Effectiveness of selected surface disinfectants in the dental clinic – A report from a tertiary training facility in KwaZulu-Natal

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A dissertation submitted to the Discipline of Dentistry, School of Health Sciences, University of KwaZulu-Natal, Westville, for the degree of Master of Medical Science.

This is a dissertation in which the chapters are written as a set of discrete research publications that has followed the Southern African Journal of Infectious Disease's format with an overall introduction and final summary. Typically, these chapters will be published in internationally recognized, peer-reviewed journals.

This is to certify that the contents of this dissertation is the original research work of Dr Swati A. Deulkar, carried out under supervision within the School of Health Sciences, Westville campus, University of KwaZulu-Natal, Durban, South Africa.

Supervisor:

Signed:  Name: **Dr. Shenuka Singh** Date: 01 December 2016

ABSTRACT

Introduction

Optimal infection control practice forms the cornerstone of quality oral health care delivery in any dental setting. There is very little published evidence on dental infection control practices in South Africa. In addition there is a paucity of evidence that specifically examined the efficacy of commonly used surface disinfectants in oral health clinical settings.

Aim

The aim of this study was to determine the effectiveness of selected surface disinfectants on specific dental environmental surfaces in an identified public oral health training facility in KwaZulu-Natal. The objectives included the identification and classification of environmental areas that are at risk for cross-contamination in the dental clinic, and comparison of the microbial count at specified times of the day after the use of three surface disinfectants.

Methods

This was a prevalence (cross-sectional), descriptive research study with a non-experimental design. Data collection included the application of three commonly used surface disinfectants (Chlorine®, Ethanol and Glutaraldehyde) on identified dental environmental surfaces in a public sector dental clinic facility in KwaZulu-Natal. The clinic consists of seventeen dental units that are numbered from one to seventeen. Systematic random sampling technique was used to select selected every second chair for the study (Dental Unit number: number: 1, 3, 5, 7, 9, 11, 13, 15, 17). The dental clinical environment was then divided into four zones: 1): the working area around the dental operator/assistant (chair, head rest, arm rest, foot rest, dental hand pieces, overhead light source, air water syringe tip, spittoon, suction hose, based of dental chair, dental stool, foot control, instrument counter and handle); 2): the area behind chair (wash basin, computer monitor, window, wall, table top, dust bin and taps): 3): the area away from chair (computer processing unit, telephone and floor); and 4): the reception area (patient chairs and reception table top). The swab samples were collected at specific time intervals (7am, 9am, 11am, and at 16.00) using a charcoal swab. Chlorine, Ethanol (70% in water) and Glutaraldehyde (2%) disinfectants were applied separately on the identified nine dental units by using a spray method. Use of the MALDE-TOF spectrometer enabled the mass spectra to be acquired and the bacteria to be identified.

Results

Out of the 312 samples taken, 262 (84%) were shown to be bacterial culture positive. More than seven microbial species were identified in which *staphylococci, Bacillus species and fungi* were present. The most contaminated areas in the dental environment were the area around the chair (86.5 %) and away from chair (92%). The results indicate that Chlorine® was not active against several bacteria because 92% samples had positive growth at the end of the day. Only 56 % of the samples using Ethanol were positive in the morning but the microbial growth increased to 96 % by the end of the day. The use of Glutaraldehyde indicated that 52% of samples were positive at 9 am but that 82% were found to be positive at the end of day. The bacterial survival rate was found to be less with the use of Glutaraldehyde.

Conclusion

The study suggests that there was an association between frequency of cleaning, the type of disinfectants used and the microbial count on the specified dental environmental surfaces in the identified oral health facility. The findings therefore indicate that disinfection processes at the identified dental centre are inadequate, sub-optimal and could contribute to the infection chain. There is an urgent need to review the current infection control procedures and protocols, including a review of the type of surface disinfectants used. The frequency of disinfection (damp-dusting and housekeeping) must be reviewed, given the number of patients that are seen on a daily basis. It is also important that simple procedures such as awareness of hand hygiene practices are implemented and prioritized. There should also be dedicated infection control monitoring and evaluation processes.

DECLARATION

DECLARATION – ANTI-PLAGIARISM

I, Swati A. Deulkar declare that

1 The research report in this dissertation, except where otherwise indicated, is my original work.

2 This dissertation has not been submitted for any degree or examination at any other university.

3 This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed:

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Name: Swati A. Deulkar

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MANUSCRIPT PREPARED FOR PUBLICATION

1. **Swati A. Deulkar, Shenuka Singh, Deelip Tiwari, Anou M. Somboro. Isolation of common aerobic bacterial pathogens from the dental environmental surfaces after the use of disinfectants: A case study at a public dental clinic.**

Swati A. Deulkar contributed to the design of the project, performed the microbiological and analytical testing and wrote the paper.

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LIST OFABBREVIATIONS

ADA:	American Dental Association
ATCC:	American Type Culture Collection
BTS:	Bacterial Test Standard
Cfu/mL:	Colony forming unit/milliliter
CDC:	Centres for Disease Control and Prevention
Da:	Dalton
DNA:	Deoxy ribonucleic acid
DUWL:	Dental unit waterline
FDA:	Food and Drug Administration
GO:	Glyoxal
LPS:	Lipopolysaccharides
MPs:	Medical practitioner
MRSA:	Methicillin-resistant Staphylococcus aureus
NCDs:	Non-communicable diseases
PMN:	Poly-morpho-nuclear leukocyte
UA:	Ursolic acid
UV:	Ultra violet
VRE:	Vancomycin-resistant Enterococcus
WHO:	World Health Organization

CHAPTER 1: INTRODUCTION

1. Introduction

Health care workers, in particular oral health care workers, are highly prone to direct contact with contaminated blood, oral saliva and other body fluids during their work engagement [1]. Most dental procedures, especially those involving the use of high speed rotating instruments, have the potential to trigger infection transfer and cross contamination through the creation of aerosols that could contain blood, water and saliva [2]. This creates multiple opportunities for the spread of infection between patients and dental staff [3].

2. Background

According to available reports, hospital acquired infections (HAIs), also called nosocomial infections, could affect more than 25% of the population that access health care services [3]. Microorganisms can be inhaled, injected, ingested or splashed onto the skin or mucosal surfaces [4]. The microbes can multiply through direct contact (person to person) or via indirect contact (via contaminated apparatus, unclean ecological surfaces or through instruments) [4]. Bacterial contamination of equipment or the atmosphere have been linked to the spread of hospital acquired infection [5]; [6]. Similarly, the health worker's cellphones or computer screen, keyboards, clothes, curtains of windows, apron, ties and wrist watches could also become a resource of bacterial contaminants [7]. Health care workers, patients and people coming into contact with blood or blood product are highly at risk of infection that are transmitted through blood such as hepatitis, Human immunodeficiency virus (HIV) and other bacterial, fungal, protozoan and yeast related infections that could be airborne or blood borne [8]. The viability of pathogens on the environmental dental work surfaces such the basin, table top, keyboard, mobile, chair light, pen, radiographic equipment, files, tap, chair, could act as a vehicle for microbe transfer [9].

The prevention of nosocomial infections require the identification and addressing of the weakest link in the chain of infection [10]. Optimal infection control practice therefore forms the cornerstone of quality oral health care delivery in any dental setting [10]. Infection control can be described as a comprehensive approach to minimize and eliminate potential cross contamination through universally accepted standards [11]. The everyday infection control measures includes proper use of disinfectants, maintenance of hand hygiene, proper cleaning, management of hospital waste, disinfection and contact isolation in order to prevent nosocomial infections [12]. Avoidance of infection transfer requires various precautionary measures to be adhered to such as, hand washing after patient contact;

use of disinfectants for clinic surface cleaning; use of personnel protective equipment (e.g. gowns, gloves, apron, face masks
and protective eye wear); appropriate handling and disposal of contaminated sharp instruments and proper management of hospital waste [10], [13], [14]. Ideally all vegetative microbes should be destroyed using heat sterilization procedures [10], [13], [14]. However, it is neither cost effective nor practical to sterilize dental working surfaces or instruments that are heat sensitive [10], [13], [14].

Disinfection provides a more practical solution for ensuring prevention of cross contamination [15]. Disinfection is characterized by killing, destroying or removal of all pathogenic microbes, except spores [15]. While it is preferable for all pathogenic organisms to be destroyed, a decrease in their quantity is acceptable [14], [15]. There are various disinfection methods, which include physical disinfection, heating and chemical disinfection [3]. The disinfectants used in most hospital settings include Aldehyde, Alcohol, Iodophors, Quaternary ammonium compounds, Alcohol ammonium, and Peroxygenated compounds [16]. The effectiveness of a disinfecting solution is dependent on factors such as the concentration and nature of contaminating microorganisms, the concentration of the chemical, the exposure time and the amount of accumulated bio-burden [17].

According to available reports, hospital acquired infections (HAIs), also called nosocomial infections, affect more than 25% of the total health care population [3]. Microorganisms could be inhaled, injected, ingested or splashed onto the skin or mucosal surfaces [3]. These microbes could further multiply through direct contact (person to person) or via indirect contact (via contaminated apparatus, unclean ecological surfaces or through contaminated instruments) [4]. Bacterial contamination of equipment or air-borne infections have been linked to the spread of hospital acquired infections [5, 6]. The health worker's cell phones, computer screen, keyboards, clothes, curtains, apron, ties and wrist watches can become a source of bacterial contaminants [7]. Health care workers, patients and people coming into contact with blood or blood products are at risk of infections that are transmitted through blood such as *hepatitis, Human immunodeficiency virus (HIV) and other bacterial, fungal, protozoan and yeast* related infections [8]. The viability of pathogens on the environmental dental work surfaces such the basin, table top, keyboard, mobile units, chair light, pen, radiographic equipment, clinical files, tap, chair, etc, act as vehicle for the transfer of microbes [9].

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Environmental surfaces are classified as clinical (medical) contact and housekeeping [18]. Clinical contact surfaces refer to those areas that the oral health worker comes into direct contact with (contaminated devices, instruments, hands, or gloves), while housekeeping surfaces are not touched directly during the delivery of dental care [18]. Patients and staff engage with the various surfaces throughout the day eg. in the waiting room, clinical area and other rooms in a dental clinic, thereby providing many opportunities for cross-contamination and infection [18]. Damp dusting is a common disinfection procedure in the dental clinical environment [19].

3. Problem Statement

Patients have the right to access oral health services within in a safe and healthy clinical environment [20]. All oral health care staff have a legal obligation to ensure optional protection for their patients, co-workers and the public at large [20]. The pre-requisites for a safe and healthy work environment

include optimal infection control practices, among other requirements [21]. However there is very little published evidence on infection control practices within dental clinics in the public health sector in KwaZulu-Natal or in South Africa. In addition there is a paucity of evidence that specifically examined the efficacy or cost-effectiveness of the commonly used surface disinfectants in oral health clinical settings [22]. There is also very scarce literature on the spread of infection through cross-contamination within dental clinics in South Africa or on the use of disinfectants in KwaZulu-Natal. The implications are that surface disinfectants are used in dental practice without supporting evidence of their efficacy and cost-effectiveness.

The common surface disinfectants used in South African hospitals are chlorine, alcohol or Glutaraldehyde [23]. However, several studies have noted that microbes were present on dental handpieces after these disinfectants were used [24]. This finding is particularly worrisome and further research is need to determine this occurrence. It is also important to note that there is no data on the presence or type of microbial count that is most likely to be found in a dental clinical environment in KwaZulu-Natal (KZN).

4. Purpose of the study

The rationale of this study is to provide data that could guide infection control practices, specifically in the area of disinfection in an oral health setting. The study has the potential to make important contributions to understanding the efficacy and effectiveness of current disinfection processes in public oral health facilities in KZN. It also hopes to provide data on the efficacy of commonly used disinfectants in the public dental clinics in the province. It is possible to extrapolate on the research findings from this study to other dental settings in the public health sector because a common procurement process is used to purchase surface disinfectants for dental use.

The study also hopes to contribute to policy and planning with regards to optimal infection control processes in public oral health facilities, and hopes to highlight the type of microbial growth that persists, despite the use of dental disinfectants, thereby having important implications for health and safety. The results of this study could also influence clinical teaching in dental infection control and therefore has the potential to influence a curriculum review. Disinfection of surfaces, such as trolleys, tables and hospital walls are easily achieved however there is no standard method to establish whether dental surfaces are infection free. The consumer needs to rely on the literature supplied by the manufacturer regarding the efficiency of a disinfectant and many disinfectant manufacturers claim that their products are broad-spectrum and can kill all microbes.

5. Aim and Objectives

The aim of this investigation was to determine the effectiveness of selected surface disinfectants on specific dental environmental surfaces by comparing the microbial count after use of the disinfectant at specific time intervals in a public oral health facility in KwaZulu-Natal.

The objectives of the study were:

1. To recognize and classify dental environmental areas that are at risk for cross-contamination in the dental clinic.
2. To identify the types of microbial count present in the dental clinic at different times of the day.
3. To determine and compare the microbial count after use of the disinfectant at a specific time interval
4. To determine and compare the microbial count on identified surfaces in the operating and reception area of the dental clinic at specific times in the day.

6. Hypothesis testing

For the null hypothesis {H0}:

- There is no dissimilarity in efficacy among the three disinfectants.
- There is no correlation between frequency of cleaning and damp dusting and the microbial count at the dental clinic.

The alternate hypothesis {H1} states:

- There is a difference in efficacy among the three disinfectants.
- There is a correlation between frequency of cleaning and damp dusting and the microbial count at the dental clinic.

7. Thesis Outline

The study is presented in the following chapters:

Chapter 2: Literature Review: this section outlined the relevant local and international literature on infection control practices in the dental clinic and the subsequent implication for patient and oral health care worker safety.

Chapter 3: Methods: This section outlined the methodology used to conduct the study.

Chapter 4: Paper/Manuscript: Isolation of common aerobic bacterial pathogens from the dental environmental surfaces after the use of disinfectants: A case study at a public dental clinic

Chapter 5: Conclusion: This chapter presented the limitations and recommendations and established the extent to which the study aim and objectives were achieved.

8. Summary

This chapter provided a concise outline of the problem statement, the existing gaps in the reviewed literature and the purpose of the study. The overall aim and objectives were also presented. The next chapter provides an overview of the current debates surrounding infection control practice in the dental clinical environment from a national and international perspective.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter sheds light on nosocomial infections, the role of the dental operatory surfaces in cross contamination, types of disinfectants used and its effect on hospital surface microorganisms. According to the literature, the dental clinic can be a high risk area for the direct and indirect transmission of infectious diseases [3]. The health worker is thus at an increased risk for exposure to these diseases [3]. This is further supported by studies on sero-epidemiology that show the concentration of antigen and antibodies for *hepatitis B* [25], *hepatitis C* [16], and *Legionella species* [10] to be more evident in hospital staff than in the general public. Additionally these staff have an increased chance of respiratory infections [11].

2.2 Nosocomial Infections

Nosocomial infections, also called hospital acquired infections, are defined as infections that occur from pathogens that gain entry in a patient while he/she is hospitalized [26]. According to Meltzer, these infectious agents may affect the health care staff, patients and visitors to the facilities [27], thereby creating multiple opportunities to spread these pathogens within and outside the hospital environment [26] , [27]. The literature further indicates that the percentage of multi-drug resistant microbial strains are increasing and this could further translate into increased nosocomial infections [28] [29].

There are many species and genera of microbes, such as viruses, fungi and bacteria containing *Klebsiella spp*, *Escherichia coli*, *Enterococcus spp*, *Proteus spp* and *Staphylococcus aureus.* These microbes are seen as the basic etiological agent of nosocomial infection [30] [21]. These agents can have considerable impact on their environment, and this can vary according to their geographic location [30] [21]. Generally, infectious sources are classified into two categories, namely endogenous and exogenous, the former being those that develop from bacteria inside the body and the latter developing from bacteria outside of the body [31]. These microbes are capable of gaining access into the body through devices such bronchoscopes, needles, pens or contact with the patient's clinical chart, computer keyboard, etc. Other sources of infection transfer could be blood, saliva, open wounds, vaginal and nasal secretions, oral droplets, etc [32].

In the European countries, the age standardized sero-positivity for HSV-1 ranges from 84% in Bulgaria and the Czech Republic, 52% in Finland, while the sero-prevalence ranges from 4% in

England and Wales, to 24% in Bulgaria for HSV-2 [2]. The prevalence of HSV-2 and HSV-1 in these countries varies according to the patient's age, sex, region and population sub-group. The sero-prevalence rate of HSV-1 is 50% in the United States and 92% in China [33, 34].

An increase in *meticillin-resistant Staphylococcus aureus* (MRSA) is regarded as the main cause of hospital acquired infections. For these agents, the hospital becomes a long-term reservoir where pathogens can remain viable for considerable periods of time [35], [36], [37], [37], and is generally found on hospital furniture, surfaces, floors and clinical apparatus [38], [39], [40], [41], [41], [42]. Some hospital areas such as lockers, beds, curtains and table countertops, tend to harbor MRSA more than other surfaces [43], [44], and it is observed that these lockers and countertops are commonly located on the right hand side of the patient [9]. The identification of MRSA is particularly important for this study and will be explained in detail in the Methods chapter [38].

It is commonly found that healthcare workers transmit the MRSA, acquired from patients to the environmental surfaces [41], [37], [45], [46]. To break this series of infection transfer, hand hygiene is very important but certain challenges are noted in practicing optimal handwashing protocols [47]. In busy hospital settings, infection control practices such as proper cleaning of instruments and surfaces, and hand hygiene could be compromised [40] , [48].

Fungus infections, mostly candidal infection, are one of the endogenous sources (i.e. infection present inside the patient's body) [49]. A molecular study of fungus showed that it is commonly found among hospital workers, staff, patients and environmental surfaces, thereby indicating that the fungus could play an important role in infection transfer of hospital borne infections [50]. In bone marrow transplant patients, *Candida glabrate, Candida parasilosis and Candida albicans* are commonly found but the mode of transmission is still not clear [51]. Fungi such as *C.albicans* and *C.parasilosis* are able to survive on environmental surfaces for up to 14 days [47]. Candidal infections can also spread through irrigating solutions or blood pressure transducers [52], [53]. The fungal strains found on patients are generally similar to that found at the hospital before the patient acquires the infection [51]. In an orthopedic ward, patients were found to be infected by a fungal infection that was found on the arm boards or bandages of patients having an intravascular catheter or surgical elasticized bandages [54].

Clostridium difficile is also commonly found on environmental surfaces in hospitals and the level of surface contamination is linked to the frequency of this microorganism [35]. New patients could acquire *C.difficile* infections from previously infected patients [36]. Proper disinfection of hospital

surfaces reduces *C.difficile* infection transfer opportunities [37, 38]. In addition, *C.difficile* transmission occurs through various medical devices, such as electronic rectal thermometers and portable bed commodes [39].

Initially, the recognition, identification and characterization of bacteria were done through phenotypic and genotypic methods, with research demonstrating that genotypic methods are seen as a better mechanism to identify the microbes [55]. Recently in laboratories, an automated or semi-automated commercial system for bacterial identification have been used, such as: APIENTEROTUBE, VITEK, PHOENIX, MALDI-TOF MS and the GENOTYPE MYCOBACTERIUM CM system for mycobacterium [56].

Four decades ago, mass spectrometry (MS) was used to identify microorganisms [57], [58] and has also proved its broad applicability in clinical epidemiology and infection control measures, by highlighting the dissemination of pathogens, in medical diagnostics, bio-defense, food quality control and environmental monitoring [57], [58]. This study will also use mass spectrometry technology, specifically MALDI-TOF MS, as explained further in the Methods chapter [59].

2.3 The Role of Dental Operatory Surfaces in Cross Contamination

The mouth is a permanent reservoir of microorganisms with more than 700 bacterial strains that has the potential to cause infection to people [60]. The literature indicates that life threatening diseases such as HIV, tuberculosis and hepatitis could spread through dental procedures [61]. It is therefore imperative that optimal infection control practices is reiterated in the dental setting [61]. If cross infection control procedures not follow strictly, it may endanger both patients and dental staff [61]. The transmission of pathogens from one person to other patients is referred to as cross-contamination, and the resultant contamination is referred to as cross-infection.

The oral cavity is densely populated with a wide variety of microbes that are transferred to the environment by various procedures such as coughing, scaling, tooth cutting [62]. The expulsion of bacteria from the oral cavity is increased by sneezing and coughing during the periods of dental treatment [63]. With regard to the latter, many of the procedures carried out in the dental clinical environment results in the production of aerosols (particle s<50 Um in diameter) and splatter (particles>50 um in diameter) e.g. tooth preparation, ultrasonic, scaling and tooth polishing [64]. These particles in the aerosols are small but potent enough to stay on surfaces for long periods and can gain entry within a susceptible patient [63]. In contrast, particles present in splatter do not remain airborne

for long periods and quickly settle on surrounding surfaces [65]. Microbes present in the oral cavity (originating from the respiratory tract and mouth) may be associated with either type of particle described and can therefore be transmitted directly to other individuals or to patients and clinical personnel [66]. The avoidance of both of these means of transmission is a key feature of measures designed to prevent cross contamination in dental clinical settings [67]. While the literature is clear on the role of aerosols in cross contamination in the dental clinical environment [68, 69], little is known about the type or quantity of microbes that contaminate the dental clinical environmental surfaces.

2.4 Dental Environmental Surfaces

Environmental surfaces of the dental unit are classified into two components: housekeeping surfaces and clinical contact surfaces [70]. Housekeeping surfaces include walls, sinks, floors and table tops while the surfaces directly comes in contact with the patient, devices, hands, gloves and contaminated instruments are called clinical contact surfaces or medical equipment surfaces [71].

These surfaces comes into direct contact with dental health care personnel's (DHCP) hands or with gloves, which results in surfaces becoming contaminated with blood and oral fluids [70]. The air turbines in the dental clinic, such as spray or splashes from the hand pieces, scaler and air water syringe can contaminate dental clinic surfaces by transferring contaminants all over the clinic [71]. The clinical contact surfaces should be pathogen free, or if this is not possible, a patient safety barrier protection should be used to avoid contact with microbes [72]. Not all surfaces are able to use barrier protection methods, in which case these need to be made pathogen free through cleaning and disinfection [72]. If dental unit waterlines (DUWs) are contaminated, the clinic is further compromised from a health and safety perspective therefore necessitating that the evacuation lines must be flushed with a cleaner so as to reduce debris and microorganisms [72].

Clinical contact surfaces are more important than housekeeping surfaces in respect of contamination and need to be pathogen free [73]. Dental radiographic equipment, switches, knobs, handle, drawer handles, chair side computers, digital impression devices, nitrous oxide equipment are some examples of surfaces that may be considered clinical contact surfaces [74]. Barrier methods such as effective disinfectants are used to keep clinical contact surfaces, pathogen free (Figure 1) [2]. Barrier measures should be changed between patients, and carefully disposed in a manner that does not further support the prospect of cross-infection. The used of hand gloves while handling barriers, is very important [71].

2.5 Housekeeping surfaces

Housekeeping surfaces do not directly comes into contact with the patient and has less capacity for infection transfer, therefore requires less harsh procedures in comparison to the clinical contact surfaces [71]. Housekeeping surfaces should be cleaned on a regular basis using soap and water. Research has shown that use of disinfectant, soap and water are equally effective in cleaning housekeeping surfaces [71]. Studies on housekeeping surfaces are limited, with few recommendations, and studies show that either disinfectant or water and detergent are indicated for cleaning floors in hospital settings [75]. The study concludes that the capacity of housekeeping infection transfer is largely based on the level of contamination and the likelihood of transferring pathogen from the floor surface to the patient. Vertical surfaces such as walls are not suspected as contaminants and do not require regular disinfecting and cleaning [71].

2.6 Routes of Infection Transfer in Dental Clinics

The mouth is the usual home for many microbes, and is the permanent source of a large number of pathogenic microorganisms, whose outcome is cross-infection and possibly, cross-contamination [56] In dental practices, time consuming appointments, open wounds and invasive dental procedure increase the risk of exposure to microorganisms [76].

It is very much important to understand the pathway of infection transfer for hospital worker and it is generally bidirectional [77]. Generally, infection transfer may take place from dental staff to patient or from patient to the dental team member through the hands, instruments, equipment or from contaminated surfaces used during dental procedure [77]. If the dental unit water line (DUWL) contains contaminated water, there is further risk for cross contamination [78].

The transfer of infection commonly starts from infected patients to susceptible patients [79]. In their daily practice, dentists come into contact with surfaces such as x-ray machines, electric plugs, counter tops, scalers, light sources, countertops and the dental chair. The routes of cross contamination can be summarized as follows [80]:

1. Patient comes into contact with the hospital surfaces, and if contaminated with microbes, then the pathogen is easily transferred to the patient.
2. The microbes (pathogens) are viable on environmental surfaces and instruments.
3. Health care persons' hand and gloves become contaminated while coming into contact with contaminated surfaces of the dispensary.

4. If hospital surfaces frequently come into contact with contaminated hand and gloves, the chance of contamination is increased.
5. In the chain of infection transfer, pathogens transmitted to surfaces are then transmitted to subsequent patient or to staff, or from infected staff to surface and to the patient.
6. New patient admitted to the hospital acquires the pathogen from other contaminated patients (e.g. *VRE, MRSA, Acinetobacter, C.difficile*), which results in an increased probability of them being infected.

The chain of infection transfer starts commonly from infected patients to susceptible patients, through infected medical equipment, environment surfaces, or through water and air, while the hands of health staff can be indirectly or directly involved in the spread pathways [81]. The pathway of infection transfer is presented in Figure 2.1, which provides the basis for developing interventions to disrupt transmission. The literature supports the possible communication of communicable infection through inhaled aerosols and its settling on in animate surfaces [81].

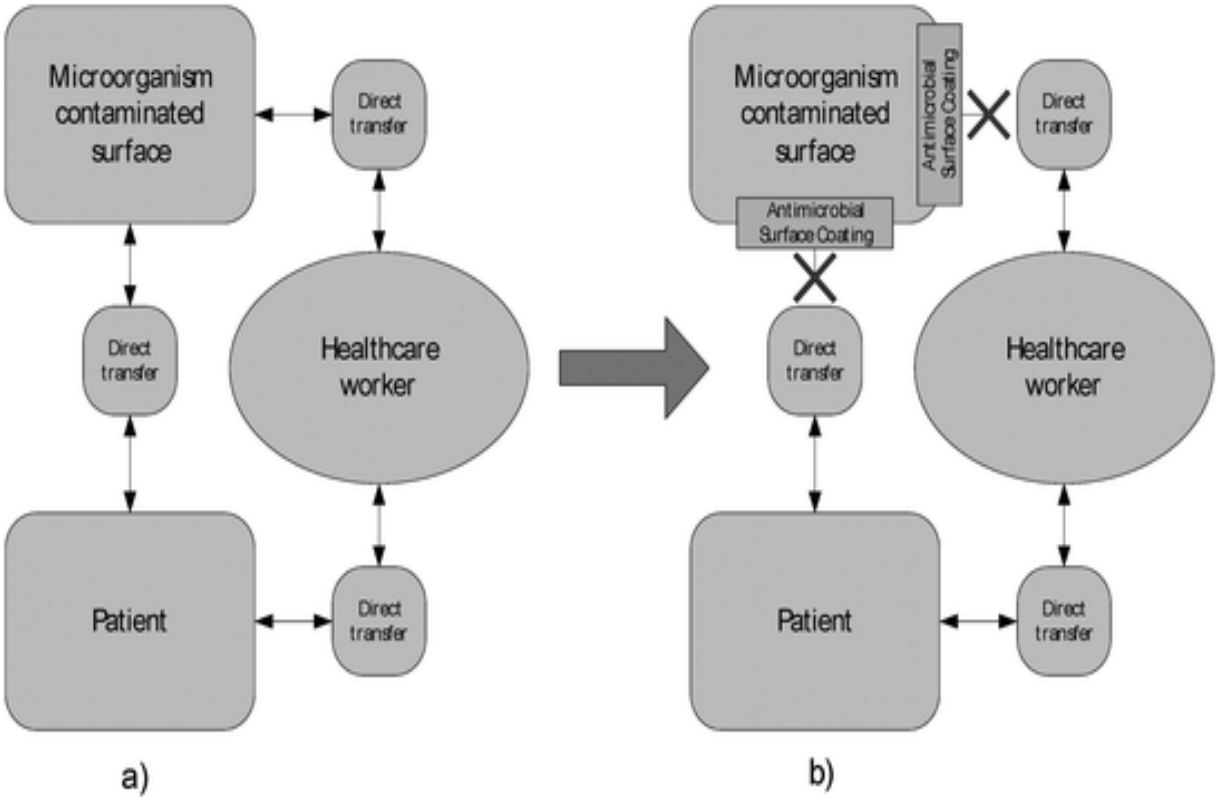

Figure 2.1: Cycle of infection transfer [82]

2.7 Methods of Preventing Cross Contamination from Surfaces

A number of general methods to control microbial pathogens have been developed by experts and by professional societies that have been made public in position statements and other documents that provide recommendations [57], [58], [59] [60]. Proper aseptic precautions should be used for a suspected patient i.e. use of an isolation room, using personal protective devices until the patient become asymptomatic for at least for 48 hours to 78 hours [83]. Alcohol is commonly used as a disinfectant, but is not sporicidal, with soap or detergent being mandatory for hand washing. It is scientifically proven that thoroughly cleaning hand with soap and water is better than using alcohol as a disinfectant to remove microbes. [61]. To remove spores, soap and water, and chlorhexidine have been shown to have good results, although the latter is not sporicidal [84]. Use of personal protective devices, such as hand gloves, reduces cross contamination in hospital staff, and hand rubbing with alcohol containing preparation can also help to reduce infection [62].

Some additional strategies applied in hospital settings to reduce infection are useful, such as creating "self-disinfecting" surfaces by applying coats of silver, liquid compound and copper, as they have persistent antimicrobial activities [85]. There are a number of no touch technology available such as aerosolized-vapor systems, and ultraviolet C radiation systems [85]. These systems are based on generating and spreading small disinfectant-containing particles throughout the patient care environments (patient rooms, operating rooms, etc.) [86]. These particles then land on the environmental surfaces and establish contact with microorganisms on these surfaces and ultimately deactivate by continuously emitting ultraviolet (UV-C) light, high-intensity narrow-spectrum (405 nm) light and a pulsed-xenon UV light system [86]. This assists in reducing infection transfer and is referred to as "no-touch" (automated) decontamination technologies [86]. These "no-touch" technologies have been shown to reduce bacterial contamination of surfaces [86].

Proper supervision of staff cleaning contaminated surfaces in dental clinics is very important, as some surfaces become repeatedly contaminated. According to the Center for Disease Control and Prevention (CDC) guideline of USA [71], the working surfaces are divided according to levels of risk. The guidelines provide further information on disinfection and sterilization processes in the dental clinic [87].

Interventions to control surface contamination [61]

To control infection transfer, the following six factors should be considered:

- Patient check-up to establish the source of pathogen transfer and to remove the route of infection.

- Personnel protection devices with blockade techniques have been developed to protect the eyes, mouth and hands of dental persons from infected blood or other infectious material, e.g. eye protection devices. Eye protection is recommended for dental staff where they are at risk of getting infections through exposure through their eyes. Hand coverings are intended to be discarded after a single use and are worn to prevent the transfer of infection between the patient and dental staff.
- Instrument autoclaved, including sterilization control.
- Disinfection of equipment and surfaces.
- Asepsis in the laboratory – the No Touch method: during dental treatment, dental health care worker should not touch any surface in the dental clinic.
- Proper management and disposal of infected waste [61].

2.8 Disinfection

Although there are six factors for infection control in hospitals, our main focus on disinfection use and its effectiveness. In practice, the effectiveness of the disinfectant is considered more important than how it is utilized [61]. Disinfectants are generally applied with water to remove blood and organic contaminants [61]. The following table shows the ideal requirement of disinfectant for hospital use (Table 2.1).

Table 2.1: Ideal requirement of disinfectant

Properties of disinfectant	**Importance**
Wide spectrum	Should be bactericidal on multiple microbes
Rapid acting	Should start effect immediate
Strong	Should be strong enough in presence of organic contaminant (e.g. saliva, sputum & blood and friendly with detergent, other chemicals and soap
Harmless and non-allergenic	Should be safe to the user
Surface compatibility	Should not damage or corrosive to metallic surfaces, nor weaken cloth, rubber and plastic
Residual effect	Should effective for long time leave an antimicrobial film
Easy to apply	Should easy and clear direction of use
Fragrance-free	Should have pleasant smell or odorless
Ecofriendly	Should not damage environment on discarding.
Cost-effective	Should be cheap

Ideal properties of disinfectants [89]

Table 2.2: Commonly used disinfectants in dental clinics.

Type of Disinfectant	**Application**
a. Iodophors	Hands, Surfaces
b. Aldehyde	Surfaces (mild aldehyde not irritate skin)

c. Alcohol/ Alcohol ammonium	Surfaces (reduced cleaning properties)
d. Quaternary ammonium compounds	Quaternary ammonium compounds
e. Peroxigenated compounds	Peroxygenated compounds
f. Peracetic acid	Surfaces

The most Commonly used chemical containing disinfect disinfectant in dentistry [24].

a. **Iodophors:** Iodophors are intermediate-level disinfectants and used at a dilution of 30-50 ppm. These are not active against spores but are active against fungi, lipophilic viruses, some hydrophilic viruses and bacteria [90].

b. **Aldehyde:** Aldehyde, especially formaldehyde and glutaraldehyde, are the most exclusively checked aldehyde, although they have killing activity, including succinaldehyde, glyoxal and benzaldehyde and ortho-phthaldehyde (OPA). Glutaraldehyde is a strong disinfectant at 2% concentration and has the capacity to destroy bacteria spores [8] and M. Tuberculosis. Glutaraldehyde does not affect rubber and plastic surfaces of equipment and is non-corrosive to metal. At very much low concentrations, Glutaraldehyde irritates the eyes, nose and skin of the health worker, even at 0.2 ppm vapour. Alcohol acts as intermediate disinfectant at 60-80%, specifically ethanol and isopropanol, which are used on surfaces. Some allergic reaction, asthma, epistaxis, rhinitis and contact dermatitis are commonly seen in health-care workers who are frequently exposed to glutaraldehyde [91].

c. **Alcohols**; In the medical field, alcohol and alcohol ammonium use as a disinfectant is very common and has been used from centuries for cleaning surfaces or as a hand rub [92]. Ethanol and isopropanol are the most frequently used agents. Alcohols are an intermediate level of disinfectant and are generally used at 60-90% concentration. Alcohols are used to disinfect small surfaces and are not able to destroy spores, while in the presence of water their activity is reduced [93].

d. **Quaternary ammonium:** These disinfectants exhibit antimicrobial activities and were recently introduced as surface disinfectant. These are noncorrosive, odorless, non-staining, and relatively non-toxic in nature. Didecyldimethylammonium chloride, benzalkonium chloride and alkyldimethylbenzyl ammonium chloride are products of quaternary ammonium that are used as surface disinfectants. Quaternary ammonium compounds are odorless, noncorrosive, non-staining and relatively non-toxic[24].

e. **Hydrogen peroxide** Hydrogen peroxide is a strong oxidizer and this quality increases its disinfectant activity, enabling it to be used as a high level disinfectant. It is bactericidal at 3% concentration but is not effective against vancomycin resistant enterococci. Chlorine and peracetic acid are stronger sporicidal than the 3% hydrogen peroxide. It is neither toxic to the environment nor carcinogenic or mutagenic to humans. Concentrated solutions may cause skin allergies and it may irritates the eyes [94].

f. **Peracetic acid** is a stronger germicidal and sporicidal agent than hydrogen peroxide. It is a strong active agent, which has been proved through vitro activity [95]. It is used as a high level disinfectant, as confirmed by the USA's Federation Dental Association (FDA) and remains active in the presence of organic matter.

   The study on the microbiological evolution of dental air turbines [24] was conducted to investigate microbial growth on dental hand pieces after various disinfectant procedures in an area of cross contamination. In that study nearly four disinfectants namely 1% peroxygen compounds and organic acids, alcohol, quaternary ammonium compound and in 100 g: 35 g 1-propanol, 25 g ethanol, 50 mg glutaraldehyde, 10 g 2ethylhexanal were used by spray technique. The study concluded that the disinfectant containing biguanide, quaternary ammonium compounds and alcohol have weak bacteria killing activity [96].

In a study where hospital surface contamination was evaluated in seven hospitals, the environment was found to be very complicated and considerable care was needed to maintain infection free surfaces. If proper universal principles of infection care are not followed, such as hand hygiene, proper cleaning and disposal of hospital waste, the chances of nosocomial cross contamination on hospital environmental surfaces may increase the amount of MRSA and pseudomonas isolates[97].

A study on the air-born microbiological contamination of surfaces in dental hospitals showed that a wide variety of cutaneous, oral, respiratory and environmental bacteria can be detected on settele plates cultivated on routine media in laboratory. *P. acnes, M. luteus and s. epidermis* are the most likely microbes to be found on contaminated surfaces in a dental clinic via the airborne route. The findings were that some pathogens were resistant to several antibiotics, and highlights the need for effective surface disinfection practices [98].

2.9 Methods of identification of bacteria

Select bacteria identification methods need to consider ease of method, accuracy, cost effectiveness, the required handling of sample processing after incubation, time required, reliability on result, skill level requirement, and the availability and extent of the databases [32].

This study will use mass spectrometry technology, specifically MALDI-TOF MS, which is relatively simple, correct, and rapid and has low consumable costs [35]. MALDI-TOF MS requires only overnight culture of bacteria data, which are available within 16 hours to identify microbes, and uses limited sample volume and with low reagent costs. This has been recently developed as a dominant device for recognizing clinical bacterial strains [63]; [64]; [65]; [66]; [67]. Various methods are currently used to detect bacteria, most of them being phenotypic.

2.10 MALDI-TOF MS approach

Hospital surfaces are contaminated with large amounts of bacteria, some of which are pathogenic and some are non-pathogenic [99]. Some of the bacteria, such *as Enterobacteria, Pseudomonas, Bacillus spore, Staphylococci, Klebciall, as methicillin-resistant, Staphylococcus MRSA, Acitobacteria, vancomycin-resistant Enterococcus (VRE), vancomycin-resistant and specially STREAK bacteria*, have the capacity to survive on surfaces for a long time, even in dry weather [99]. The frequent testing of hospital surfaces are seldom recommended as they are neither warranted nor cost effective. Surfaces sampling is frequently done to check the epidemiology of hospitals and to determine which microbes are present on their surfaces, which areas of dental units are more infected [99]. To identify the bacteria in this study the MALDI- TOF approach was used, as it is relatively simple, rapid and within 16 hours a cost-effective result can be achieved with significantly lower consumables compared to other identification approaches [100].

Microbes are very small and are not visible to the naked eye, thus two methods are used to study them: 1. growing them on media to become visible with the necked eye, and 2. using microscopy to identify them, in combination with stain to make them observable. When the bacteria grows on requisite media, they multiply by binary fission to create a characteristic mass of genetically identical bacteria, which are called a colony, and considered to be identical in shape, color, consistency, margins and elevation for specific of the same microbes [101]. These masses are genetically same and called a colony which is as pure, identical in shape, color, consistency, margins and elevation of these colonies are same for specific microbes [102].

For the use of MALDI to identify bacteria, fresh colonies of bacteria of 16 hours old are selected and their protein extracted by treating it with chemicals[103]. Checking the protein takes place because the genome of every bacterial species is unique, as spectra for the specific bacteria may vary with an increase in the number of veritable snapshot of that organism's proteome. For bacterial identification, the protein mass is checked and masses in range of 2000 to 20,000 kDa is taken into consideration 102]. The snapshot reveals a level of exclusivity that may be used for detection purposes.

CHAPTER 3: METHODS AND MATERIALS

3.1 Introduction

This chapter outlines the methods and materials used to conduct the study. The study was conducted at the Oral and Dental Training Centre (ODTC) in King George Hospital (King DinuZulu Hospital). The clinic operates five days a week (from Monday to Friday: 7.30 to 16.00). This dental training centre is affiliated with the University of KwaZulu-Natal (UKZN) and is an accredited site for undergraduate dental clinical training for dental therapy and oral hygiene students. The centre is also used as a clinical placement site for student dental assistants at the Durban University of Technology. The staff complement at this training centre includes eight dentists, four dental therapists, three oral hygienists, 14 dental assistants, four admission clerks and six general assistants. Approximately 150 patients visit the centre on a daily basis. Approximately 80 undergraduate dental therapy and oral hygiene students are placed in the centre for service-based clinical training, and use the clinic on different days of the week. There are 17 dental units available for clinical training and a further seven units for oral health service delivery, and are also used by the dental professional staff when the students are not in the clinic. The dental operatory surfaces are damp dusted with surface disinfectants after every patient use. The floors are cleaned once a day at about 8.00 in the morning.

3.2 Study design

This was a cross-sectional, descriptive study using a non-experimental design.

3.3 Data Collection

The study consisted of two components, the first being the application of three commonly used disinfectants on identified dental environmental surfaces in a public dental clinic facility in KwaZulu-Natal. The second consisted of samples being taken at specific time intervals to determine the microbial growth following surface disinfection. The samples were collected over a period of one month (March 2016). Only the 17 dental units allocated to the undergraduate training programme were used for the study, with systematic random sampling being used to select nine units for the study. All dental units are numbered and those numbered: 1, 3, 5, 7, 9, 11, 13, 15, 17 were selected for the study. The dental clinical environment was divided into four zones:

1. working area around the dental operator/assistant (chair head rest, arm rest, foot rest, dental hand pieces, overhead light source, air water syringe tip, spittoon, suction hose, based of dental chair, dental stool, foot control, instrument counter and handle)
2. area behind chair (wash basin, computer monitor, window, wall, table top, dust bin, taps),

3. area away from chair (computer processing unit, telephone, floor) and
4. Reception area (patient chairs, reception table top).

All these areas added up to 26 surfaces that were sampled at each of the nine units.

The process of data collection was as follows:

1. Swabs were collected from the 26 identified areas at 7.00 in the morning prior to the normal infection control and cleaning procedures that are conducted in the clinic.
2. Chlorine, ethanol (70% in water) and Glutaraldehyde (2%) disinfectants were applied on all 26 surfaces of the nine dental units on the days in which the swabs were taken for that disinfectant. This was done by using a spray method, as per the manufacturer instruction on the 26 and wiped with a cloth for one minute.
3. The charcoal swabs were taken at specific times during the day (7am, 9am, 11am, and at 16.00) and stored below 23 °C, after which they were taken to the microbiology laboratory for plating on plates of nutrient agar and incubated at 37 °C for 24 hours to enable microbial.
4. Colony forming unit (C.F.U.) counts of plates were checked for the highest number of different colonies after use of the disinfectant.
5. 312 swabs from nine dental units and the reception area in the following manner:

Day 1: Chlorine:

Chair 1: 26 swabs at 7am after cleaning and at 9am,

Chair 3: 26 swabs at 11 am

Chair 5: 26 swabs at 4 pm.

Day 2: Ethanol:

Chair 7: 26 swabs at 7am after cleaning and at 9am,

Chair 9: 26 swabs at 11 am

Chair 11: 26 swabs at 4 pm.

Day 3: Glutaraldehyde

Chair 13: 26 swabs at 7am after cleaning and at 9am,

Chair 15: 26 swabs at 11 am

Chair 17: 26 swabs at 4 pm.

This resulted in 104 swabs being collected after use of one disinfectant, with a total of 312 for the three disinfectants.

3.3.1 Collection of bacterial strains

The 312 swab samples were taken from the various environmental surfaces by following the CDC guideline for surface sampling [67]. The procedure of obtaining the swab samples was done with dipping cotton tipped in autoclaved water, hand-held applicators (Sterilin, England), which were premoistened with autoclaved normal saline. These applicators were gently agitated /rotated over the desired environmental surface. To evaluate the quality of our work randomly swabbing of autoclaved surfaces of instrument was checked from hospital which acts as regulator.

Normally after every patient treatment, the chair was wiped with a paper napkin for one minute and the identified sites were swabbed at the appropriate times. The cotton tip was immediately plated on nutrient agar media for a contact time of one minute. After swabbing the nutrient agar plates are transported to micro laboratory through cool box and after that all plates are immediately placed in incubator for 24 hours whose temperature is carefully maintained at 37°C [68]. After 24 hours bacterial growth was checked if it was sufficient then and then only colony forming units (CFUs) counting was done otherwise plates were kept in incubator another 24 hours. Then colony forming unit in the range of 1-100 were counted, and the data was represented as the number of CFU per cubic meter of surface sampled. The microbial colonies were differentiated by observation and sub culturing was done either in bacterial[69] or fungal species (16) and other criteria such growth on the specific growth media used, in addition to the MALDI TOF (Becton Dickinson Diagnostic Instrument System, Sparks, MD) [7]. All bacterial isolates were saved at very low temperature i.e. at -80 °C in trypticase soya broth with 10% glycerol and recovered at 37 °C in Nutrient Agar medium prior to use. Primary subcultures were used for MALDI-TOF MS analysis. The spectra which called as mass spectra were deposited in triplicate batches for each new strain. The same experiment was repeated for following day for the reputability test.

3.3.2 Preparation of intact bacterial cell

All strains of bacteria were sub-cultured in selective medium (Sigma) at 37 °C for a day. New bacterial strains were transfer into eppendorf tubes containing 300 µL autoclave MilliQ water that has a density of OD600 = 0.8, and were then pipetted into a new eppendorf tube and mixed with 900 µL of 100% ethanol (HPLC grade, Sigma Aldrich Germany). The mixture was centrifuged for two minutes at 13000 rpm, which that supernatant was removed and again centrifuged at 13000rpm for two minutes, and the ethanol removed completely. The pellet after removal of the ethanol mixture was again centrifuged for two minutes at 13000 rpm followed by vortex. An aliquot of 1 µL of the supernatant was applied directly onto a polished still plate of MALDE-TOF machine. After aliquot allow it to dry for two minutes, 1 µL of the saturated matrix solution containing acetonitrile, trifluoroacetic acid and sterile MilliQ water (HPLC

grade respectively) with α-4-cyanohydroxycinnamic acid (Bruker Daltonics, Germany) was applied onto the sample. The prepared target was air dried prior to the MALDI-TOF MS analysis. Mass spectra were acquired in triplicate batches for each isolate. The same experiment was repeated the following day for the reputability test.

3.3.3 Mass Spectrometry Tests

The spectra were obtained using the spectrometer of a MALDI-TOF (Bruker Daltonics) that had been prepared with the nitrogen laser. Spectra were assigned in the linear mode of positive ions (20 kV) in the range of molecular masses 2000 - 20000 Dalton (Da). The spectra checked Bruker Bacterial Test Standard (BTS) which act as regulator obtained from Bruker Daltonics Germany and include carefully manufactured extract of E. coli DH5 alpha with which contain two extra high molecular pecks by this calibrant mixture. The increase of detection sensitivity was achieved by matrix excess removal of six laser impulses at 40% power output, followed by subsequent data acquisition at laser power from 30-40%. Every spectrum was obtained with 200 laser impulses, with the spectra with a resolution exceeding 400 being obtained. The programmed software (flex control version 3.4 build 119, flex analysis version 3.4 build 70) was used to treat, obtain and analyse the spectra to identify the isolates at the strain level. Identification was using the Biotype 3.1 software of MALDE, which contains 4613 microbial spectra as a reference library for various types of microbes. Genus identification was considered reliable, when the required score being in the range of 1.999 to 2.299. The score from 2.000 to 2.299 provided reliable genus identification only (Table3.1)

3.4 Pilot study

The ODTC at the King DinuZulu Hospital, has two areas one where student work and another where the skilled dentist treat complicated patient. The pilot study was conducted in the area used by the skilled dentists, with ethanol being used as the disinfectant to check the affectivity of the project. Each unit contains a chair, computer, table top, material cabinet and two units separated by four feet wall. Surface samples were collected at 26 sites over a 20 minute period. As mention in Table 3.1, 26 sites are located in the active dental treatment area. Samples were collected before and after disinfectant use at different time interval. Microbial growth was noted in each of these, which assisted in establishing the possibility, cost, time, unpleasant actions, and affect size (statistical variability) of the study. After the pilot study had been conducted, the full study was undertaken, as described above.

3.5 Swab analysis

The swabs obtained before the application of the disinfectant from unit and reception areas were the control group, all of which showed 100% microbial growth for the presence of six types of bacteria. The samples from the sites disinfected with chlorine, ethanol and glutaraldehyde were analyzed using a standardized process of laboratory methods. Various data analysis tools were employed for the optimization in this study.

The first phase of the analysis was evaluating the bacteria visually from agar plates by their morphology followed by sub-culturing. Subculture colonies were grown on selective media with respect to bacteria, such as MacConkey agar as selective media for Gram-negative bacteria. S. aureus was isolated using Manitol salt agar from other Staphylococcus species, Enterobacteria identification done by Bile salt agar. Isolation and identification of microorganisms were done according to the standard procedure [13].

The second phase of the analysis was the quantification of the bacteria in the collected samples using serial dilutions, plating and counting methods. This is an appropriate method for microbial enumeration, and entails comparing the colony forming units (C.F.U.) of the sample to the dilution factor. This experiment identified the number of distinct colony and (C.F.U.) count.

The third phase of this analysis was the species identification of the unknown isolates via MALDI Biotyping. To analyze experimental group strain we use, *E. coli* ATCC 25922 and BTS were used as celebrants while checking unknown isolates, so that we were confident on identification capacity of this technique.

3.6 Data analysis

The data analytical processes are presented in line with the objectives of the study. Firstly, the bacterial growth in the samples was checked before the application of the disinfectant, with the data showing all swabs samples were 100% contaminated (i.e. 100% bacteria growth) which became measure to check other data . That data was compared with the four areas (chair side area, area behind chair, area away from chair and reception area) in that all areas showed bacterial growth, with the reception area showing 100% positive culture samples, even after the application of different disinfectant it is more risky. The area which shoes more percentage positive samples had more potential to transfer microbes and favorable for cross-contamination in the dental clinic. Chi square statistical analysis was used to calculate the probability of contamination.

The colonial growth on the MALDE-TOF machine was checked and compared to the experimental bacterial spectra with regulator group spectral bacteria (*Bruker Bacterial Test Standard (BTS) and E. coli DH5 alpha).*

The microbial count was determined and compared after use of disinfectant at a specific time interval (the 7am data was compared to the 11 am results). The viable colony forming unit count (C.F.U.) in the range of 10 to 100 was considered to create infection. We compared all data before the application of disinfectant with the data at different time interval of the respective disinfectant. Univariate descriptive statistics such as frequency and mean distribution were conducted for all variables. An inferential technique such as the Pearson chi-squared test was used to determine a relationship between the use of the specified disinfectants and the positive bacterial growth. Commonly for biological research, a level of $p<0.05$ was established as being significant.

In order to determine and compare the microbial count on identified surfaces in the operating and reception area of the dental clinic at specific times in the day, we compared the viable C.F.U. count of the operating area before the application of disinfectant with the microbial count of the reception area. Chi-square analysis was used to calculate relationship between the level of contamination of reception and operating area.

3.7 Reliability and Validity

The key quality assurance process was the identification of each colony forming unit's molecular masses which was then compared to the bio-information repositories MALDI-TOF. This comparison of biofilm cultures with known bio-information of bacteria (that act as a reference) added to the validity of the study. The reliability of the results was achieved by repeating each test three times to eliminate any findings that had outliers.

3.8 Data Management- storage and access

Access to the data was restricted to authorized users (the researcher, supervisors and statistician) only. Written data will be handed to the supervisor and securely stored in a locked cupboard for a period of five years after which it will be destroyed by shredding. The electronic data was stored on digital versatile discs (DVD's) and stored in a locked cupboard at the Discipline of Dentistry offices (Westville campus of the University of KawaZulu Natal) where it will be stored for five years after submission of the final dissertation, after which it will be physically destroyed and then burnt.

3.9 Ethical considerations

Ethical approval was obtained from the Biomedical Research Ethics Committee (BREC Reference number: 068/16) and the KZN Department of Health (Reference number: 26/16 KZ-2015RP12-306. All records collected will be kept at the University of KawaZulu Natal (Discipline of Dentistry).

3.10 Time frame

Activity	Time
Submission of Research Proposal	March to November of 2015
Data Collection	March and April of 2016
Data Analysis	May to August of 2016
Submission of First draft	October of 2016
Submission of Final Project	November of 2016

3.11 Budget for the Project

Activity	Cost
Stationary	R 3000
Manuscript printing	R 7000
Email access	R 2000
Transport	R 3000
Lab Equipment	R 15000
Total	**R 30,000**

3.12 Dissemination of results

The results of the study will be made available to the Department of Health, KZN; The Head of the Discipline of Dentistry, UKZN and the Clinical and Medical Managers in charge of the Oral and Dental Training Centre at King DinuZulu Hospital. A feedback session, in the form of an oral presentation will be held with the dental therapy students, dental assistants and oral hygiene students to ensure that corrective measures are in place for infection control practice. The results will also be disseminated via conference presentations and journal publications.

3.13 Summary

The hospital environment is a complex ecology and many interventions are required to ensure the highest standard of infection control. The knowledge of surface bacteria and the effectiveness of disinfectants is equally important. There are many disinfectants available and almost all manufacturers claim that theirs

are active against a wide-range of microbe agent suitable for diverse applications [5]. The study focused on Chlorine, Ethanol and Glutaraldehyde to determine the effectiveness as disinfectants.

CHAPTER 4: MANUSCRIPT

Isolation of common aerobic bacterial pathogens from the dental environmental surfaces after the use of disinfectants: A case study at a public dental clinic

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Abstract

Introduction: Cross infection in the dental clinical environment remains a low priority in conversations around oral health care despite the high risk of exposure to blood and airborne infections.

Aim and objectives: The aim and objectives of this study was to determine the effectiveness of selected surface disinfectants on specific dental environmental surfaces by comparing the microbial count after use of disinfectant at specific time intervals at a public oral health facility in KwaZulu-Natal.

Methods: This was a prevalence (cross-sectional) descriptive case study with a non-experimental design. The dental clinical environment was divided into four zones and 9 dental units were systematically selected. Swabs were collected from the 26 identified areas at specific time intervals (7am, 9am, 11am, and at 16.00) after the use of chlorine, ethanol (70% in water) and glutaraldehyde (2%) as surface disinfectants. The collected swab samples were cultured in nutrient agar media for two days at 37°C. The colony forming units were then examined and characterized using the **MALDI-TOF** spectrometer.

Results: Out of the 312 samples taken, 262 (84%) were shown to be bacterial culture positive. The most contaminated areas in the dental environment were around the chair area (86.53%) and the area away from the chair (92%). Glutaraldehyde was found to be more effective than chlorine and ethanol.

Conclusion: The study suggests that there was an association between frequency of cleaning, the type of disinfectant used and the microbial count at the specific dental environmental surfaces in the identified oral health facility.

Keywords: infection control; disinfectants; dental environment; bacteria; cross-infection (contamination).

Introduction

Cross-contamination in the health (oral) clinical environment should be a major public health concern given the high risk of exposure to blood and airborne infections however this remains a low priority in conversations around oral health care. The greatest potential for cross-infection is among dental operators, assistants and patients, due to the presence of a combination of blood, saliva and contaminated instruments[1]. Apart from these possible sources of cross contamination, the environmental work surfaces and water lines of dental units could pose a potential risk unless optimal infection control measures are in place. Patients have the right to access oral health treatment in a safe and healthy clinical environment[3]. All oral health care staff have a legal obligation of caring for their patients, co-workers and the general public to create a safe and healthy work environment that includes optimal infection control practices[4]. Universal precautions in infection control include hand washing/disinfection; use of personnel protective equipment (e.g. gowns, protective eye wear, and gloves); use of disinfectants for clinic surface cleaning; and appropriate handling and disposal of contaminated sharp instruments[5-7].

Ideally all vegetative microbes should be destroyed using heat sterilization procedures. However, it is neither cost effective nor practical to sterilize dental working surfaces or instruments that are heat sensitive[8].

Disinfection provides a more practical solution for ensuring prevention of cross contamination[1]. Disinfection is characterized by killing, destroying or removal of all pathogenic microbes, except spores[8, 9]. While it is preferable for all pathogenic organisms to be destroyed, a decrease in their quantity is deemed acceptable[9]. There are various disinfection methods, which include physical disinfection, heating and chemical disinfection [10]. The disinfectants used in most hospital settings include Aldehyde, Alcohol, Iodophors, Quaternary ammonium compounds, Alcohol ammonium, Peroxigenated compounds [11]. The effectiveness of a disinfecting solution is dependent on factors such as the 'concentration and nature of contaminating microorganisms, the concentration of the chemical, the exposure time and the amount of accumulated bio-burden' [12].

Environmental surfaces are classified as clinical (medical) contact and housekeeping[13]. Clinical contact surfaces are those that comes in contact with contaminated devices, instruments, hands, or gloves, while housekeeping surfaces are not touched directly during the delivery of dental care [13]. Patients and staff engage with the various surfaces throughout the day, in the waiting rooms, clinics, and other rooms in a dental clinic, thus providing many opportunities for cross-contamination and

cross-infection[14]. Damp dusting is a common disinfection procedure in the dental clinical environment[15].

This study arose of the need to investigate the effectiveness of commonly used surface disinfectants on dental environmental surfaces. There is a paucity of evidence that specifically examined the efficacy or effectiveness of commonly used surface disinfectants in oral health clinical settings[16]. There is also very scarce literature on the spread of infection through cross-contamination in dental clinics in South Africa or on the use of disinfectants in KwaZulu-Natal province. There is no standard method to establish if dental surfaces are infection free. The consumer generally relies on the information supplied by the manufacturer regarding the efficacy of a disinfectant and many disinfectant manufacturers claim that their products are broad-spectrum and can kill all microbes.

The aim and objectives of this study was to determine the effectiveness of selected surface disinfectants on specific dental environmental surfaces by comparing the microbial count after use of disinfectant at specific time intervals at a public oral health facility in KwaZulu-Natal. The null hypothesis was that there is no correlation between frequency of cleaning and damp dusting and the microbial count at the identified dental clinic. The study site was a public oral health facility in Durban. This facility is also used as a clinical teaching platform for undergraduate oral therapy and dental hygiene training. The facility is quite busy and registers an excess of 100 patient visits on a daily basis. There are 17 dental units used for undergraduate clinical training and 5 dental units for oral health service delivery. Damp dusting of the dental operatory surfaces occurs after every patient and overall cleaning of the floors occurs once a day.

Methods

This was a cross-sectional descriptive case study using a non-experimental design. Ethical approval was obtained from the Biomedical Research Ethics Committee (BREC Reference number: 068/16) and the KZN Department of Health (Reference number: 26/16 KZ-2015RP12-306). Systematic random sampling was use to select 9 dental units for the study purpose. All dental units used in the facility are numbered and those numbered: 1, 3, 5, 7, 9, 11, 13, 15, 17 were selected for the study. The dental clinical environment was divided into four zones:

5. working area around the dental operator/assistant (chair head rest, arm rest, foot rest, dental hand pieces, overhead light source, air water syringe tip, spittoon, suction hose, based of dental chair, dental stool, foot control, instrument counter and handle)

6. area behind chair (wash basin, X-ray monitor, window, wall, table top, dust bin, taps),
7. area away from chair (computer processing unit, computer monitor, telephone, floor) and
8. Reception area (patient chairs, reception table top).

All these areas added up 26 surfaces that were sampled at each of the nine units.

Swabs were collected from the 26 identified areas at 7.00 in the morning prior to the normal infection control and cleaning procedures that are conducted in the clinic. Chlorine, ethanol (70% in water) and Glutaraldehyde (2%) disinfectants were applied on all 26 surfaces of the nine dental units on the days in which the swabs were taken for that disinfectant as indicated below.

Day 1: Chlorine:

Chair 1: 26 swabs at 7am after cleaning and at 9am,

Chair 3: 26 swabs at 11 am

Chair 5: 26 swabs at 4 pm.

Day 2: Ethanol:

Chair 7: 26 swabs at 7am after cleaning and at 9am,

Chair 9: 26 swabs at 11 am

Chair 11: 26 swabs at 4 pm.

Day 3: Glutaraldehyde

Chair 13: 26 swabs at 7am after cleaning and at 9am,

Chair 15: 26 swabs at 11 am

Chair 17: 26 swabs at 4 pm.

This was done by using a spray method, as per the manufacturer instruction on the 26 sites and wiped with a cloth for one minute. The procedure of obtaining the swab samples was done with dipping cotton tipped in autoclaved water, hand-held applicators (Sterilin, England), which were pre-moistened with autoclaved normal saline. These applicators were gently agitated /rotated over the desired environmental surface.

The charcoal swabs were taken at specific times during the day (7am, 9am, 11am, and at 16.00) and stored below 23 °C, after which they were taken to the microbiology laboratory for plating on plates of nutrient agar and incubated at 37 °C for 24 hours to enable microbial. Colony forming unit (C.F.U.) counts of plates were checked for the highest number of different colonies after use of the disinfectant. This resulted in 104 swabs being collected after use of one disinfectant, with a total of 312 swabs for the three disinfectants.

The microbial colonies were differentiated by observation and sub culturing was done either in bacterial or fungal species and other criteria such growth on the specific growth media used, in addition to the MALDI TOF (Becton Dickinson Diagnostic Instrument System, Sparks, MD). All bacterial isolates were saved at very low temperature i.e. at -80 °C in trypticase soya broth with 10% glycerol and recovered at 37 °C in Nutrient Agar medium prior to use. Primary subcultures were used for MALDI-TOF MS analysis. The spectra which called as mass spectra were deposited in triplicate batches for each new strain. The same experiment was repeated for following day for the reputability test.

A pilot study was conducted on dental units that were not a part of the identified clinical area so as to iron out any challenges in the data collection process.

Data Analysis

The first phase of the analysis was evaluating the bacteria visually from agar plates by their morphology followed by sub-culturing. Subculture colonies were grown on selective media with respect to bacteria, such as MacConkey agar as selective media for Gram-negative bacteria.

The second phase of the analysis was the quantification of the bacteria in the collected samples using serial dilutions, plating and counting methods.

The third phase of this analysis was the species identification of the unknown isolates via MALDI Biotyping.

Firstly, the bacterial growth in the samples was checked before the application of the disinfectant, and this became the control measurement. This data was compared with the four selected areas (chair side area, area behind chair, area away from chair and reception area).

The colonial growth on the MALDE-TOF machine was checked and compared to the experimental bacterial spectra with regulator group spectral bacteria (*Bruker Bacterial Test Standard (BTS) and E. coli DH5 alpha).* The microbial count was determined and compared after use of disinfectant at a specific time interval (the 7am data was compared to the 11 am results). The viable colony forming unit count (C.F.U.) in the range of 10 to 100 was considered to create infection. Univariate descriptive statistics such as frequency and mean distribution were conducted for all variables. An inferential technique such as the Pearson chi-squared test was used to determine a relationship between the use of the specified disinfectants and the positive bacterial growth. A level of $p<0.05$ was established as being statistically significant.

The key quality assurance process was the identification of each colony forming unit's molecular masses which was then compared to the bio-information repositories MALDI-TOF. This comparison of biofilm cultures with known bio-information of bacteria (that act as a reference) added to the validity of the study. The reliability of the results was achieved by repeating each test three times to eliminate any findings that had outliers.

Results

Most contaminated areas of the dental clinic

Altogether 312 swabs samples were collected from various sites and 260 (83.33%) were found bacterial positive. Almost ten different species of bacteria and fungi in which *staphylococci and Bacillus spp* were common isolates, were identified (Table 4.1 and 4.2). However, we did not check multidrug-resistant bacteria.

Table 4.1: *Microbial count (C.F.U. count) present at different surfaces after use of three common disinfectants at different time interval in operating area.(- = No bacterial growth, N = More than 100 colonies)*

Area Time	Chlorine								Ethanol								Glutardlhyde							
	No. of colonies at 7AM	C.F.U. count 7 AM	No. of colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day	different colonies at 7AM	C.F.U. count 7 AM	different colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day	different colonies at 7AM	C.F.U. count 7 AM	different colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day
Chair Side Area	**Out of 156 samples135 had bacteria (86.53 % contamination rate)**																							
Head rest	4	20	4	60	4	30	4	60	4	50	3	20	4	30	5	50	5	N	1	1	4	50	4	50
Arm rest	6	30	5	25	6	N	5	25	6	40	3	10	6	N	6	70	3	10	3	50	3	50	3	50
Foot rest	5	20	6	100	4	N	6	100	5	25	5	25	3	N	6	90	3	20	2	-	3	50	4	50
Aerotar	5	25	-	-	4	40	6	20	5	40	-	-	3	0	5	20	6	50	-	-	-	-	1	3
Light source	2	3	-	-	3	4	5	40	4	3	0	0	3	60	4	20	3	3	2	5	3	50	3	10
Air water syringe tip	1	1	-	-	4	N	3	40	3	3	-	-	5	50	5	60	3	20	-	-	4	20	1	5
Spittoon	4	20	3	20	6	60	4	20	5	30	4	30	-	-	5	30	4	100	3	10	2	50	4	60
Counter top	10	20	4	N	0	0	4	10	2	30	3	-	3	-	4	30	4	10	-	-	-	-	-	-
Suction hose	3	N	-	-	5	0	7	N	5	N	2	-	3	20	7	N	4	50	-	-	3	20	4	50
Chair base	3	30	7	N	2	10	3	20	3	30	3	30	2	N	5	50	5	50	-	50	3	10	3	20
Dental stool	8	30	3	20	3	15	3	30	7	30	4	25	3	20	4	20	4	50	5	10	-	-	3	10
Foot controller	3	N	3	30	7	70	7	N	4	N	3	20	7	60	7	N	7	N	2	25	4	20	4	50
Handle	4	4	7	N	4	10	5	16	3	4	4	30	4	30	4	30	4	40	3	-	-	-	3	10

Table 4.1: *Microbial count (C.F.U. count) present at different surfaces after use of three common disinfectants at different time interval in operating area.(- = No bacterial growth, N = More than 100 colonies)*

Area Time	Chlorine								Ethanol								Glutardlhyde							
	different colonies at 7AM	C.F.U. count 7 AM	different colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day	different colonies at 7AM	C.F.U. count 7 AM	different colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day	different colonies at 7AM	C.F.U. count 7 AM	different colonies at 9AM	C.F.U. count 9 AM	different colonies at 11AM	C.F.U. count 11 AM	different colonies at end of day	C.F.U. count at end of day
Area behind chair	**Out of 84 samples 59 samples are bacteria +that is 70.23% contamination rate**																							
Wash basin	4	25	5	50	4	50	5	50	5	30	4	40	4	20	5	30	3	10	2	4	3	10	3	20
X-ray monitor	5	N	0	0	0	0	0	0	5	N	0	0	0	0	0	0	4	30	-	-	-	-	3	20
Window	3	20	0	0	0	0	0	0	4	24	0	0	0	0	2	30	3	30	-	-	-	-	-	-
Wall	1	2	3	5	1	9	3	5	1	2	0	0	0	0	3	5	5	28	-	-	1	B	3	50
Table top	3	4	4	20	3	10	4	20	3	6	0	0	5	N	4	30	6	50	2	3	1	B	3	10
Dust bean	8	N	3	20	7	N	3	20	7	N	0	0	7	N	6	90	4	20	2	B	3	5	3	50
Tap	2	20	2	5	7	N	2	5	2	30	0	0	0	0	5	50	2	50	-	-	-	-	2	10
Area away from chair	**Out of 48 samples44 samples are bacteria +that is 92% contamination rate**																							
CPU	5	30	6	40	5	N	6	40	6	36	0	0	2	10	3	30	1	N	-	-	3	5	3	5
Computer Screen	3	50	6	30	4	N	6	30	4	50	0	0	2	10	4	20	3	50	-	-	-	-	-	-
Telephone	3	70	4	15	2	3	4	5	4	50	2	10	3	10	4	20	4	50	3	B	4	5	3	50
Floor	8	N	7	150	7	N	7	150	8	N	5	100	7	N	7	N	6	70	3	10	2	50	4	50
Reception area	**Out of 24 samples24 samples are bacteria +that is 100% contamination rate**																							
Sitting chair	4	N	5	40	4	80	7	N	6	N	2	10	5	60	7	N	5	100	4	50	4	60	6	100
Reception table top	4	20	4	20	4	60	7	N	4	20	2	12	4	50	7	N	6	N	7	60	6	100	5	60

The most contaminated areas in the dental environment were as follows: around the chair area was 86.53 % and the area away from chair was 92% (Table 4.1). It should be noted that the area around the chair was damp-dusted after every patient but that the contamination levels were still quite high despite infection procedures. The second most contaminated area observed, was the area away from the dental chair i.e. 92%, such as the computer, telephone, reception and floor.

Table 4.2: *Evaluation of bacterial isolates by the MALDE-TOF Biotyping method.*

Organism (best match)	**Score Value**	**Organism (second best match)**	**Score Value**
Enterococcus faecalis	2.051	*Enterococcus faecal*	1.91
Escherichia coli	2.148	*Escherichia coli*	2.146
Bacillus megaterium	2.000	*Bacillus megaterium*	1.977
Klebsiella pneumonia	2.212	*Klebsiella pneumonia*	2.102
Staphylococcus aureus	2.037	*Staphylococcus aureus*	1.778
Neisseria perflava	2.32	*Neisseria perflava*	2.025
Pantoea ananatis	2.008	*Pantoea ananatis*	1.95

Types of microbes identified in dental clinic

The dental chair, especially the spittoon and the floor of the dental unit, were one of the highest recorded contaminated sites in each samples it shows bacterial growth which shows 100% contamination rate. The results were classified into two parts: samples before disinfectant use and samples after disinfectant use. Gram- positive cocci (CoNS, *Enterococcus spp and S. aureus*,) and Gram-negative *enteric bacilli (pneumonia, Enterobacter spp and Klebsiella)* were the majority frequent isolates (Table 4.2).

Bacteriological species detection of all selected stains [18, 19] was established by MALDI-TOF MS profiling. The *E-coli, Enterobacteria, Bacillus megaterium, Nisseria, Klebsiella pneumoniae, Enterobacter cloacae, and Staphylococcus aerus* were analyzed on the present Biotyper method database (Figure 4.1).

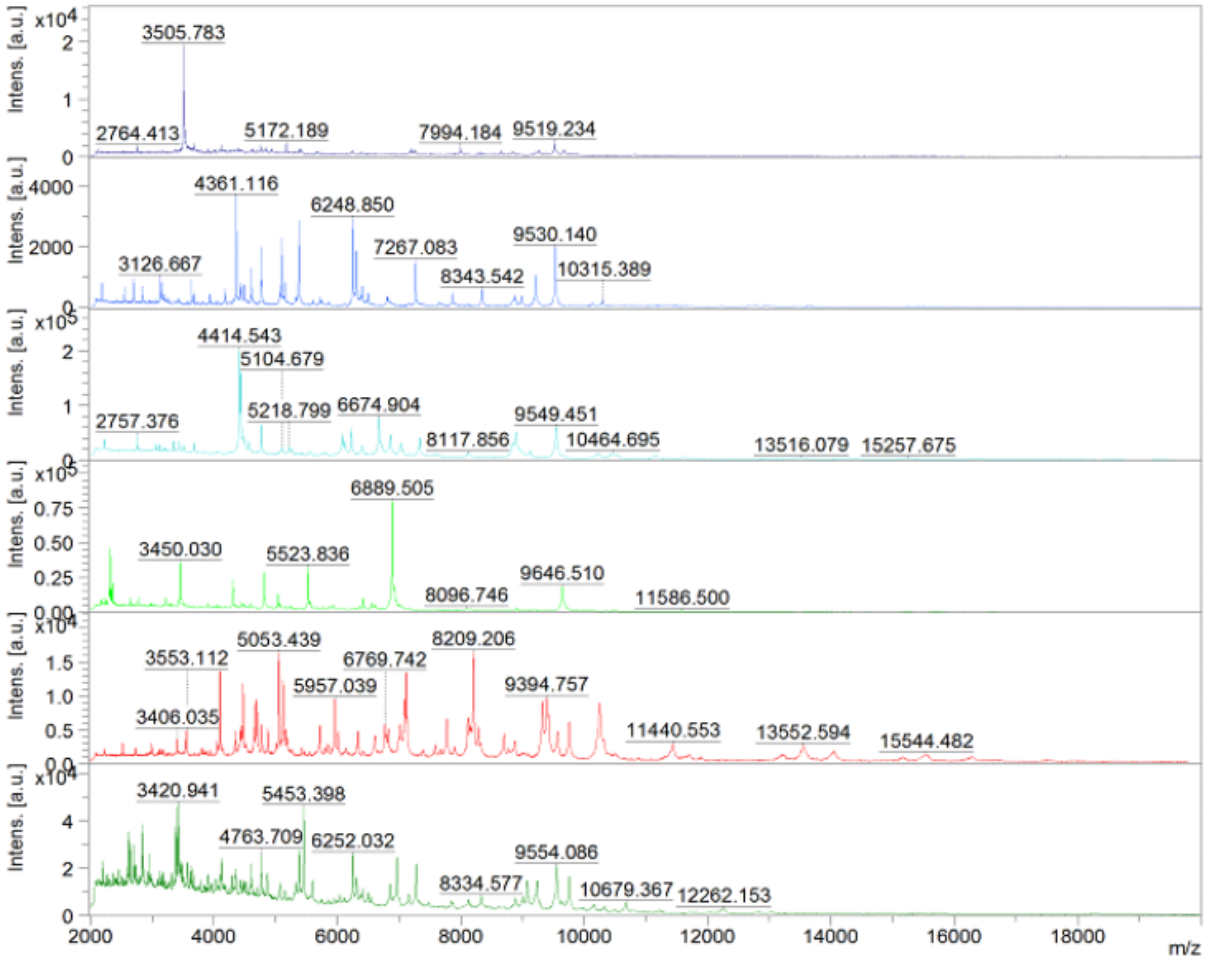

Figure 4.1: MALDI-TOF mass spectra of *Enterococcus faecalis* (dark blue), *Escherichia coli* (light blue), *Bacillus megaterium* (sky blue), *Klebsiella pneumonia* (light green), *Staphylococcus aureus*(red),*Neisseria perflava*(dark green) respectively.

A main spectrum (MSP) of the standard control strain was created to ensure that the create formation procedure include all peaks that were recognized as limited between the genera. An MSP is the basic of classification using MALDI Biotyper. It is a reference spectrum (normally an average of six to eight combined spectra) or more precisely mention peak list that is registered to strain or species. The ideal detection method gives a score of 2.9 indicating false positive for the genus and species detection. The bacterial isolates were scanned for identification by MALDI-TOF machine coordinated to give complete scores for their entries (score of 3.000). The differences of scores between the first matched strains and second matched strains were 0.617 to 0.957 which are considered as reliable in terms of genus identification.

Microbial count after use of disinfectant at specific time interval

The percentages of microorganism growth after disinfection procedures at specific time intervals are indicated in below Table 3. Glutaraldehyde and Ethanol had the more in vitro bacteria killing activity. The results indicate that chlorine® was not active against several bacteria because it showed that 92% samples had a positive growth at the end of day and its mean value is 22.66 which is quite more than the ethanol and Glutaraldehyde . Ethanol was a more effective in initial period and gradually reduced its effectiveness. Ethanol was more effective only 56 % samples were positive at 9am but at the end of day this increased to 96 % its mean value is 20.33 which is less than chlorine but more than Glutaraldehyde so can consider as intermediate disinfectant. Glutaraldehyde was found to be more effective than Chlorine and Ethanol its mean value is very less than chlorine and Glutaraldehyde. The results indicate that 52% samples were positive at 9 am and 82% of samples were found to be positive at the end of day. Bacterial survival rate was found to be less with the use of Glutaraldehyde.

The microbial count on identified surfaces in the operating and reception area at specific time interval:

The C.F.U count before application of disinfectant was high. After application of disinfectant count was very less in Glutaraldehyde and more in chlorine, but count increases at the end of day which mentioned in Table no 4.1. The microbial count in the reception area before the application of the disinfectants was high, and remained high despite the application of disinfectant. In the reception area, every samples showed positive bacterial growth, even after application of disinfectant, which indicates that the reception area is more contaminated than the operating area (Table 4.2).

More effective disinfectant

With respect to objective 5 there was positive bacterial growth recorded after use of all three disinfectants. As mention in above table (Table 4.3 and 4.4) the highest bacterial growth was recorded after use of Chlorine® which is (92% of samples) at the end of day. The use of Glutaraldehyde indicated that 52% of samples were positive for bacterial growth at 9 am and 82% of samples were found to be positive at the end of day. The bacterial survival rate was found to be lesser with the use of Glutaraldehyde so we considered it is more effective than Chlorine and ethanol.

Table4.3: *Bacterial growth after use of disinfectant at specific time*

Observation	**Chlorine**	**Ethanol 70%**	**Glutaraldehyde 2%**
Positive culture before disinfectant use at 7 am in %	26(100%)	26(100%)	26(100%)
Positive culture at 9 am in %	21(80%)	15(56%)	13(52%)
Positive culture at 11am in%	23(88%)	21(80%)	19(74%)
Positive culture at end working day in %	24(92%)	25(96%)	21(82%)
Frequency of number of positive samples after application of respective disinfectant	68	61	53
Mean value after application of respective disinfectant	22.66	20.33	17.66
Test of statistics	Value	Df	P
Likelihood ratio of Chi-square	9.08	2	0.010673

Here we calculate test of significance for efficacy among three disinfectants in which our p value is lower than our significance value (>0.05) it indicate there is a difference in efficacy among the three disinfectants and percentage of bacterial growth in 9am and 11am samples are less than the percentage of 7am and end of day are less which proved there is a correlation between frequency of cleaning and damp dusting and the microbial count at the dental clinic.

Table 4.4: *The frequency of number positive samples showing probable most contaminated area after disinfectant use*

Areas	**No Bacterial growth**	**bacterial growth**	**Total**	**Ratio**
Chair side area	21	135	156	86.53%
Area behind chair	25	59	84	70.23%
Area away from chair	04	48	72	92.00%
Reception area	00	24	24	100.00%
Total	50	262	312	
Test statistics	Test statistics	Df	P	
Likelihood ratio Chi-square	**9.000**	3	0.029291	

Here we calculate hypothesis related to reception and operating area in which our p value is lower than our significance value (>0.05) it indicate there is a difference in microbial count of operating and percentage shows reception area and reception is more contaminated than area.

Discussion

The results indicate that the microbial count on the dental environmental surfaces before disinfection use was fairly high [20]. *Staphylococcus* species were found on surfaces of the dental unit. This could be due to the use of dental instruments that create a propelling force such as a high-speed dental hand pieces, or a drill combined with a water spray which create numerous airborne infectious microbial agents [21].The aerosols which form because of hand pieces add to the infectious agent transfer and places the dental staff and patients at risk of cross infection [22-28]. The aerosols which form in dental office containing microbes are considered dangerous to HIV and other immunosuppressed patients [20].

Microbial presence in the dental environment

The presence of *Staphylococcus* species in this study could be attributed to its presence in the human body and it may reflect poor hand hygiene procedures or disinfection procedures. Although the *Staphylococcus coagulase* negative species was not common, *S. aureus* was the more common Gram+ve bacteria found in the clinic, which is of considerable concern [29]. *P. aeruginosa* and *Escherich coli* and are the most common Gram-ve bacteria found in hospital infections [30]. *Escherich coli* causes both gastrointestinal and extra intestinal disease such as bloodstream and pneumonia, meningitis, urinary tract, wound and abdominal s infections [31].

The fact that these organisms can survive in the hospital environment increases the risk to immuno-compromised patients and to other patients. *Staphylococcus* species survive for four weeks to seven month, *Pseudomonas* can survive six to 16 months, *Klebsiella* is able to alive for 2 h to nearly about 30 months, and *E. coli* is viable for five to 16 months, while *Enterococcus species* can be live from five days to 14 months [32, 33]. Survival of aerosolized gram negative bacteria including, *Enterobacter* and *Pseudomonas species*, as well as *Klebsiella,* can survive for a long time in humid conditions and at less temperatures [34]. It is therefore essential that clinic surfaces should be cleaned with long lasting disinfectants. This is particularly important in a country like South Africa given the number of immunosuppressed patients (*HIV and tuberculosis*) seen in the public dental clinics.

Effectiveness of disinfectants

Regarding Objective 5, Glutaraldehyde® and Ethanol® had the most effect on the vitro bacteria, and with six types of microbial growth being present before disinfection use. Glutaraldehyde appeared to control most of the bacteria, except *staphylococcus* species. Ethanol also controlled most of the bacteria, except *Bacillus* and *staphylococcus* [35], mainly due to its alcohol content [36]. Ethanol is a good

low-level and intermediate surface disinfectants for environment and equipment [37]. Chlorine® was not active against many bacteria, and it shows six different bacterial species, which is higher than ethanol and Glutaraldehyde. Chlorine can be considered the weakest disinfectant for bacteria, with resistant being due to its continuous use in the hospital. In literature indicates that chlorine as a disinfectant acts on different enzymes of bacteria and is active against sulfur-containing and aromatic amino acids[38]. However, it not effective against cytoplasmic enzymes, signifying that reaches bimolecular variety within the bacterium. Chlorine generally targets the cell wall, but its effect on gram–ve and on spore-forming bacteria is not understand properly, which have no outer membrane[39]. The results of this study indicate that Chlorine® is therefore the weakest disinfectant, with bacterial resistant possibly having formed due to its continuous use in the hospital. When selecting a disinfectant, many factor should considered, such as its toxicity, chemical content, stability, cost, degree of microbial killing required, potential damage to instrument and the ability to kill microorganisms rapidly [40]

Awareness of disinfection procedures in the dental clinic

In this study, 84% of clinic surfaces were contaminated. Therefore cleanliness of dental clinic and regular use of disinfectant is important to maintain optimal infection practice in the clinic. The results further suggest that chlorine is a weak disinfectant in comparison with Ethanol and Glutaraldehyde or possibly that the bacteria becomes resistant as result of long-term use at low-level concentrations [41].

The study findings therefore indicate that disinfection processes at the identified dental centre is inadequate, sub-optimal and could actually to contributing to the infection chain. There is an urgent need to review the current infection control procedures and protocols, including a review of the type of surface disinfectants used. The frequency of disinfection (damp-dusting and housekeeping) must be reviewed, give the number of patients that are seen every day basis. It is also imperative that simple procedures such as awareness of hand hygiene practices are implemented are prioritized. There should in turn be dedicated infection control monitoring and evaluation processes. It is also imperative that the undergraduate training programme includes dedicated training and monitoring of students' conduct and attitudes towards infection control, in particular disinfection processes.

Conclusion

The study suggests that there is an association between frequency of cleaning, the type of disinfectant used and the microbial count at the specific dental environmental surfaces in the identified oral health facility.

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

Optimal infection control procedures are fundamental to preventing cross-contamination in the dental clinic. There is very little published evidence on infection control practices in South Africa. In addition there is a paucity of evidence that specifically examined the efficacy or cost-effectiveness of the commonly used surface disinfectants in oral health clinical settings. We succeeded to achieving our aim in that Glutaraldehyde was seen to be a more effective surface disinfectant on specific dental environmental surfaces in an identified public oral health training facility in KwaZulu-Natal.

The results of Objective 1 indicate that, with respect to identifying and classifying environmental areas that are at risk for cross-contamination in the dental clinic, all areas of operating room had potential of cross contamination but the most contaminated areas in the dental environment were the area around the chair (86.5 %) and the area away from chair (92%). Regarding Objective 2, over seven different bacterial species were isolated, of which the most common was *staphylococci and Bacillus species*. The results from Objective 3 suggested that there was positive bacterial growth recorded after use of all three disinfectants, specifically after the use of Chlorine® (92% of samples). The use of Glutaraldehyde indicated that 52% of samples were positive for bacterial growth at 9 am and 82% were positive at the end of the day. Objective 4 mentions that the microbial count on identified surfaces in the operating area was greater before than after application of the disinfectant. The bacterial survival rate was found to be less with the use of Glutaraldehyde in Objective five. The microbial growth in the reception area was greater before and even after the application of the disinfectants, which suggests that the reception area is highly contaminated, possibly due to this being the area where all the patients and other accompanying persons wait. The findings revealed a difference in efficacy of the three disinfectants, which was due to an association between the frequency of cleaning and damp dusting, and the microbial count at the dental clinic. This supports our hypothesis that there is a correlation between frequency of cleaning and damp dusting and the microbial count at the dental clinic.

5.2 Study Limitations

The study had a number of limitations, including focusing exclusively on bacterial growth, with other microbes, including viruses, yeasts and Protozoans, being excluded. It included only commonly occurring bacteria and did not probe for resistant strains. Despite these limitations, the study does provide valuable

insight into the use of surface disinfectants in the dental clinic. In our study, 84% samples shows positive bacterial growth, and the remaining 16% indicate that the disinfectants are more effective after their initial application period and its effectivity decreases with time , as an increase in time increases is associated with a decrease in effectiveness, which makes a long-acting surface disinfectant essential.

The study suggests that there is an association between the frequency of cleaning, the type of disinfectant used and the microbial count at the specific dental environmental surfaces in the identified oral health facility. The study findings therefore indicate that disinfection and cleaning processes used at the identified dental centre are inadequate, sub-optimal and could actually be contributing to the infection chain. There is an urgent need to review the current infection control procedures and protocols, including a review of the type of surface disinfectants used. The frequency of disinfection (damp-dusting and housekeeping) must be reviewed, given the number of patients that are seen on a daily basis. It is also important that simple procedures, such as awareness of hand hygiene practices, are implemented and prioritized. There should in turn be dedicated infection control monitoring and evaluation processes.

5.3 Significance of the Study

These findings suggest the following:

- The study raised awareness on infection control practices at the identified site.
- The study also raised awareness on selection and use of disinfectants at the identified site.
- The study also contributed to raising awareness of the need to reiterate infection control practice in undergraduate clinical training.

5.4 Recommendations

The following recommendations are made:

- These experiments on the efficacy of commonly used surface disinfectants need to be conducted on regular basis. There is a further need to ensure that the potency of these disinfectants is not compromised either chemically or through improper application.
- The infection control procedures at the identified oral health facility need to be reviewed.
- A review of the type of surface disinfectants used in dental and other government sector clinics that use the same disinfectants needs to be conducted.
- Greater emphasis needs to be placed on the high risk areas for cross contamination in the dental clinic.
- Breaking the chain of infection is important; therefore hand washing is imperative before entering in operating area.

- There is also a need for regular disinfection of the dental reception area.
- More research is required to determine the effect of these surface disinfectants on other microbes.
- There is need for a curriculum review in terms of instructions on infection control.
- The results of the study will be made available to the dental assistance, dental therapy students and oral hygiene students to ensure that corrective measures should be place for infection control practice.

The study findings suggested that there was an association between frequency of cleaning, the type of disinfectant used and the microbial count at the specific dental environmental surfaces in the identified oral health facility. These findings should be considered for oral health planning within the identified oral health facility.

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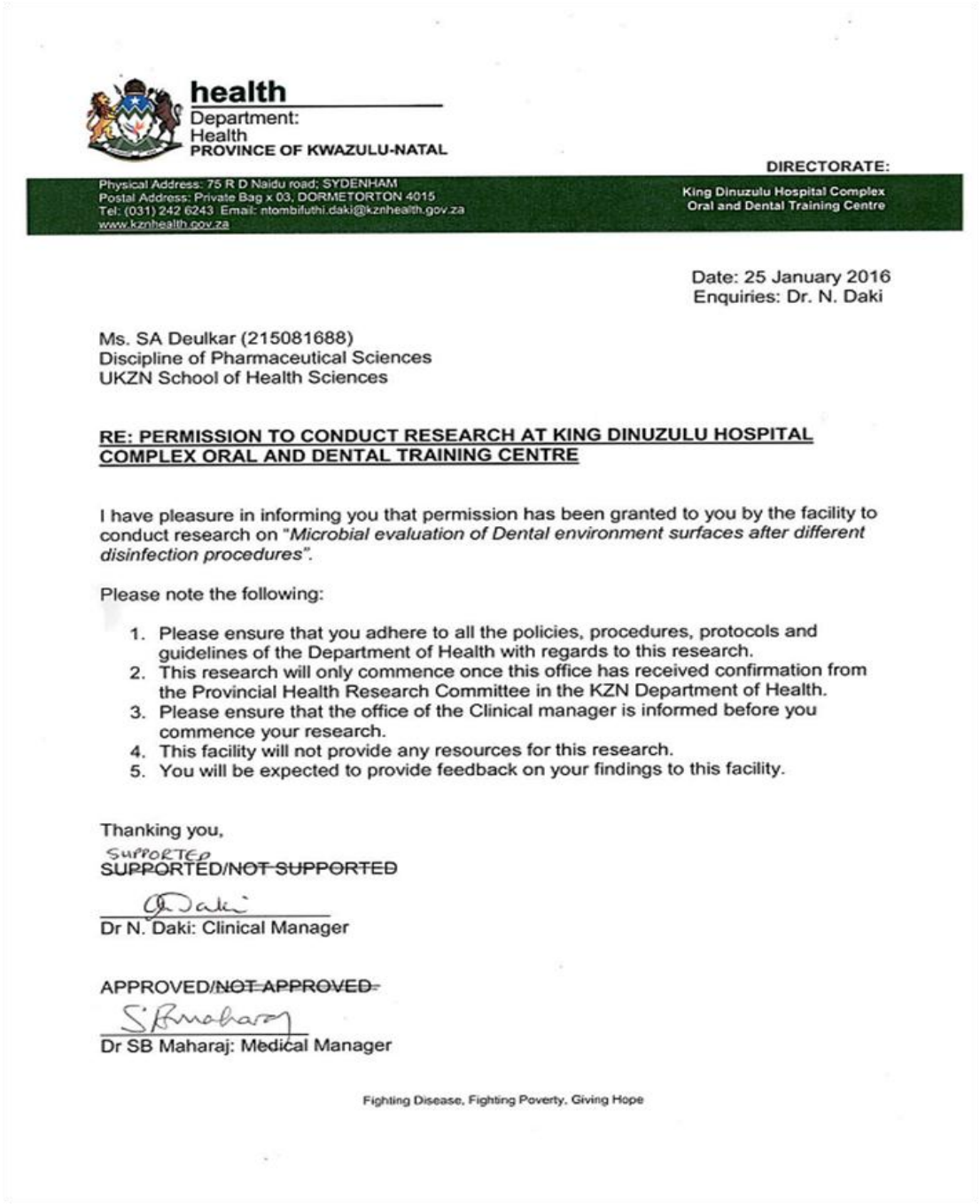
APPENDICES

1. **Permission letter from hospital.**
2. **BREC Ethical Clearance letter**
3. **Permission letter from department of health**



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Oral and Dental Training Centre

Date: 25 January 2016
Enquiries: Dr. N. Daki

Ms. SA Deulkar (215081688)
Discipline of Pharmaceutical Sciences
UKZN School of Health Sciences

RE: PERMISSION TO CONDUCT RESEARCH AT KING DINUZULU HOSPITAL COMPLEX ORAL AND DENTAL TRAINING CENTRE

I have pleasure in informing you that permission has been granted to you by the facility to conduct research on "*Microbial evaluation of Dental environment surfaces after different disinfection procedures*".

Please note the following:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN Department of Health.
3. Please ensure that the office of the Clinical manager is informed before you commence your research.
4. This facility will not provide any resources for this research.
5. You will be expected to provide feedback on your findings to this facility.

Thanking you,

SUPPORTED
~~SUPPORTED/NOT SUPPORTED~~

Dr N. Daki: Clinical Manager

APPROVED/~~NOT APPROVED~~

Dr SB Maharaj: Medical Manager

Fighting Disease, Fighting Poverty, Giving Hope

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

25 February 2016

Ms SA Deulkar (215081688)
Discipline of Pharmaceutical Sciences
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Protocol: Microbial evaluation of dental environment surfaces after different disinfection procedures.
Degree: MMed
BREC reference number: BE512/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 09 December 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 25 February 2016 to queries raised on 24 February 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from **25 February 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **08 March 2016.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

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Reference: 26/16
KZ_2015RP12_306

Date: 15 February 2016

Dear Ms SA Duelkar
Email: deulkarswati@gmail.com

Approval of research

1. The research proposal titled **'Microbial evaluation on dental environmental surfacers after different disinfection procedures'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at king Dinuzulu Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 17/02/16

Fighting Disease, Fighting Poverty, Giving Hope