Resistance in Healthcare Settings: Why a Campaign? Atlanta, GA: Centers for Disease Control and Prevention; 2001. Available at www.cdc.gov/drugresistance/healthcare/problem.htm

Centers for Disease Control and Prevention. National Nosocomial Infection Surveillance (NNIS) System report: data summary from January 1992-June 2001. Am J Infect Control 2001b; 29:404-421

Centres for Disease Control and Prevention. HAI data and statistics. Healthcare-associated infections. Atlanta, Georgia: CDC; 2016. [Online] Available from:http://www.cdc.gov/HAI/surveillance/index.html[Accessed on 10 August 2016.

Centers for Disease Control and Prevention. Infection control: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Available at: https://www.cdc.gov/coronavirus/2019-ncov/infection-control/control-recommendations. html. Accessed on 9 March 2020.

Chabi, R and Momtaz, H.2019. Virulence factors and antibiotic resistance properties of the *Staphylococcus epidermidis* strains isolated from hospital infections in Ahvaz, Iran. Journal of *Tropical Medical Health*, 47- 56.

Chu, V.H., Cabell, C.H., Abrutyn, E., Corey, G.R., Hoen, B., Miro, J.M., Olaison, L., Stryjewski, M.E., Pappas. P., Anstrom, K.J., Eykyn, S., Habib, G., Benito, N., Fowler, V.G Jr. International Collaboration on Endocarditis Merged Database Study Group. Native valve endocarditis due to coagulase-negative staphylococci: report of 99 episodes from the International Collaboration on Endocarditis Merged Database. 2004. *Journal of Clinical Infectious Diseases*, 39 (10), 1527-30.

Coelho, V.H.M., Venancio, G.N., Cestari, T.F., Alves de Almieda, M.E and Da Cruz, .B.N. 2016. Microbial contamination of a university dental clinical in Brazil. *Brazilian journal of Oral Science,* 15(4), 248-251.

Damasceno, JL., Dos Santos, RA., Barbosa, A.H., Casemiro, LA., Pires, RH and Martins, CHG. 2017. Risk of fungal infections to dental patients. *The Scientific World Journal,* 2017, 1-8.

Da Silva, P.T.G., Vicente, C.V.V and Alves. D.P. 2014. Performance of the Vitek 2 system software version 5.03 in the bacterial identification and antimicrobial susceptibility test: evaluation study of clinical and reference strains of Gram-positive cocci. *Revista da Sociedade Brasileira de Medicina Tropical, 47*(3), 377-381.

Decreane, V., Ready, D., Pratten, J and Wilson, M. 2008. Air-borne microbial contamination of surfaces in a UK dental clinic. *Journal of Applied Microbiology*, 54, 195-203.

Diegritz, C., Manhart, J., Bucher, K.,Grabeinz, B. Schuierer, G., Kuhnisch, J., Kunzelmann, K., Hickel, R and Fotiadou, C. 2020. A detailed report on the measures taken in the Department of Conservative Dentistry and Periodontology in Munich at the beginning of the COVID-19 outbreak. *Journal of Oral Clinical Investigations,* 24, 2931-2941.

Faden, F. 2019. Methicillin- resistant *Staphylococcus aureus* (MRSA) screening of hospital dental clinic surfaces. *Saudi Journal of Biological Sciences*, 26, 1795-1798.

Freires, I.A., Avil´es-Reyes, A., Kitten, T. 2017. Heterologous expression of *Streptococcus mutans* Cnm in *Lactococcus lactis* promotes intracellular invasion, adhesion to human cardiac tissues and virulence. *Virulence Journal*, 8 (1), 18–29.

Fotedar, S and Ganju, S. 2015. Microbial contamination of dental unit water lines in H.P Government Dental College, Shimla. *The Saudi Journal for Dental Research,* 6, 129-132.

Funke, G., Monnet, D., DeBernardis, C., Von Graevenitz, A and Freney, J. 1998. Evaluation of the VITEK 2 System for Rapid Identification of Medically Relevant Gram-Negative Rods. *Journal of Clinical Microbiology,* 36 (7), 1948-1952.

Ge, Z., Yang, L., Xia, J., Fu, X and Zhang, Y. 2020. Possible aerosol transmission of COVID-19 and special precautions in dentistry. *Journal of Zhejiang University-SCIENCE B (Biomedicine and Biotechnology),* 1-8.

Gowtham, S and Deepthi, E. 2014. Bioaerosol contamination in the dental clinic: A potential health hazard. *Annals and Essences of Dentistry,* 6 (1), 33-36.

Gravenitz, A. V. 1993. Revised nomenclature of *Alloiococcus* otitis. *Journal of Clinical Microbiology,* 31:472. (Letter).

Grenier, D. 1995. Quantitative analysis of bacterial aerosols in two different dental clinic enviroments. *Applied and Enviromental Microbiology,* 61 (8), 3165-3168.

Gungor, N.D., Kadaifciler, DG and Peker, OO. 2014. Investigation of the bacterial load and antibiotic susceptibility of dental units. *Journal of Environmental monitoring and Assessment,* 186, 1847-1853.

Gunnar, K. 2017. EUCAST propose to change the definition and usefulness of the susceptibility category "intermediate", *Journal of clinical microbiology and infection,*23 (12), 894-895.

Hallier C., Williams D.W., Potts A.J.C and Lewis M.A.O. 2010. A pilot study of bioaerosol reduction using an air cleaning system during dental procedures. *British Dental Journal,* 209, 1-4.

Harrel, S.K and Molinari, J. 2004. Aerosols and splatter in dentistry: A brief review of the literature and infection implications. *Journal of the American dental association,* 135, 429-437.

Haque, M., Sartelli, M and Haque, S.J. 2019. Dental infections and resistance-Global health consequences. *Dentistry Journal,* 7(22), 1-19.

Jain, M., Athur, A., Mathur, A., Mukhi, P.U., Ahire, M and Pingal, C. 2020. Qualitative and quantitative analysis of bacterial aerosols in dental clinical settings: Risk exposure towards dentist auxiliary staff, and patients. *Journal of Family Medicine primary Care*, 9, 1003-1008.

Jenkins,D.R. 2017. Nosocomial infections and infection control. *Journal of Medecine,* 45(10), 629-633.

Khan, H.A., Baig, FK and Mehboob, R. 2017. Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pacific Journal of Tropical Biomedicine,* 7(5), 478-482.

Kimmerle, H., Wiedmann-Al-Ahmed, M., Pelz, K., Wittmer, A., Hellwig, E and Al-Ahmad, A. 2012. Airborne microbes in different dental environments in comparison to a public area. *Archives of oral biology*, 57, 689-696.

Koboza, J., Pastusszka, J.S and Bragoszewska. 2018. Do exposures to aerosols pose a risk to dental professionals? *Journal of Occupational medicine.* 68, 454-458.

Kruskal, W. 1952. "Use of ranks in one criterion variance analysis". *Journal of the American Statistical Association,* 47(260), 583-621.

Kurita, H., Kurashina, K and Honda, T. 2006. Nosocomial transmission of methicillin-resistant Staphylococcus aureus via the surfaces of the dental operatory. *British Dental Journal,* 201(5), 297-300.

Laheij, A.M.G.A., Kistler, J.O., Belibasakis, GN., Valimaa, H and de Soet, JJ. 2012. Healthcare-associated viral and bacterial infections in dentistry. *Journal of Oral Microbiology,* 4 (1), 17659.

Lasserre, J.F., Brecx, MC and Toma, S. 2018. Oral Microbes, Biofilms and their role in Periodontal and Peri-implant Diseases. *Journal of Materials Science and Engineering,* 11, 1802.

Leggat, P.A and Kedjarune, U. 2001. Bacterial aerosols in the dental clinic: A review. *INTERNATIONAL DENTAL JOURNAL*, 51, 39-44.

Levesque, S., Dufresne, PJ, Soualhine, H., Domingo, M., Bekal, S., Lefebvre, B and Tremblay, C. 2015. A Side by Side comparison of Bruker Biotyper and Vitek MS: Utility of MALDI-TOF MS Technology for microorganism Identification in a Public Health Trefernce Laboratory. *PLoS ONE*, 10 (12), 1-21.

Ligozzi, M., Bernini, C., Bonora, M.G., De Fatima, M., Zuliani, J and Fontana, R. 2002. Evaluation of the VITEK 2 System for Identification and Antimicrobial Susceptibility testing of Medically Relevant Gram-positive Cocci. *Journal of Clinical Microbiology,* 40 (5), 1681-1686.

Ling, T.K.W., Liu, Z.K and Cheng, A.F.B. 2003. Evaluation of the Vitek 2 system for Rapid Direct Identification and Susceptibility Testing of Gram-Negative Bacilli from Positive BloodCultures. *Journal of Clinical Microbiology*, 41(10), 4705-4707.

Manarte-Monteiro, P., Carvalho, A., Pina, C., Oliveira, H and Manso, MC. 2013. Air quality assessment during dental practice: Aerosols bacterial counts in a universitary clinic. *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial*, 54 (1), 2-7.

Mann, H .B and Whitney, D.R. 1947. On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics,* 18 (1),50-60.

McCormack, M.G., Smith, A.J., Akram, AN., Jackson, M., Robertson, D and Edwards, G. 2015. *Staphylococcus aureus* and the oral cavity: An overlooked source of carriage and infection. *Journal of Infection,* 43, 35-37.

MacGowan, A and Macnaughton, E. 2017. Antibiotic resistance. *Journal of Prevention and Control of infection,* 45, (10), 622-628.

Meng, L., Hua, F and Bian, Z. 2020. Coronavirus disease 2019 (Covid-19): Emerging and future challenges for dental and oral medicine. *Journal of Dental Research, 99 (5),481-487*

Micik, R.E., Miller, R.L., Mazzarella, M.A and Ryge, G. 1969. Studies on dental aerobiology: I. Bacterial aerosols generated during dental procedures. *Journal of dental research,* 48, 49-56.

Miltiadous, G and Elisaf, M. 2011. Naïve valve endocarditis due to Micrococcus luteus;A case report and review of the literature. *Journal of Medical CASE REPORTS,* 5, 251.

Monaghan,N.P. 2016. Emerging infections-implications for dental care. *British Dental Journal,* 221, 13-15.

Nakasone, I., Kinjo, T., Yamane, N., Kisanuki, K and Shiohira, CM. 2007. Laboratory-based evaluation of the colorimetric VITEK-2 Compact system for species identification and of the Advanced Expert System for detection of antimicrobial resistances: VITEK-2

Compact system identification and antimicrobial susceptibility testing. *Journal of Diagnostic Microbiology and infectious disease,* 58, 191-198.

Pankhurst, CL and Johnson NW. 1998. Microbial contamination of dental unit waterlines: the scientific argument. *International Dental Journal,* 48, 359-368.

Pankhurst, CL and Coulter, WA. 2007. Do contaminated dental unit waterlines pose a risk of infection? *Journal of Dentistry,* 35, 712-720.

Paramashivaiah, R., Prabhuji, MLV and Narayanan, R. 2016. Dental unit water lines decontamination with the aid of nanotechnology. *Indian Journal of Dental Research,* 27, 91-99.

Pasquarella, C., Pitzurra, O and Savino, A. 2000. The index of microbial air contamination. *JOURNAL OF HOSPITAL INFECTION*, 46 (4), 241-256.

Pasquarella, C., Veronesi, L., Napoli, C., Castiglia, P., Liguori, G., Rizzetto, R., Torre, I., Righi, E., Farruggia, P., Tesauro, M., Torregrossa, MV., Montagna, MT., Colucci, ME., Gallè, F., Masia, MD., Strohmenger, L., Bergomi, M., Tinteri, C., Panico, M., Pennino, F., Cannova, L., Tanzi M; SItI Working Group Hygiene in Dentistry. 2012. Microbial environmental contamination in Italian dental clinics: A multicenter study yielding recommendations for standardized sampling methods and threshold values. *Science of the Total Environment,* 420, 289-99.

Peng, X., Li, X., Cheng, L., Zhou, X and Ren, B. 2020. Transmission routes of 2019-nCoV and controls in dental practice. *International Journal of Oral Science*, 12 (9)

Polednick, B. 2014. Aerosol and bio aerosol particles in a dental office. *Journal of Enviromental Research*, 134, 405-409.

Rautemma, R., Nordberg, A., Wuolijoki-Saaristo, K and Meurman, J.H. 2006. Bacterial aerosols in dental practice: a potential hospital infection problem. *Journal of Hospital Infection,* 64, 76-81.

Rodrigues, S., Suvarna, S., Suvarna, J., Saralaya, V., Saldanha, S and Shenoy, VK. 2017. Microbial assessment of Dental Unit Waterlines in an industrial setup in Karnataka, South India. *Indian Journal of Dental Research,* 28, 555-559.

Rodrigues, S., Shenoy, V and Joseph, M. 2005. Changing face of infection control: Dental unit water lines. *Journal of Indian Prosthodontic Society,* 5 (4), 170-174.

Sawhney, A., Venugopal, S, Girish Babu, RJ, Garg, A., Matthew, M., Yadav, M., Gupta, B and Tripathi, S. 2015. Aerosols: How dangerous they are in Clinical Practice. *Journal of Clinical and Diagnostic Research,* 9 (4), 52-57.

Shraschchandra, BM and Ambika, P. 2014. "Dental Aerosols: - Role of its reduction in infection control-An important review. *International Journal of Innovative Research and Review, 2* (4), 83-89.

Singh, A., Shiva Manjunath, RG., Singla, D., Bhattacharya, HS., Sarkar, A., Chandra, N. 2016 Aerosol, a health hazard during ultrasonic scaling: A clinico-microbiological study. *Indian Journal of Dental Research,* 27(2):160-162.

Singh, S., Shah, S.J., Podar, R and Shetty, R. 2020. Combating respiratory hazards in dentistry: A comprehensive review. *Journal of Dental Research,* 7, *86-90.*

Smith, A., Jackson, M.S and *Kennedy,* H. 2004. Antimicrobial susceptibility of Viridans group Streptococcal blood isolates to eight antimicrobial agents. *Scandinavian Journal of Infectious Diseases,* 36, 259-263.

Spanu, T., Sanguinetti, M., Ciccaglione, D., D'Inzeo, T., Romano, L., Leone, F and Fadda, G. 2003. Use of the Vitek 2 system for Rapid Identification of Clinical Isolates of Staphylococci from bloodstream infections. *Journal of Clinical Microbiolgy,* 41(9), 4259-4263.

Swaminathan, Y and Thomas, J. 2013. "Aerosol"-A Prospective Contaminant of Dental Enviroment.*Journal of Dental and Medical Sciences*, 11 (2), 45-50.

Szymanski, J. 2007. Dental Bioaerosol as an occupational hazard in a dentist's workplace. *Journal of Annals of Agricultural and Environmental medicine,* 14, 203-207.

Szymanski, J and Dutkiewicz, J. 2008. Concentration and species composition of aerobic and facultatively anaerobic bacteria released to the air of a dental operation area before and after disinfection of dental unit waterlines. *Ann Agric Environ Med,* 15, 301-307.

Tellier, R., Li, Y., Cowling, B.J and Tang, J.W. 2019. Reconition of aerosol transmission of infectious agents: a commentary. *Journal of Biomed Central infectious diseases,* 19, 101-109.

Timmerman, M.F., Menso, L., Steinfort, J., Van Winkelhoff, A.J and Van der Weijden. G. 2004. Atmospheric contamination during ultrasonic scaling. *Journal of Clinical Periodontology, 31*, 458–462.

Toroglu, M.S., Haytac, M.C and Koksal, F. 2001. Evaluation of aerosol contamination during debonding procedures. *Journal of Angle Orthodontics,* 71, 299–306.

Umar, D., Basheer, B., Husain, A, Baroudi, K, Ahamed, F and Kumar, A. 2015. Evaluation of Bacterial Contamination in a Clinical Enviroment. *Journal of International Oral Health*, 7 (1), 53-55.

Ural, S., Yurtsever, S.G., Ormen,B., Turker,N., Kaptan, F., El, S., Akyildiz, Z.I., Nejat Ali Coskun,N.A.2014. *Gemella morbillorum* Endocarditis, *Case Reports in Infectious Diseases,* 1-3.

Veena, H.R., Mahantesha, S., Joseph, P.A., Patil, S.R and Patil, S.H. 2015. Dissemination of aerosol and splatter during ultrasonic scaling: A pilot study. *Journal of Infection and Public Health*, 8, 260-265.

Volgenant, CMC and Persoon, IF. 2019. Microbial water quality management of dental unit water lines at a dental school. *Journal of Hospital infection,* 103 (1), 115-117.

Wallet, F., Loiez, C., Renaux, E., Lemaitre, N and Courcol, RJ. 2005. Performances of VITEK 2 Colorimetric Cards for Identification of Gram-Positive and Gram-Negative Bacteria. *Journal of Clinical Microbiology*, 43 (9), 4402-4406.

Yadav, K; Prakash, S; Yadav, N.P; Sah, R.S. 2015. Multi-Drug Resistance of Bacterial Isolates among Dental Caries Patients. *Janaki Medical College Journal of Medical Science*, 3, 37–44.

Zawadzki, P.J., Perkowski, K., Padzik, M., Mierzwinska-Nastalska, E., Szaflik, J.P. Conn, DB and Chomicz, L. 2017. Examination of Oral Microbiota Diversity in Adults and Older Adults as an Approach to prevent spread of risk factors for human infections. *International Journal of Biomedical Research,* 1-7.

Zemouri, C., De Soet,H., Crielaard,W and Laheij, A. 2017. A scoping review on bio-aerosols in healthcare and the dental environment.*Plos One,* 12(5): e0178007.

Zemouri, C.,Volgenant, C.M.C., Buijs, M.J., Crielaard, W., Rosema, N.A.M., Brandt, B.W., Laheij, A.M.G and De Soet, J.J. 2020. Dental aerosols: Microbial composition and spatial distribution. *Journal of Oral Microbiology*, 12(1), 1762040.

Appendixes

Appendix 1. VITEK® antimicrobial assay

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VITEK 2™ — technology **AST-ST03** IVD

Streptococcus Susceptibility Card

SUMMARY AND EXPLANATION

The VITEK® 2 Streptococcus Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an in vitro test to determine the susceptibility of ***S. pneumoniae*, beta-hemolytic Streptococcus, and Viridans Streptococcus** to antimicrobial agents when used as instructed in the Product Information manual.

INSTRUCTIONS FOR USE

A package insert is provided in the kit or downloadable from **www.biomerieux.com/techlib**

See the Product Information manual for additional Instructions for Use.

STORAGE CONDITIONS

Store at 2° to 8° C.

CONTENTS OF THE CARD

Antimicrobic	Code	Concentration §	Calling Range ≤	Calling Range ≥	FDA Indications for Use
Ampicillin [NS2]	AM	0.5, 1, 4, 8	0.25	16	grpBetaHS**, grpVIR**
Benzylpenicillin [NS2]	P	0.06, 0.12, 0.5, 2	0.06	8	grpC/G**, *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, grpVIR**
Cefotaxime	CTX	0.25, 0.5, 1, 2	0.12	8	*S. pneumoniae*, SpyA**, *Streptococcus* spp.
Ceftriaxone [NS2]	CRO	0.12, 0.25, 1, 4	0.12	8	grpVIR**, *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*
Chloramphenicol	C	1, 4, 8	1	16	N/A**
Clindamycin [c]	CM	CM 0.12, CM 0.25, CM 0.5, CM/E 0.5/0.1	0.25	1	SP(Pen(S))**, *S. pyogenes*, *S. agalactiae*, *S. mitis*, *S. oralis*
Erythromycin	E	1, 2, 4, 16	0.12	8	*S. pneumoniae*, *S. pyogenes*, grpVIR**
Gentamicin	GM	512, 1024	64	512	N/A**
Inducible Clindamycin Resistance *S. agalactiae*, *S. pyogenes*	ICR	CM 0.5, CM/E 0.25/0.5	- NEG	- POS	*S. agalactiae*, *S. pyogenes*
Levofloxacin	LEV	1, 2, 4, 16	0.25	16	SP+MDRSP**, *S. pyogenes*, grpC/F**, grpG**, *S. agalactiae*, grpVIR**, *S. milleri*
Linezolid [NS]	LNZ	2, 4	2	8	SP+MDRSP**, *S. agalactiae*, *S. pyogenes*
Moxifloxacin	MXF	0.5, 1, 4, 8	0.06	4	*S. anginosus*, *S. constellatus*, SP+MDRSP**, *S. pyogenes*, *S. agalactiae*, grpVIR**
Rifampicin	RA	0.015, 0.03, 0.12, 0.5	0.06	4	N/A**
Teicoplanin	TEC	0.5, 1, 4	0.12	4	N/A**
Tetracycline	TE	0.12, 0.5, 1, 4	0.25	16	*S. pneumoniae*, *S. pyogenes*, grpVIR**
Tigecycline [NS]	TGC	0.12, 0.25, 0.5	0.06	1	SP(Pen(S))**, *S. anginosus* grp. (includes *S. anginosus*, *S.intermedius*, and *S. constellatus*), *S. agalactiae*, *S. pyogenes*
Trimethoprim/Sulfamethoxazole [c]	SXT	8/152, 16/304, 64/1216	10 (0.5/9.5)	320 (16/304)	*S. pneumoniae*
Vancomycin [NS]	VA	0.5, 1, 2, 4	0.12	8	grpVIR**, *S. pyogenes*, *S. agalactiae*

Numerical values are expressed in µg/ml.

§ Equivalent standard method concentration by efficacy.

[NS2] = Beta-hemolytic Streptococci: The current absence of resistant isolates precludes defining any results other than susceptible. Isolates yielding MIC results suggestive of Nonsusceptible category should be submitted to a reference laboratory for further testing.

**grpBetaHS = Beta-hemolytic group Streptococcus species

Appendix 2. Statistical power analysis.

23 April 2018

To whom it may concern

Sonia Bredenkamp consulted with me on the 23 November 2017 regarding her research protocol requirements, specifically sample size and statistical planning of the study.

1. For the sample size estimation, the sample size of 300 samples was given by the researcher. This represents 50 patients with 6 repeated measurements each. Of interest was the power that such a sample could provide to test the hypotheses. The objective used in the power calculation was the comparison of bacterial concentration between scaling and drilling considering distance to the plate and timing of the sample. The between subjects effect was scaling and drilling (with 2 levels) and the two within-subjects effects were the distance to the plate (2 levels) and timing of the sample (3 levels). Repeated measures ANOVA power analysis was performed in PASS version 12 using a full factorial design – see attached. The power is given as 1-beta where the beta values for all estimated effect sizes were <=0.2, ensuring adequate power with the stipulated sample size.
2. Organisation of the data. The data should be captured in MS excel or equivalent spreadsheet or statistical package. Categorical variables should be coded numerically and numerical variables should be captured with the numeric value only. Each participant should have multiple rows of data corresponding to the number of within subject variables. Each participant should be identified by a numeric code only.
3. Statistical analysis will be done in Stata version 15. Repeated measures ANOVA testing will be done to test the hypothesis of effect of within and between subject variables on the outcome of bacterial concentration.

Yours sincerely

Tonya Esterhuizen

Senior lecturer/Biostatistician Biostatistics Unit

Division of Epidemiology and Biostatistics

Faculty of Medicine and Health Sciences

University of Stellenbosch