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Evaluating the Surface Hygiene in the Doornfontein Homeopathic Health Training Centre

A dissertation submitted to the
Faculty of Health Sciences, University of Johannesburg,
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Master's Degree in Homoeopathy

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DECLARATION

I, Agnes Kalubi, declare that this dissertation is my own, unaided work, except where otherwise indicated in the text. It is being submitted for the Master's Degree in Technology: Homeopathy at the University of Johannesburg. It has not been submitted before for any degree or examination in any other University.

Signature of Candidate________________________

On this_____ day of____________________________2019
DEDICATION

To My Heavenly Father, Thank you for your undeserved kindness in my life.

To My father Kalubi-Katenda Djunes, Thank you for your unconditional love and support.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my Heavenly Father for giving me the strength, ability and opportunity to undertake this research study and to persevere and complete it satisfactorily.

I also would like to acknowledge a number of important people who played key roles in making this work a reality.

I sincerely thank Dr. R Razlog, my supervisor, for giving me the necessary suggestion to better this study.

I’m gratefully acknowledging Prof. TG Barnard and Dr. A Singh, my co-supervisors, for their patience, support and encouragement. Thank you for your valuable critiques. This study would have not been a success without the both of you.

Related thanks to Raihaanah and Pfano from the water and health laboratory for helping collect and culture samples.

Finally, and without hesitation, I’d like to thank my family and friends for their support, prayers and belief in me.

I would like to send my gratitude to my parents for their guidance, unconditional love and financial support. Thank you for all the opportunities you’ve presented me with.

To my siblings, thank you for believing in me. Special thanks to Yvette and Dorcas for their constant love and support throughout the peaks and valleys of our lives.

The love of my life, Pierce, thank you for always having my back, always believing in me and showering me with a human love that is quite uncommon in this world.
ABSTRACT

Hygiene practices has been a matter of concern in the healthcare system in South Africa and across the world. The patient environment in healthcare settings has continually proven to be a reservoir of potentially harmful and even lethal multi-drug resistant organisms (MDROs). Hand hygiene is the primary element of infection control activities that should be performed in order to reduce cross-contamination. Lack of proper hand hygiene leads to contamination of surfaces by healthcare workers, patients and visitors. Nevertheless, recent studies indicate that on average only 30-50% of healthcare professionals comply with hand washing protocols.

To this end, no study has been conducted at the Homeopathic Health Training Centre (HHTC), at the University of Johannesburg, South Africa. Therefore, this study provides an initiative to investigate surface hygiene in the HHTC; to determine if there are any pathogens that could possibly lead to nosocomial infections. This is necessary since nosocomial infections can worsen the patients’ presenting complaints or even act as an obstacle to cure.

This study was an exploratory study with a quantitative research design using objective (microbial enrichment) data to evaluate the surface hygiene in the HHTC.

Seven (7) consulting rooms in the HHTC at the University of Johannesburg (UJ) Doornfontein campus that are frequently used by practitioners and their patients were assessed. Samples were collected from the surface areas of vinyl beds (n=21), wooden tables (n=21), steel cupboards (n=14) and steel cabinets (n=21), within each room. Therefore, the total number of surfaces sampled was n=77. Surface samples were taken using bioMerieux Count-Tact range. The samples were obtained by briefly depressing the 65mm
irradiated CT3P agar contact plates for 10 seconds under 500g pressure with the Count-Tact® applicator over the three standardized areas for the tables, beds and steel cupboards and two standardized areas for the steel lockers. One sample of each set was incubated for up to 3 days (for bacteria) and the other incubated for up to 5 days (for fungi). The samples were taken after the clinic's normal hours of operation so that none of the patients, students, or clinicians were aware of the study and thus could not change their normal habits.

Colonies per plate (25cm) were counted and the results in colony forming unit (CFU) per cm were reported. Bacteria isolates were identified via the VITEK instrument and the fungal isolate were sent to the University of Free State for identification through sequencing.

The result of the surface sampling demonstrated that all of the surfaces sampled at the HHTC contained microorganisms, with the highest bacterial counts on tables and fungal counts on beds. The results also highlighted that the surfaces are indeed a potential reservoir of bacteria such as; *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Bacillus spp.*, *Kocuria spp.*, *Micrococcus luteus*, *Sphingomonas paucimobilis* and *Gardnerella vaginalis*, as well as fungi such as; *Aspergillus spp.*, *Fusarium spp.*, *Cladosporium spp.* and *Penicillium*.

These findings are consistent with the studies done at the Chiropractic UJ Clinic. Although most of the microorganisms isolated on the surfaces were harmless skin bacteria and/or environmental fungi, they are opportunistic which pose a direct threat to the patients, practitioners and possibly the community. Disinfection protocols must therefore address removal of microorganisms from these surfaces to prevent potential horizontal transmission or nosocomial acquisition.
Overall, the information gathered in this study both supports and emphasizes the need for an effective disinfection protocol for the prevention of bacterial and fungal build-up on the surfaces in the HHTC.

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Chapter 1: INTRODUCTION

1.1 Problem Statement

Hygiene practices has been a matter of concern in the healthcare system in South Africa and across the world. The patient environment in healthcare settings has continually proven to be a reservoir of potentially harmful and even lethal multi-drug resistant organisms (MDROs) (Chemaly et al., 2014). Hand hygiene is the primary element of infection control activities that should be performed in order to reduce cross-contamination. Lack of proper hand hygiene leads to contamination of surfaces by healthcare workers, patients and visitors. Nevertheless, recent studies indicate that on average only 30-50% of healthcare professionals comply with hand washing protocols (Marra and Edmond, 2014; Tenna et al., 2013).

Homeopathy is a primary healthcare profession focusing on the diagnosis, treatment and prevention of almost any disease and/or disorder (De Schepper, 2010). During consultation, the practitioner performs physical examination creating the opportunity for the potential exchange of harmful microorganisms. Based on related research findings, it can be presumed that the protocols of hand hygiene and surface hygiene are not followed appropriately at the Homeopathic Health Training Centre, and this can lead to a continuous cycle of contamination between hands and surfaces. As a result, microorganisms growing on the surface can potentially affect the health of patients during physical examination. These adverse effect ranges from minor complaint to major health issues (Hamilton, 2013; Scherbaum et al., 2014).

To this end, no study has been conducted at the Homeopathic Health Training Centre in Johannesburg, South Africa. Therefore, this study provides an initiative to investigate surface hygiene in the Homeopathic
Health Training Centre (HHTC); to determine if there are any pathogens that could possibly lead to nosocomial infections. This is necessary since nosocomial infections can worsen the patients’ presenting complaints or even act as an obstacle to cure.

1.2 Nosocomial Infections

According to the Center of Disease Control and Prevention (CDC, 2017a), millions of individuals around the world are affected by nosocomial infections each year and this continues to be a significant issue regardless of all the precautions taken. One in seven patients entering South African hospitals may become infected with nosocomial infections (Revelas, 2012). Nosocomial infections are influenced by many factors; two of these are the general health of the patient and the environment. Bacteria, fungi and viruses are the cause of nosocomial infections. The most common pathogens associated with nosocomial infections are Staphylococcus aureus, Escherichia coli, Clostridium difficile, Pseudomonas aeruginosa, Klebsiella Pneumoniae and Candida spp. Pathogens such as Clostridium difficile and Escherichia coli, for example, have a strong relationship between surface contamination and transmission (CDC, 2017a; Hamilton, 2013).

1.3 Aim

The aim of this study was to assess the surface hygiene of identified furniture in the Homeopathic Health Training Centre by investigating the presence of the total culturable microbial populations in the homeopathic consultation rooms at the University of Johannesburg, Doornfontein Campus, Homeopathic Health Training Centre.

1.4 Outcomes of the Study

Possible outcomes of this study are as follows:
• Insight on the microbial load with current hygiene practice used at the Homeopathy Health Training Centre
• Aid in the research needed to understand the possible spread of infections in the Homeopathic Health Training Facility.
CHAPTER 2: LITERATURE REVIEW

2.1 Nosocomial Infections

According to the World Health Organization (WHO) nosocomial infections, otherwise known as hospital acquired infections, are defined as “infections occurring in patients in a hospital or other health care facility in whom the infections were not present or incubating at the time of admission” (WHO, 2015). This may also include infections appearing 48 hours after admission or after discharge. These infections are also believed to spread among staff of the facility (CDC, 2017a; Hefzy et al, 2016; Hamilton, 2013).

Infectious diseases represent the leading aggregate cause of human death worldwide, with multi-drug resistance organisms (MDRo) in particular, constituting an important and growing threat to human health (Tanwar et al, 2014; Singh, 2013). Nosocomial infections represent a large subset of the group of antibiotic-resistant pathogens, as more than 70% of the bacteria that cause these infections are resistant to at least one of the drugs most commonly used to treat these infections (Van Duin and Paterson, 2016).

2.1.1 Aetiology of Nosocomial Infections

Epidemiological studies report that nosocomial infections are caused by pathogenic bacteria, viruses and fungi present in the air, on surfaces and on equipment (CDC, 2017a; Khan et al, 2017; Revelas, 2012). On initial admission, the patient is free from any of these pathogens and they are most likely to acquire infection through direct contact between person-to-person and mostly during invasive procedures. Nosocomial infections can also be acquired through indirect contact (Boyce, 2014). The World Health Organization (WHO, 2015) has identified hygiene protocols, like hand
washing techniques to prevent transmission between healthcare workers, their patients and their surroundings.

The most common cause of nosocomial infections is *Staphylococcus aureus* (*S. aureus*), a bacterium that may cause pneumonia and endocarditis as well as serious infections of the skin, soft tissues and bloodstream. Methicillin-resistant *S. aureus* (MRSA) is an antibiotic-resistant form of this pathogen that was identified shortly after the introduction of methicillin into clinical use and practice in the United Kingdom (Stryjewski and Corey, 2014).

2.1.2 Prevalence of Nosocomial infections

According to Kamunge *et al* (2014), there is significant literature on the prevalence, incidence, and risk factors related with nosocomial infections; however, the gross morbidity, mortality and extra costs of healthcare associated with these infections are still rising (Storr *et al*, 2017; Kamunge *et al*, 2014; Scherbaum *et al*, 2014). Nosocomial infections affect about 5-15% of hospitalized patients and more than 50% of patients in intensive care unit (ICU) in the developed countries, while it affects many more patients in developing countries. The exact estimation of affected individuals in developing countries is unknown due to lack of research; however, the figure is predicted to be much higher than in developed countries (Khan *et al*, 2017; Cheng *et al*, 2015; Scherbaum *et al*, 2014).

2.1.3 Impact of nosocomial infections

Nosocomial infections negatively affect the healthcare system by placing strain on it, thereby resulting in the degradation and/or loss of human lives (Hamilton, 2013; Kamunge *et al*, 2014; Revelas, 2012). Several studies have discovered that the foremost burden of nosocomial infection is loss of human lives and the financial impact (Byrd, 2016; Kamunge *et al*, 2014). Nosocomial infections usually result in an increased length of hospital stay and an
increased risk of mortality. This puts pressure on health systems to establish surveillance mechanisms to quantify and evaluate the magnitude of the problem in order to assist in justifying resource mobilization and improving infection control systems in healthcare settings (Salah et al, 2017).

In South Africa (SA), the surveillance of nosocomial infections is somewhat neglected and poorly resourced (Dramowski et al, 2017). The true burden of nosocomial infection is undisclosed, in spite of the fact that it is largely accepted that it is greater in the public sector than in the private sector and would likely range between 10-20%. Due to poor resources, more detailed analysis and reporting of nosocomial rates are required (Lowman, 2016).

2.1.4 Nosocomial Infections and the Environment

Many pathogens have surfaced since the late 1980s that contribute to the spread of nosocomial infections worldwide. There are countless studies demonstrating the presence of Multiple Drugs Resistance Organisms (MDROs) in the patient care environment (Chabni et al, 2018; Lee et al, 2018; Van Duim, 2016; Ahmed, 2014; Lim et al, 2014). Pathogens associated with nosocomial infections are causing increases in mortality and morbidity due to microbial resistance (Chemaly et al, 2014).

It is well proven that pathogens can survive in healthcare environment for a long duration (Russotto et al, 2015). The exact survival period of different pathogens varies depending on the temperature and morphology of the implicated pathogen. Both Gram-positive and Gram-negative bacteria can survive up to 4-5 months on dry surface, with longer persistence under humid and lower-temperature conditions (Russotto et al, 2015; Chemaly et al, 2014). Under conditions likely to occur in healthcare facilities, Clostridium difficile spores, vancomycin resistant Enterococcus (VRE), Methicillin-resistant
**Staphylococcus aureus** (MRSA) (Leas and Oakley, 2014), *Acinetobacter baumannii* and *Escherichia coli* have been recovered after 4 to 5 months, with endospores typically lasting longer than vegetative bacteria (Chemaly et al, 2014). A summary of organisms implicated with their survival period is illustrated in **Table 2.1**.

**Table 2.1**  Summary of survival time for nosocomial infections

<table>
<thead>
<tr>
<th>Organism</th>
<th>Survival time*</th>
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</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>7 days to &gt;12 months</td>
</tr>
<tr>
<td>Vancomycin-resistant Enterococci</td>
<td>5 days to &gt;46 months</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6h to 16 months</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>3 days to 11 months</td>
</tr>
<tr>
<td>Carbapenem-resistant Enterobacteriaceae</td>
<td>19 days</td>
</tr>
<tr>
<td>Norovirus</td>
<td>8 to 7 days</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>6 to 60 days</td>
</tr>
</tbody>
</table>

*Survival times of MDROs on dry inanimate objects.

Adapted from Chemaly et al (2014).

As mentioned, multiple studies have substantiated the presence of pathogens in the patient’s environment (Van Duim and Paterson, 2016; Ahmed, 2014; Lim et al, 2014). In one, MRSA was cultured from 43 % of beds of individuals that were not MRSA positive. Birnbach et al (2015) concluded that operating rooms, while considered to be the hallmark of cleanliness, are not a sterile environment; studies have shown that known pathogens such as MRSA, VRE, *Clostridium difficile*, and multidrug-resistant Gram-negative bacilli have been found on many operating rooms surfaces after standard cleaning was performed (Birnbach et al, 2015). Most likely, this contamination is due to viability of organisms shed by previous occupants, but it could also be due to horizontal transmission from healthcare workers, visitors or asymptomatic carriers, as well as migration of the organisms through air flow or other means (Creamer et al, 2014).
Studies have investigated the benefit of proper cleaning practices of high-touched surfaces to be paramount when it comes to preventing infections. Although not validated, microbial standards for a safer healthcare environment have been proposed as less than 5 colony forming units (CFU)/cm² on surfaces (Dancer, 2004). Maintaining counts below these thresholds may assist in reducing nosocomial infections (Claro et al, 2015).

2.1.5 Factors Influencing Infection

Multiple factors contribute to the transmission of infectious organisms in healthcare facilities. Microbes requires a favourable nutritional and environmental condition for survival. These factors determine if exposure to a microbe will result in prolonged colonization and can be divided into three categories; host characteristics, microbial properties and environmental factors (Perdijk, 2017). It is the responsibility of the facility to develop policies and procedures designed to interrupt the transmission of infectious organisms from the source to patients (Khan et al, 2017; Mehta et al, 2014).

2.1.5.1 Host characteristics

In this category, there are many factors that increases the risk of nosocomial infections. Age, gender, ethnicity, health status and socioeconomic standing are factors that contribute to the health and wellbeing of patients (Hamilton, 2013).

Mostly, hospitalized neonates and children who are admitted in intensive care unit (ICU) are affected. Infants under the age of 2 months were identified to be more susceptible to nosocomial infections than older ones, and bloodstream infections were the most common throughout this age group (Kamunge et al, 2014). Adults, especially the elderly, are more prone to respiratory and urinary tract conditions (Revelas, 2012). Geriatric patients are at a higher risk of developing nosocomial infections due to decreased
immunity, functional impairment and presence of chronic disease such as diabetes mellitus and hypertension, to name a few (Revelas, 2012).

It has been reported that males are more susceptible to nosocomial infection due to greater bacterial colonization on their skin or weak adherence of wound dressing due to coarser, thicker hair (Mythri and Kashinath, 2014). Females on the other hand have marked humoral and cellular immune responses and a better developed thymus during procreative years (Taub, 2008).

A study conducted in the United States among patients hospitalized with acute cardiovascular disease, pneumonia and undergoing major surgery found that Asians and Hispanic patients had significantly higher rates of nosocomial infections than white and black patients due to language barrier. The study concluded that it was unlikely to be due to economic and educational differences (Bakullari et al., 2014).

Patients that are immuno-compromised are at a much higher risk of nosocomial infections, especially those infections that are caused by airborne or water borne microorganisms (Mehta et al., 2014). The three most common infections that affect immuno-compromised patients are meningococcal meningitis, pneumococcal pneumonia and tuberculosis. It has been documented that these infections can be sometimes difficult to treat due to antimicrobial resistance (Green et al., 2017; Mehta et al., 2014). Diabetic patients have significantly higher rate of nosocomial infections than non-diabetic patients (Al Zayer, 2017).

Other risks that are associated with nosocomial infections are alcoholism, smoking and malnutrition. Malnutrition has been proven to be a factor that impose one to be at a greater risk of nosocomial infections. However, there is no literature to show that improvement of dietary intake will prevent
nosocomial infections (Thibault et al, 2015). Obesity is however a risk factor due to under-dosing of anti-microbial medication in the treatment or prevention of nosocomial infection (Perdijk, 2017).

Cigarette smoking has a direct influence on innate immunity (Khan et al, 2017). When smoking, ciliary epithelium undergoes histological changes; making it difficult for clearance of the airways. Smoking cigarettes suppresses the immune system. Cigarette smoke also promotes the microbial virulence and antibiotic resistance (Feldman and Anerson, 2013).

Alcohol consumption directly affects the immune system leading to an increased risk of nosocomial infections (Mihaly et al, 2016). Substantial clinical evidence suggests that alcohol abuse suppresses both innate and adaptive immune responses leading to an increased risk for infections, and delayed recovery from trauma (De Wit et al, 2012; Weisfelt et al, 2010). According to De Wit et al. (2012), patients with alcoholism who undergo a variety of elective operations have an increased risk of infections that may lead to death. Malnutrition and/or malabsorption is a risk factor for nosocomial infection, as discussed before. On the other hand, malnutrition is associated with chronic alcohol abuse and the two becomes an important contributor to immuno-suppression and increased susceptibility to infections (Mihaly et al, 2016). Another study done by Weisfelt et al (2010) explained that heavy alcohol consumption was associated with an increased risk of nosocomial infection in men who underwent general surgical procedures.

2.1.5.2 Environmental factors influencing survival of microorganisms

The patient’s environment in healthcare settings has continually proven to harbor a reservoir of potentially harmful and even lethal multidrug resistant
organisms (MDROs) (Chemaly et al, 2014). Many microbial organisms can survive for weeks to months in the absence of decontamination (Claro et al, 2015). Several studies demonstrate that healthcare associated pathogens frequently contaminate the patient environment, including both porous surfaces (such as curtains) and hard, nonporous surfaces (such as bed rails and medical equipment) (Al-Abdi and Baiu, 2016). Outbreak reports have provided additional evidence that patients are infected by organisms acquired from the inanimate environment. However, the extent to which healthcare environment contributes to nosocomial infections is still controversial because many infections appear to be attributable to the endogenous flora of the patient and/or direct transmission via hands of healthcare providers, rather than to inanimate objects (Doll et al, 2018; Singh, 2013). Changes in temperature and humidity are also proven to influence the development of infection (Van Duim and Paterson, 2016).

2.1.5.3 Microbial Agent

The patient is exposed to many different microorganisms during their stay in healthcare facilities (Oswald, 2015). Contact between the patient and a microorganism does not by itself certainly result in the development diseases, other factors influence the nature and frequency of nosocomial infections. The probability of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount of infective material (Hottel, 2015; Gaulke, 2014)

As mentioned, many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person (cross-infection) or may be caused by the patient’s own flora (endogenous infection) (Thompson, 2014). Some
organisms may be acquired from an inanimate object or substances recently contaminated by another person (Turnage, 2017; Shiang, 2013).

### 2.1.6 Modes of Transmission

Infectious agents enter the body through various portals, including the mucous membrane, skin, the respiratory and gastrointestinal tracts (Fuller, 2015; Khan et al, 2015). The skin acts as a barrier to infection. However, any open injury to the skin invites the entrance of pathogens; this can be caused by tubes placed in body cavities (catheters) or punctures caused by invasive procedures (WHO, 2015).

A method of transmission is the movement or the transmission of pathogens from a reservoir to a susceptible host. Once a pathogen has exited the reservoir, it needs a mode of transmission to the host through a portal entry. It was formerly presumed that coming into direct contact with an infected individual was necessary to cause nosocomial infections, but due to the fact that proper hygiene is not followed accordingly, these pathogens are left on medical equipment, beds, other surfaces and in-patient restrooms even after cleaning (CDC, 2017a; Hamilton, 2013). Microorganisms are transmitted by four main routes; contact, airborne, vector-borne and vehicle (WHO, 2015).

Contact is by far the most frequent mode of transmission of nosocomial infections (Dramowski et al, 2017; Fuller, 2015; Kimble, 2013). Contact is divided into direct and indirect contact. Direct contact is person-to-person transmission of pathogens through touching, biting, kissing, sexual intercourse or trans-placental (Marra and Edmond, 2014; Tenna et al, 2013). Indirect contact involves a contaminated object. This is often a result of unclean hands contaminating an object or environment. The pathogen remains on the inanimate object to be picked up by the next person (Khan et al, 2017; Hamilton, 2013).
Airborne transmission occurs either by airborne droplets nuclei (small particles of 5 mm or smaller in size) or by dust particles containing microorganisms generated during coughing, sneezing and talking precipitate in the air. These microorganisms land on another person, entering that new person's system through contact with his/her mucous membrane. These microorganisms are relatively large and travel only short distances (up to 6 feet or 2-metres). Microorganisms carried in this manner remain suspended in the air for long periods and they can be dispersed widely by air currents (Frenstrom and Goldblatt, 2013). Because of this, there is risk that all the air in a room may be contaminated (Hefzy et al, 2016).

Vehicle transmission involves vehicles such as water and food. WHO estimates that contaminated drinking water is responsible for more than 500,000 deaths each year. Food contaminated through poor handling or storage can lead to foodborne transmission of disease (WHO, 2013).

Lastly, infections can be transmitted by a mechanical or biological vector, an animal that carries the disease from one host to another. Mechanical transmission is facilitated by a mechanical vector. For example, a fly may land on faecal matter and later transmit bacteria from the faeces to food that it lands on; a human eating the food may develop dysentery. Biological transmission occurs when the pathogen reproduces within a biological vector that transmit the pathogen from one host to another. Malarial transmission is an example thereof (Jung et al, 2019).

2.1.7 Pathogens implicated in Nosocomial Infections

In general, different strains of bacteria, viruses and fungi are associated with different diseases. Some of the more common bacterial and fungal pathogens includes *Streptococcus* spp., *Acinetobacter* spp., *Enterococci* spp,
*Pseudomonas aeruginosa*, *S. aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*) and *Candida spp* (*Candida spp.*) (Dagher et al, 2017; Khan et al, 2015; Maczulak, 2012). Typical nosocomial infections include: Urinary infections (*E. coli*), which are the most common type; Surgical site infections (*S. aureus*); Nosocomial pneumonia (*K. pneumoniae*); Nosocomial bacteraemia (multi-resistant coagulase-negative *Staphylococcus* and *Candida spp.*); Gastroenteritis (*Clostridium difficile*, *E. coli* and Rotavirus) (Mundhada and Tenpe, 2015; Mehta et al, 2014). The National Healthcare Safety network with the Centre for Disease Control for surveillance has classified nosocomial infection sites into 13 types, with 50 infection sites, which are specific on the basis of biological and clinical criteria. A summary of some of the bacteria that persist on environmental surfaces, associated and their transmission routes is given in **Table 2.2**.

**Table 2.2** Common pathogens associated with nosocomial infection

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Affected patients</th>
<th>Transmission</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Extremely ill, hospital bound patients</td>
<td>Person to person contact and airborne</td>
<td>Spellberg &amp; Bonomo, 2013</td>
</tr>
<tr>
<td>Enterococci</td>
<td>Debilitated, immunocompromised</td>
<td>Medical devices and Direct contact</td>
<td>Loftus et al, 2015</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococci</em> (CoNS)</td>
<td>Immunocompromised</td>
<td>Medical devices</td>
<td>Becker et al, 2014</td>
</tr>
<tr>
<td>Legionella</td>
<td>Patients with compromised immunity</td>
<td>Contaminated water</td>
<td>Llewelly et al., 2017; Prussin et al., 2017</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Immunocompromised</td>
<td>Direct/ indirect contact of infected body fluid</td>
<td>Normann and Poirel, 2014</td>
</tr>
</tbody>
</table>

The more common pathogens are discussed below.
2.1.7.1 Escherichia coli

*E. coli* is a short Gram-negative, non-spore forming, peritrichous and fimbriated bacillus. It belongs to the family Enterobacteriaceae and is the only member of the genus Escherichia. *E. coli* can be subdivided into stable biotypes based on a variety of properties. It is normally part of the intestinal flora of warm-blooded animals and is found in its primary habitat, which is the gastrointestinal tract (Rivas *et al.*, 2015). *E. coli* is reported as one of the most common agents for hospital acquired infections, and since it is present in the faeces of all warm-blooded animals, is a good indicator of faecal pollution, and by extension poor hygiene practices (Dagher *et al.*, 2017). When found in nature, either in the soil, water or elsewhere, it is derived from the primary habitat, usually by faecal contamination. *E. coli* strains can cause a number of different types of diseases; including diarrhoea, dysentery, haemolytic uraemic syndrome, bladder and kidney infections, septicaemia, pneumonia and meningitis. In general, different *E. coli* strains are associated with different diseases (Khan *et al.*, 2015; Rivas *et al.*, 2015; Wattal and Khardori, 2014)

2.1.7.2 Klebsiella pneumoniae

*Klebsiella pneumonia* is a Gram-negative bacterium that has been implicated in causing different types of nosocomial infection including pneumonia, wound/surgical site infection, meningitis and blood stream infections (McNeil, 2014). In humans, *Klebsiella* is usually found in the gastrointestinal tract and in faeces where they do not cause diseases. Patients receiving treatment in healthcare settings are susceptible to *Klebsiella pneumoniae*, especially those in ICU. Healthy people usually do not get Klebsiella infections (Gorrie *et al.*, 2017).
Klebsiella bacteria mostly spreads through person-to-person contact or less commonly by contamination of the environment. Klebsiella infection can be prevented by following specific infection control precautions. Some of these precautions include strict adherence to hand hygiene and wearing gowns and gloves when coming into contact with infected individuals (CDC, 2017a). Healthcare facilities must also follow strict cleaning procedures in order to prevent the spread of Klebsiella. Uncomplicated Klebsiella infection can be successfully treated with antibiotics (Byrd, 2016)

2.1.7.3 Staphylococcus aureus

Out of many species of Staphylococcus genus, S. aureus is accountable for most of the nosocomial infections encountered in healthcare facilities. It is Gram-positive cocci, non-spore forming, catalase- and coagulase-positive, immotile, facultatively anaerobe (Tanwar et al, 2014). S. aureus causes a wide range of diseases from mild to life-threatening conditions. It is one of the most prevalent causes of nosocomial bacteraemia, hospital-acquired pneumonia, and surgical site infections. Additionally, S. aureus has a high potential to acquire antimicrobial resistance (Denis, 2017).

S. aureus is not only a disease-causing organism but also plays its role as commensal, or non-disease forming bacteria. It is found in both the environment and in normal human flora, located on the skin and mucous membranes (most often the nasal area) of healthy individuals (Khan et al, 2015). S. aureus does not normally cause infection on healthy skin; however, if it enters the bloodstream or internal tissues through open wounds, these bacteria may cause a variety of potentially serious infections (Price et al, 2017; Creech et al, 2015). Immunocompromised patients are more prone to S. aureus infections (McNeil, 2014). Transmission is typically from direct contact; however, some infections involve other transmission methods (Denis, 2017; Knox et al, 2015)
2.1.7.4  *Pseudomonas aeruginosa*

*P. aeruginosa* contributes to 11% of all nosocomial infections (Khan *et al*, 2017). It is Gram-negative, non-fermenter causing diseases especially among immune-compromised people. The sites of colonization are kidney, urinary tract and upper respiratory tract (Lamas-ferreiro *et al*, 2017). *Pseudomonas aeruginosa* infections are associated with high morbidity and mortality rate as they tend to produce invasive infections making them complicated and life threatening (Eves, 2016). Transmission is from direct contact. Common reservoirs for its contamination include sinks, incubators and breast pumps (Russotto *et al*, 2015).

2.1.7.5  *Clostridium difficile* (*C. difficile*)

*C. difficile* is the leading cause of health-care-associated infective diarrhoea (Khan *et al*, 2015). It is a Gram-positive bacillus, spore-forming anaerobic bacteria. It usually colonizes in intestinal tract and serves as part of normal microbiota (Mundhada and Tenpe, 2015; Maczulak, 2012). Diseases caused by toxins produced by *C. difficile* are colitis and it is responsible for 15–25% of diarrhoea cases (Maczulak, 2012).

2.1.8  Multiple Drug Resistance Organisms

Almost all pathogens (bacteria, fungi, virus, and parasite) have shown some degree of resistance to antimicrobial drugs with enhanced morbidity and mortality (Tanwar *et al*, 2014). Multidrug resistance (MDR) is defined as insensitivity or resistance of a microorganism to the indicated antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite the fact that it was sensitive to it before (Singh, 2013). According to Tamwar *et al* (2014) and Van Duin and Paterson (2016),
inappropriate use of antimicrobial drugs has led to the emergence of multidrug resistance in spite of the fact that resistance development is a natural phenomenon. Inadequate sanitation has been known to encourage the spread of lethal infectious diseases (Van Duin and Paterson, 2016).

The ability of a microorganism to survive at a given concentration of an antimicrobial agent at which the normal population of the microorganism would be killed is Antimicrobial Resistance I. This is called the epidemiological breakpoint. The ability of a microorganism to survive treatment with a clinical concentration of an antimicrobial agent in the body is Antimicrobial Resistance II. This is called the clinical breakpoint. It is important to define resistance using clinical breakpoints to determine appropriate anti-microbial therapy for achieving improved clinical outcomes (Singh, 2013). Table 2.3 gives a summary of mechanism use for antimicrobial resistance.

The most recent study from WHO on antimicrobial resistance report have shown very high rates of antimicrobial resistance in bacteria (Table 2.3) such as E. coli resistance against cephalosporin and fluoroquinolones, K. pneumoniae resistance against cephalosporin and carbapenems and S. aureus resistance against methicillin to name a few (Tacconelli et al, 2018).
Table 2.3  Summary of the antimicrobial resistance mechanisms as described by Munita and Arias (2016)

<table>
<thead>
<tr>
<th>Antimicrobial resistance method</th>
<th>Explanation and examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A)  Genetic basis of AMR</td>
<td></td>
</tr>
<tr>
<td>1) Mutational resistance</td>
<td>Mutations in gene(s) often associated with the mechanism of action of the compound</td>
</tr>
<tr>
<td>2) Horizontal gene transfer</td>
<td>Acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer</td>
</tr>
<tr>
<td>I. Transformation</td>
<td>Incorporation of naked DNA</td>
</tr>
<tr>
<td>II. Transduction</td>
<td>Phage mediated</td>
</tr>
<tr>
<td>III. Conjugation</td>
<td>Gene transfer.</td>
</tr>
<tr>
<td>B)  Mechanistic basis</td>
<td></td>
</tr>
<tr>
<td>1) Modification of antibiotic molecule</td>
<td>Produce enzymes that inactivate the drug by adding specific chemical moieties to the compound or that destroy the molecule itself</td>
</tr>
<tr>
<td>I. Chemical alterations</td>
<td>Biochemical reactions include i) acetylation (aminoglycosides, chloramphenicol, streptogramins), ii) phosphorylation (aminoglycosides, chloramphenicol), and iii) adenylation (aminoglycosides, lincosamides).</td>
</tr>
<tr>
<td>II. Destruction of antibiotic molecule</td>
<td>Example is beta-lactam resistance by producing beta-lactamase to destroy beta-lactam ring.</td>
</tr>
<tr>
<td>2) Decrease antibiotic penetration and efflux</td>
<td>Decreasing uptake of the antimicrobial molecule to prevent the antibiotic from reaching its intracellular or periplasmic target</td>
</tr>
<tr>
<td>I. Decrease permeability</td>
<td>Production of bacterial machineries to extrude a toxic compound out of the cell</td>
</tr>
<tr>
<td>II. Efflux pumps</td>
<td>Protection of the target (avoiding the antibiotic to reach its binding site) and modifications of the target site that result in decreased affinity for the antibiotic molecule</td>
</tr>
<tr>
<td>III. Change in target sites</td>
<td>Example target protection mechanism like tetracycline resistance determinants Tet(M) and Tet(O).</td>
</tr>
<tr>
<td>a) Target protection</td>
<td></td>
</tr>
<tr>
<td>b) Modification of target site</td>
<td>Example of mutational resistance is the development of rifampin (RIF) resistance</td>
</tr>
<tr>
<td>I. Mutations of target site</td>
<td>Example methylation of the ribosome catalysed by an enzyme encoded by the erm genes (erythromycin ribosomal methylation), which results in macrolide resistance</td>
</tr>
<tr>
<td>II. Enzymatic alteration of target site</td>
<td>Examples include methicillin resistance in S. aureus due to the acquisition of an exogenous PBP (PBP2a) and vancomycin resistance in enterococci through modifications of the peptidoglycan structure mediated by the van gene clusters</td>
</tr>
<tr>
<td>III. Replacement of bypass of target site</td>
<td>Examples are development of resistance to daptomycin (DAP) and vancomycin (low-level in S. aureus) resistance phenotypes that are the result of a global cell adaptive response to the antibacterial attack.</td>
</tr>
<tr>
<td>c) Resistance due to global cell adaptations</td>
<td></td>
</tr>
</tbody>
</table>
The most common types of multi-drug resistant organisms include:

1. Methicillin-resistant *Staphylococcus aureus* (MRSA)

*Staphylococcus aureus* is the most common bacterium associated with nosocomial infection around the world. Penicillin resistance was encountered only a few years after it was introduced to clinical practice (Chambers and Deleo, 2009). Methicillin became the drug of choice; however, over time, *S. aureus* strains formed resistance to it. This has created an ever-changing challenge to find new therapies. The prevention of MRSA involves isolating the infected patient, diligent hand hygiene practices, proper surface cleaning and antimicrobial stewardship (CDC, 2014).

2. Vancomycin-resistant *enterococci* (VRE)

*Enterococci* are harmless bacteria that are normally found in the gastrointestinal tract of animals and humans, and in the female genital tract; however, they can pose a threat to immunocompromised patients, causing endocarditis, urinary tract, bloodstream, wound and intra-abdominal infections (Byappanahalli et al., 2012). Vancomycin-resistant enterococci are specific strains of enterococci that have developed resistance against the antibiotic Vancomycin. It is believed that heavy usage of antibiotics in hospitalized patients causes the resistance of enterococci to vancomycin (kampmeier *et al.*, 2018). VRE are spread directly through contaminated hands and indirectly via the environment (Jones *et al.*, 2013). VRE are able to survive on surfaces for a longer period but they can be readily removed by thorough cleaning and disinfection of the surfaces (Benamu and Deresinski, 2018). Together with clean environment, proper hand hygiene practices and correct use of antibiotics can prevent the number of infections (Rubinstein & Keynan, 2013).
3. Multi-resistant Gram-negative bacilli (MRGN)

Gram-negative bacilli are normally found in the intestinal tract of humans and most animals where they are essential for proper digestive processes. They are however capable of causing infections when they are introduced into normally sterile body sites; such as bladder or deep tissues, mostly during surgery (Exner et al., 2017). Gram-negative bacilli are referred as Multidrug Resistant when they are resistant to Carbapenems, Fluorquinolones, Ureidopenicillins and cephalosporins (Exner et al., 2017; Ruppe et al., 2015). Resistance occurs by the mechanisms explained in Table 1.3.

Because these organisms are found in the intestine, any environmental surfaces that comes into contact with faecal material can become contaminated and serve as a reservoir for cross-infection. Furthermore, these organisms prefer a wet environment, and can quickly colonise sink drains and taps (Mundhada and Tenpe, 2015). They have also been found to contaminate diluted disinfectants and detergent solution used for cleaning. Hand washing is the most and paramount prevention of MRGN (Hawkey et al., 2018).

2.1.9 Infection Control and Prevention

Prevention of nosocomial infections is crucial and improvement in compliance with hygiene protocols reduces the spread of nosocomial Infections (Revelas, 2012). Acknowledging the importance, the environment plays in the transmission of microorganisms, the Centres for Disease Control and Prevention and the Health Care Infection Control Practices Advisory Committee recommend surfaces in proximity to patients, which are frequently touched, be properly cleaned and disinfected and that health care facilities ensure its professionals adhere to such procedures (Chemaly et al., 2014).
Despite evidence of the transmission of infectious organisms from environment to patient, the role of a clean environment in healthcare settings remains controversial. It is still unclear as to which extent the environmental contamination contributes to nosocomial infections. Surface cleaning cannot be substituted for other infection control practices such as hand washing, limiting medical device usage, and gowning or gloving when indicated. However, routine effort to decrease the overall bioburden of the healthcare environment via cleaning is most likely the basis to other efforts; lower levels of infectious organisms on surfaces translates to less contamination of healthcare provider’s hands and patients care objects as they make contact with the healthcare environment (Doll et al, 2018)

2.1.9.1 Hand Washing and Sanitizing

Nosocomial infections have taken a toll on patients, their loved ones, hospital staff members and society; physically, mentally, and financially (Salah et al, 2017). Multiple studies have found that good hand hygiene practices, positive attitudes about hand hygiene, and/or addressing hand hygiene barriers positively impact the rate of Hospital acquired infections (HAIs). For example, according to Tippin (2015), good hand hygiene is one of the easiest and most important actions that healthcare professionals can carry out to decrease the overall spread of infectious diseases and safeguard the health of patients. Mathur (2011) also supports this notion by stating that hand hygiene is the simplest and most cost-effective strategy that can decrease nosocomial infections.

As the hands of healthcare workers are the most important means of transmission of nosocomial infections and MDROs, the importance of hand hygiene cannot be overstated. Several studies have shown that improvements in hand hygiene are associated with lower healthcare-associated infection rates, and/or reductions in MDRO transmission and
acquisition. Nonetheless, compliance with hand hygiene recommendations is often poor, with studies reporting rates as low as 30-50% compliance (Marra and Edmond, 2014; Tenna et al, 2013).

### 2.1.9.2 Environmental Cleaning and Disinfection

Multiple studies have found that cleaning and/or disinfecting environmental surfaces reduces contamination, and consequently, contributes to reducing the occurrence of infection (Wattal and Khardori, 2014). Environmental cleaning (EC) is a fundamental principle of preventing infection in the hospital and healthcare setting. Both porous surfaces (e.g., mattresses) and nonporous surfaces (e.g., bed rails) in patient rooms are highly susceptible to bacterial contamination with dangerous pathogens, including *C. difficile*, and antibiotic-resistant organisms such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and multiple species of *Acinetobacter*. Hard, nonporous surfaces, which include common items such as furniture, bed rails, and medical equipment, as well as fixed spaces like floors and bathrooms facilities, form part of the environmental reservoir that can lead to significant microbial contamination (Chemaly, 2014).

Antiseptics and disinfectants are broadly used in healthcare settings for disinfecting various surfaces. The Centre of Disease Control and prevention (CDC) is guide to selection and use of disinfectants recommends which chemicals might be most appropriate or effective for specific microorganisms and settings when disinfecting. Careful attention must be paid to product selection and concentration as certain disinfectants can be corrosive. Alcohols have rapid broad-spectrum antimicrobial activity against vegetative bacteria, viruses and fungi. Hydrogen peroxide (H₂O₂) is a broad-spectrum antiseptic that has greater effects against Gram-positive than Gram-negative bacteria. Quaternary ammonium compounds (QACs) are the most useful antiseptics as they will kill most bacteria, viruses and fungi, including MDRO
from surfaces and are excellent for hard-surface cleaning (Wilson et al, 2018).

Appropriate cleaning of these surfaces is an important part of an overall strategy to reduce the risk of nosocomial infections. However, little consensus exists for optimal approaches to environmental cleaning. This is achieved by implementing adequate methods for cleaning, disinfection and sterilization (CDC, 2017a; Metha et al, 2014).

2.1.9.3 Cleaning Standards for Prevention on Nosocomial Infections

Achieving good cleaning outcomes is paramount to maintain a low risk of nosocomial infections. The healthcare facilities are required to have the following support programs in place to ensure the hygiene standards in the facility are up to standard.

- Organizational chart and governance structure.
- A facility map providing the outlined detailed floor plan of all functional areas.
- Site-specific cleaning schedule to ensure that high touched surfaces are cleaned appropriately.
- Staff member are required to be trained by a suitable qualified person to ensure that they are skilled accordingly and have adequate knowledge on cleaning procedures.
- All cleaning chemicals and equipment used in the facility must be fit for purpose and undergo a thorough risk assessment prior to usage.
- All cleaning techniques must be documented in relevant cleaning policies and standard operating procedures.
- Maintaining records allows cleaning stuff, supervisors and managers to ensure that all cleaning activities are being completed and in the event
that it has been missed, the matter can be rectified without delay (Allen and Frye, 2008).

2.2 Homeopathy

2.2.1 Introduction to homeopathy

The word homeopathy is derived from the Greek word “homos” which means “like” and “pathos” which means “suffering” (Jayasuriya, 2010). Homeopathy is a medical philosophy and practice based on the law of similar which involves administration of ultra-diluted remedies prepared according to the homeopathic pharmacopeias methods. When a person has a disease, the remedy prescribed will match the disease “picture”, as well as take into account the mental and emotional state of the person. The remedies stimulate the body’s natural ability to heal itself and promote cure (Kayne and Kayne, 2007).

Homeopathic practitioners takes a holistic approach towards the sick and focus on treating the whole patient rather than treating symptoms. The main goal of homeopathy is to improve the performance of the immune system which in turn brings back the equilibrium of the individual at all three levels; namely the physical, emotional and mental planes (Kayne and Kayne, 2007).

2.2.2 History of Homeopathy

Christian Samuel Hahnemann, the founder of homeopathy, was born on the 10th of April 1755 in Meissen, Germany. He was an exceptional student who had remarkable interest in language and sciences. He completed his medical studies in 1779 and while translating Cullen’s Materia medica in 1796, discovered that if a healthy individual would take a dose of quinine, they will develop the symptoms that were known to be curable by quinine. Curious
with his observational result, he experimented further, identifying trends, postulating theories and refining his approach, until eventually he formed the laws which govern homeopathy and thus grounded this healing art (Jayasuriya, 2010; De Schepper, 2010).

2.2.3 The Homeopathic Consultation

Homeopathy is a primary healthcare profession aiming at diagnosing, treating and preventing disease and/or disorder (De Schepper, 2010). Observational evidence has suggested that the environment may play a significant role in the transmission of nosocomial infections. Contamination of environmental surfaces like tables, beds and medical equipment has been documented (Leas and oakley, 2014). A typical homeopathic consultation room is similar to hospital consultation rooms; consisting of a table, chairs, bed, bookshelves and a cupboard. These non-porous surfaces pose a greater risk of harboring bacteria due to its ability to retain oxygen and moisture.

During the consultation process, both patient and practitioners come into contact with all the various furniture in the room. The practitioner takes a case history and performs physical examination to arrive at a diagnosis. Patients are required to uncover certain parts of their body for examination purposes (Homeopathic 5th year study guide). As a result, the surfaces that comes into contact with patients' skin are likely to bid as reservoirs from which patients and healthcare workers may encounter and transfer microbes.

It has been proven that healthcare practitioners don’t always comply with hand and surface hygiene (Claro et al, 2015); therefore, it is safe to assume that the protocols of hand hygiene and surface hygiene in the Homeopathic Health Training Centre at the University of Johannesburg is not followed appropriately. As a result, microorganisms growing on the surface can affect
the health of patients during physical examination. These adverse effect ranges from minor complaint to major health issues (Adisa et al, 2016).

The transmission of microorganisms on patients during physical examination creates an opportunity for infections to move from patients to practitioners and so on (Allen and Frye, 2008). Thus, calling for the need to know if the pathogens are really present on the surfaces in Homeopathy Health Training Centre, and the potential risks they pose.

2.3 Related Studies

Past research conducted at the Chiropractic Clinic, University of Johannesburg, reported that all the sampled surfaced of the chiropractic treatment beds carried microorganisms. The results highlighted that the chiropractic treatment beds are indeed a potential reservoir of bacteria such as; *Pseudomonas* spp., *Klebsiella* spp., *E coli* spp., and *Enterobacter* spp. As well as fungi such as; *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Rhicopus* spp., and *Cryptococcus neoforman* (Perdjik, 2017; Kruger, 2017). Kruger (2017) concluded that microbe accumulation can be avoided by disinfecting the treatment surfaces with alcohol-based disinfectant. Perdjik (2017) concluded that additional hygiene training within the University of Johannesburg chiropractic-teaching clinic needs to be implemented.
CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter describes the study design, objective measurements, the data evaluation techniques, statistical analysis tools, and ethical considerations.

3.2 Study Design

This study was an exploratory study with a quantitative research design using objective (microbial enrichment) data to evaluate the surface hygiene in the Doornfontein Homeopathic Health Training Centre.

3.3 Sampling Site

Seven (7) consulting rooms in the Homeopathic Health Training Centre (HHTC) at the University of Johannesburg Doornfontein campus that are frequently used by practitioners and their patients were assessed. Samples were collected from the surface areas of vinyl beds (n=21), wooden tables (n=21), steel cupboards (n=14) and steel cabinets (n=21), within each room. Therefore, the total number of surfaces sampled was n=77.

3.4 Inclusion Criteria

Only rooms that were frequently used for consultation by student and practitioners were included in the study. Only tables, beds, steel cupboards and steel cabinet were sampled. No other equipment was sampled.
3.5 Sample Approach

Figure 3.1 is a flow diagram to provide a clear understanding of the methodology.

![Flow diagram of methodology]

3.6 Sampling Equipment- Count-Tract range

The bioMerieux Irradiated Count-Tact® 3P™ (CT3P) agar contact plates were used according to manufacturer's instruction (APPENDIX A). The plates were developed for the monitoring of microbial contamination in industrial and hospital cleanroom environments. The irradiated CT3P agar contact plates are proven to be a valid and reliable method of testing for microbial contamination due to its design. The convex agar meniscus allows for direct application to the test surface for hygiene monitoring. Each plate has a
diameter of 65 mm and a grid scored on the base for easy colony counting (Biomerieux, 2007).

A single CT3P agar contact plate slides onto a Count-Tact® applicator which enables the standardization of surface sampling in terms of time (10 seconds) and pressure (500g) (Biomerieux, 2007).

3.6.1 Sample Collection

Objective data was collected by means of the bioMerieux irradiated CT3P agar contact plates. Double samples (n=154) were taken in the following order; right to left for tables, top to bottom for steel cupboards, lockers and beds.

The samples were taken by briefly depressing the 65mm irradiated CT3P agar contact plates for 10 seconds under 500g pressure with the Count-Tact® applicator over three standardized areas for tables, beds and steel cupboards and two standardized areas for steel lockers (n=154): (1) the right, middle, left of the table (n=42), (2) the top and bottom surface of the lockers (n=28), (3) the top, middle and bottom surface of the steel cupboard (42), and (4) the top, middle and bottom of the beds (n=42) (Figure 3.2). One sample of each set was incubated for up to 3 days (for bacteria) and the other incubated for up to 5 days (for fungi). Colonies per plate (25cm) were counted and the results in colony forming unit (CFU) per cm were reported.

3.6.2 Primary Organism Isolation

After the incubation period, the contact plates that had bacterial growth were further tested. Bacterial isolates were then plated onto sheep blood agar plates (National Health Laboratory Services) for characterization. A representative number of bacterial growth morphologies and lysis patterns were selected as representation of the bacteria present in the sample. They
were then further characterized using VITEK 2 compact (bioMerieux) using the methods and consumables specified by the manufacturer.

Plates containing fungal growth were sent to the University of the Free State for isolation, purification and identification using sequencing of the appropriate genes to facilitate identification.

Figure 3.2  The furniture sampled included the desks (top left), steel cupboard (top right), bedside tables (bottom left) and patient bed (bottom right).

3.7 Validity and Reliability
The in vitro experiment was conducted in duplicate per isolate and repeated to ensure that the method was reliable and reproducible, and yielded accurate results. All experiments were conducted under the supervision of a qualified laboratory technician in a controlled environment to ensure reliable results. Appropriate controls were included to confirm the reliability and trustworthiness of the results.

Irradiated Count-Tact™ 3P is an improvement of the Count-Tact irradiated agar, enabling improved growth of microorganisms encountered in the environment. This agar is recommended for monitoring surfaces by applying the agar manually and for monitoring equipment and personnel. Use of this agar is described in the ISO 14698-1 standard (Biomerieux, 2007).

The VITEK 2 COMPACT instrument and VITEK 2 software provides reliable microbial identification. The instrument also enhances laboratory efficiencies with reduced hands-on time and rapid reporting capabilities (Biomerieux, 2007).

### 3.8 Ethical Consideration

All aspects of the study were conducted in accordance to the declaration of Helsinki and conformed to international ethical standards with Higher Degree and ethical approval. An ethical approval (Appendix B) was obtained from the Research Ethics Committee of faculty of Health Sciences, University of Johannesburg. The manager of the UJ Doornfontein Campus Health Training Centre, Dr. Pieter Els and the clinician in charge were emailed to request permission to conduct the study (APPENDIX C).

### 3.9 Data analysis
The data were analyzed according to the one sample $t$-test. The statistical analysis was conducted in IBM SPSS Statistics by STATKON. Frequencies and descriptive statistics of the whole sample were used to interpret the data, by performing an exploratory data analysis. Non-parametric testing was exploited. The Kruskal-Wallis test was then performed to calculate the mean rank. Because there were more than two groups of data, the Mann-Whitney U test was done to determine the differences between the groups.
CHAPTER 4: RESULTS

4.1 Introduction

This chapter provides findings and analysis of the data collected by the researcher in order to gain insight of the microbial load on the surfaces of certain furniture in the Homeopathic Health Training Centre.

4.2 The microbial loads found on the surfaces at the HHTC

Results from the surface sampling indicate that surfaces which come into contact with patients and practitioners do harbor microbes (Table 4.1). Considering that samples were collected at the end of the day, it would be safe to assume that patients’ collective flora had been deposited on the surfaces.

A total of 144 samples were taken for bacteria (n=77) and fungi (n=77): 21 from tables, 21 from beds, 21 from steel cupboard and 14 from lockers. A total of 66 (85%) of the samples had growth for bacteria and 72 (92%) for fungi, respectively. Table 4.1 shows the average number of colony-forming units (cfu/cm²) found on each of the sampled surfaces. Both bacterial and fungal cfu/cm² were found on all sampled surfaces (Figure 4.1 and Figure 4.2).

Table 4.1 Mean aerobic colony count (cfu/cm²) on each surface
<table>
<thead>
<tr>
<th>Surface</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table</td>
<td>21.56</td>
<td>16.94</td>
</tr>
<tr>
<td>Locker</td>
<td>3.78</td>
<td>3.99</td>
</tr>
<tr>
<td>Steel cupboard</td>
<td>6.99</td>
<td>13.52</td>
</tr>
<tr>
<td>Bed</td>
<td>26.83</td>
<td>69.99</td>
</tr>
</tbody>
</table>

**Figure 4.1**  Bacterial growth on contact plate after incubation
The beds had the highest average bacterial (26.83 cfu/cm², 45%) and fungal (69.99 cfu/cm², 67%) count per area tested. This was followed by tables (21.56 cfu/cm², 16.94 cfu/cm²), steel cupboard (6.99 cfu/cm², 13.52 cfu/cm²) and lockers (3.78 cfu/cm², 3.99 cfu/cm²) respectively (Figure 4.3).

When comparing the areas of the sampled surfaces (Table 4.2), there were significant differences in the number of microbial cfu/cm² on these surfaces. The top and middle surface of the beds had the highest average of bacterial counts whereas the top and bottom surface had the highest concentration of fungi. The highest amount of fungi at the bottom of the bed shows that fungal colonies are mostly from patients is feet.

**Table 4.2** Microbial load of the different surfaces of the sampled areas

<table>
<thead>
<tr>
<th></th>
<th>Bacterial count (cfu/cm²)</th>
<th>Fungal count (cfu/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampled surfaces</td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>Table Right</td>
<td>13.42</td>
<td>8-27</td>
</tr>
<tr>
<td>Table Middle</td>
<td>30.28</td>
<td>11-80</td>
</tr>
<tr>
<td>Table Left</td>
<td>21</td>
<td>6-46</td>
</tr>
<tr>
<td>Locker Top</td>
<td>4.28</td>
<td>0-15</td>
</tr>
<tr>
<td>Locker Bottom</td>
<td>3.28</td>
<td>0-6</td>
</tr>
<tr>
<td>Steel Cupboard Top</td>
<td>18.42</td>
<td>8-30</td>
</tr>
<tr>
<td>Steel Cupboard Door 1</td>
<td>1</td>
<td>0-3</td>
</tr>
<tr>
<td>Steel Cupboard Door 2</td>
<td>1.57</td>
<td>0-4</td>
</tr>
<tr>
<td>Bed Top</td>
<td>39.23</td>
<td>0-150</td>
</tr>
<tr>
<td>Bed Middle</td>
<td>30.28</td>
<td>0-70</td>
</tr>
<tr>
<td>Bed Bottom</td>
<td>11</td>
<td>0-36</td>
</tr>
</tbody>
</table>

The tables had the second highest concentration of both bacteria and fungi; the middle surface had highest number of colonies compared to the right and left. The cupboard had the third highest microbe load concentration, especially on its top surfaces where students and practitioners put their instruments and equipment. The locker had the lowest colony counts.
Table 4.3  Evidence of the statistical difference in the bacterial loads of the sampled surfaces in the HHTC (Kruskal-Wallis)

<table>
<thead>
<tr>
<th>Surfaces</th>
<th>Number</th>
<th>Mean Rank</th>
</tr>
</thead>
</table>

Figure 4.3  Bar graph showing the mean microbial counts (colony forming units [CFU]/cm²) of surface sampled with contact plates

4.3  Statistical Analysis

Kruskal-Wallis, non-parametric test, calculated mean rank and showed a statistical significance of 0.000 across all groups (Table 4.3). When comparing the different surfaces (Table 4.4) for bacterial samples using the Mann-Whitney test, there were four significant statistical observation between table and locker (p= 0.000), bed and cupboard (p=0.043), table and cupboard (p= 0.044) and bed and locker (p= 0.001). These results suggest that bacterial contamination mostly occurs on high touched surfaces (Dancer, 2014; Doll et al, 2018), which in this case would be tables and beds.
<table>
<thead>
<tr>
<th>Samples</th>
<th>Bacteria mean</th>
<th>Bacteria p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table</td>
<td>23</td>
<td>0.000</td>
</tr>
<tr>
<td>Lockers</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Table</td>
<td>19</td>
<td>0.878</td>
</tr>
<tr>
<td>Cupboard</td>
<td>15</td>
<td>0.175</td>
</tr>
<tr>
<td>Table</td>
<td>19</td>
<td>0.044</td>
</tr>
<tr>
<td>Bed</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Locker</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Cupboard</td>
<td>17</td>
<td>0.043</td>
</tr>
<tr>
<td>Bed</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4  Evidence of the statistical difference in the bacterial loads of the sampled surfaces in the HHTC (Mann-Whitney U)

For fungal samples (Table 4.5 and Table 4.6), Kruskal-Wallis test calculated mean rank and showed a statistical significance of 0.000 across all groups (Table 4.5). Mann-Whitney U test showed three statistical significance observation between table and locker (p= 0.035), Locker and cupboard (p=0.005) and bed and locker (p= 0.003) (Table 4.6). These results suggest
that fungal contamination mostly occurs on high touched surfaces (Dancer, 2014; Doll et al, 2018), which in this case would be tables and beds.

**Table 4.5** Evidence of statistical difference in the fungal loads of the sampled surfaces in the HHTC (Kruskal-Wallis)

<table>
<thead>
<tr>
<th>Surfaces</th>
<th>Number</th>
<th>Mean Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tables</td>
<td>21</td>
<td>40.52</td>
</tr>
<tr>
<td>Lockers</td>
<td>12</td>
<td>10.07</td>
</tr>
<tr>
<td>Steel Cupboard</td>
<td>15</td>
<td>20.40</td>
</tr>
<tr>
<td>Beds</td>
<td>21</td>
<td>51.64</td>
</tr>
<tr>
<td><strong>P-Value</strong></td>
<td></td>
<td><strong>0.000</strong></td>
</tr>
</tbody>
</table>

**Table 4.6** Evidence of statistical difference in the fungal loads of the sampled surfaces in the HHTC (Mann-Whitney U)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fungal mean</th>
<th>Fungal p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Locker</td>
<td>4</td>
<td>0.035</td>
</tr>
<tr>
<td>Table</td>
<td>9</td>
<td>0.306</td>
</tr>
<tr>
<td>Cupboard</td>
<td>6</td>
<td>0.277</td>
</tr>
<tr>
<td>Table</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Bed</td>
<td>9</td>
<td>0.005</td>
</tr>
<tr>
<td>Locker</td>
<td>4</td>
<td>0.003</td>
</tr>
<tr>
<td>Cupboard</td>
<td>11</td>
<td>0.481</td>
</tr>
<tr>
<td>Locker</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Bed</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Cupboard</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Bed</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
4.4 Types of Pathogens Identified on the Surfaces

The majority of the isolated microorganisms in this study represent normal human microflora and ubiquitous environmental fungi. These findings are consistent with previous healthcare hygiene studies (Doll et al., 2018; Hefzy et al., 2016) such as the Chiropractic Clinic also housed within the Doornfontein Health Training Centre (Kruger et al., 2017; Perdjik et al., 2017). A broad variety of pathogens were identified due to the sensitivity of the identification method used for bacteria and fungi. Non-pathogenic, Gram-positive and pathogenic Gram-negative bacteria were identified, as well as ubiquitous fungi that cause infections in immunocompromised patients. A summary of isolated pathogens with relevant literature and their clinical significance is illustrated in Tables 4.8 and Table 4.9.

4.4.1 Bacteria

Bacterial samples were then taken for analysis and identification. Gram-positive isolates were predominate, followed by gram-negative bacteria. Sixty-six (66) randomly selected samples were put through the bioMerieux VITEK 2 system in order to identify the bacterial species. Table 4.7 summarizes the type of bacteria identified. Although Micrococcus luteus was the most identified bacteria, many samples (n=10) couldn’t be identified (Table 4.7).
### Table 4.7  Bacteria identified through bioMereux VITEK 2 System

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number of pathogens</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus capitis</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Staphylococcus xylosus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus lentus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus warneri</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus pumilus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bacillus fortis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bacillus galactosidilyticus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Kocuria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kocuria kristinae</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Kocuria rhizophila</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Kocuria rosea</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Kocuria variens</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>10</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Granulicatella</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulicatella adiacens</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Sphingomonas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Gardnerella</strong></td>
<td></td>
<td>Variable</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Unidentified</strong></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
4.4.2 Fungi

Laboratory analysis was performed on the collected samples to see whether the sample produced any fungal growth. The fungal growth were sent to the University of the Free State for identification using sequencing. Table 4.9 illustrates the identified fungi with their clinical relevance.
Table 4.8  Identified bacteria with their clinical significance

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Mode of Transmission</th>
<th>Diseases and clinical features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. paucimobilis</td>
<td>Indirect contact (Fomite)/ Vehicle (water)</td>
<td>Bacteraemia</td>
<td>Nandy et al (2013)</td>
</tr>
<tr>
<td>G. vaginalis</td>
<td>Indirect Contact (Fomite) / Direct Contact (Vertical) / Direct Contact (Horizontal)</td>
<td>Septic osteo-articular involvement: fever, chills, fatigue, malaise, joint pain, redness and swelling; bacterial vaginosis.</td>
<td>Horau et al (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Muzny and Schwebke (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Schwebke et al (2014)</td>
</tr>
<tr>
<td>K. rosea</td>
<td>Direct/ Indirect contact with contaminated objects and/or surfaces. Inhalation of contaminated droplets</td>
<td>Peritonitis; brain abscess; meningitis; urinary tract infection; cholecystitis</td>
<td>Sohn et al (2015)</td>
</tr>
<tr>
<td>K. kristinae</td>
<td></td>
<td></td>
<td>Domont et al (2014)</td>
</tr>
<tr>
<td>M. luteus</td>
<td>Direct/Indirect contact with contaminated hands or objects</td>
<td>Skin infections</td>
<td>Leas and Oakley (2014)</td>
</tr>
<tr>
<td>G. adiacens</td>
<td>Direct contact with infect body fluid</td>
<td>Infective endocarditis; pulmonary, CNS and ocular infection.</td>
<td>Gupta et al (2018)</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>Inhalation Direct/indirect</td>
<td>Septic arthritis; skin infection</td>
<td>Shivamurthy et al (2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clemente et al (2016)</td>
</tr>
<tr>
<td>B. cereus</td>
<td>Contaminated food Contaminated linen</td>
<td>Symptoms of diarrhoeal syndrome resemble those of clostridium food poisoning; emetic syndrome; bloodstream infection</td>
<td>Anma et al (2017)</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>Contact with infected objects</td>
<td>Brain abscess; skin infection</td>
<td>Guo et al (2015)</td>
</tr>
<tr>
<td>S. capitis</td>
<td>Direct and indirect contact</td>
<td>Skin infection; food poisoning; septicaemia; toxic shock syndrome; septic arthritis</td>
<td>Tande et al (2014)</td>
</tr>
</tbody>
</table>
Table 4.8 continued  Identified bacteria with their clinical significance

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Mode of Transmission</th>
<th>Diseases and clinical features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. xylosus</td>
<td>Direct/ indirect contact of body fluid</td>
<td>Erythema nodosum; brain abscess</td>
<td>Giordano et al (2016)</td>
</tr>
<tr>
<td>S. epidermis</td>
<td>Direct/ indirect contact</td>
<td>Bacteraemia; mediastinitis; eye keratitis</td>
<td>Namvar et al (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kahl et al (2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Harris et al (2016)</td>
</tr>
<tr>
<td>S. lentus</td>
<td>Direct contact</td>
<td>Peritonitis</td>
<td>Koller et al (2011)</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>Direct/ indirect contact with infected hands and objects</td>
<td>Shunt nephritis; urinary tract infection</td>
<td>Czekaj et al (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Barros et al (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ruzauskas et al (2014)</td>
</tr>
<tr>
<td>S. warneri</td>
<td>Direct/ indirect contact with infected hands and objects</td>
<td>Septic arthritis; bacteraemia; endocarditis</td>
<td>Legius et al (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ivić et al (2013)</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Mode of transmission</td>
<td>Clinical features</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------</td>
<td>----------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Indirect contact (Fomite) /</td>
<td>Aspergillosis cutaneous infections</td>
<td>Vermeulen <em>et al</em> (2014)</td>
</tr>
<tr>
<td></td>
<td>Vehicle (Food/Air)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cladosporiom cladosporioides</em></td>
<td>Indirect contact (Fomite) /</td>
<td>Fungal meningitis; Haemorrhagic</td>
<td>Grava <em>et al</em> (2016)</td>
</tr>
<tr>
<td></td>
<td>Vehicle (Food/Air)</td>
<td>pneumonia</td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>Inhaled Air</td>
<td>Chronic Pulmonary Penicilliosis</td>
<td>De Monte <em>et al</em> (2014)</td>
</tr>
<tr>
<td><em>Fusarium proliferatum</em></td>
<td>Indirect Contact</td>
<td>Fusariosis</td>
<td>Sandoval-Denis <em>et al</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2015)</td>
</tr>
</tbody>
</table>
CHAPTER 5: DISCUSSION

5.1 Introduction

Numerous studies have reported that surfaces which come in contact with patients and practitioners can be contaminated by different pathogens (Bryrd, 2016; Claro et al, 2015; Doll et al, 2018; De wit et al, 2012). The current study was carried out to gain insight into the potential pathogenic load on the surface in the HHTC, UJ Doornfontein campus by sampling the surfaces of some furniture. The samples were cultured accordingly, and a subset of the bacteria and fungi representing the typical isolates obtained were further identified. This chapter explore and discuss the results of the study and possible explanations of the outcomes.

5.2 Microbial Load on Surfaces

The aerobic culture results revealed that most surfaces at the HHTC were found to be contaminated by various bacteria and fungi. This finding is comparative to the studies done at the Chiropractic UJ Clinic (Kruger, 2017; Perdijk, 2017). In the present study, most of the bacterial isolates were Gram positive. The higher frequency of Gram-positive bacteria might be due to the dry conditions of the clinic environment and transmission from skin and mucus membranes of students, practitioners and patients, as described previously (Heller and Edelblute, 2018). The recovered fungi were ubiquitous (Kwon-Chung and Suguí, 2013); however, severe infections have been documented in the literature (Trovey and Grenn, 2005).

In the present study, the reported mean aerobic colony count from all the surfaces is 14.79 cfu/cm² for bacteria and 26.08 cfu/cm² for fungi (refer to Chapter 4 for detailed breakdown). These counts are higher than the acceptable limits recommended by Dancer (2004), which states that the mean aerobic count from microbial culture of surface sampled should be <5 cfu/cm². This higher recorded count may add an increased risk of
infection for patients and practitioners in the centre. Furthermore, the finding calls for the evaluation and strengthening of infection control and prevention practices, and for regular monitoring of microbial levels in the HHTC.

Higher colony counts for both bacteria and fungi were found on the beds and tables and this may be caused by patients depositing microbes during physical examination or less effective suboptimal cleaning (Claro *et al*, 2015). Kruskal-Wallis test showed a statistical significance of *p*=0.000 for both fungi and Bacteria across all groups. According to the Mann-Whitney test performed, a significant statistical observation was noticed in the number of organisms found on highly touch surfaces, supported by Ali (2017). Tables and lockers (*p*=0.000 for bacteria; *p*=0.035 for fungi), tables and cupboard (*p*=0.044 for bacteria; *p*=0.004 for fungi). This could be due to the fact that lockers and cupboards are only touched by practitioner. However, the top surface of the cupboards (18.42 cfu/cm²) were relatively contaminated by bacteria and fungi, showing that practitioners do deposit microbes on them as they are mostly touched only by practitioners. Beds were significantly infected with bacteria and fungi in comparison, to lockers (*p*=0.001 for bacteria; *p*=0.003 for fungi) and to cupboards (*p*=0.043 for bacteria; *p*=0.003 for fungi).

The results reveal that the surfaces in the HHTC are potential reservoirs for pathogens and this supports similar reports by other studies. This poses a risk since patients are examined on the beds with bare skin exposed at times and they tend to rest their hands on the tables during examination. Hand hygiene must therefore be complied with to prevent deposition of microbes on surfaces and disinfection of surfaces must be addressed and followed properly to prevent infection acquisition. (Perdjik, 2017; Claro *et al*, 2015; Doll *et al*, 2018). The next section will discuss, in more detail, some of the bacteria and fungi identified.

a. Bacteria
The human body is colonized by a wide variety of bacteria (Leas and oakly, 2014). Most of these bacteria are commensal; they benefit from the host, but they do not cause any harm to the host. Their localization in healthy individuals is normally restricted to the skin, respiratory tract, gastrointestinal tract and vagina (Kruger, 2017).

When looking at the frequency of identified isolates in Table 4.7, *M. luteus* (n=10) is the most frequent isolate found, followed by *S. epidermis* (n=4), *S. waremi* (n=3), *S. capitis* (n=2) and *S. heamolyticus* (n=1). *M. luteus* predominantly colonizes the upper arms, legs and trunk and can withstand dry conditions (Tande et al, 2014). It was discovered on both the beds and tables. It is non-pathogenic; however, it may affect immunocompromised individuals and cause intracranial abscess. *S. heamolyticus* shows high resistance to most antibiotics (Barros et al, 2012).

*Staphylococci epidermidis* is the most isolated species of the staphylococci family from human epithelia (Nguyen et al, 2017). It was found on beds. It mostly colonizes the axillae, head and nares. As part of human epithelial flora, *S. epidermidis* has a benign relationship with its host (Namvar et al, 2014). Among *Coagulase-Negative Staphylococci* (CoNS), *S. epidermidis* is responsible for the vast majority of non-specified CoNS infections. *S. epidermidis* enters the body through an indwelling medical device and accounts for 22% of bloodstream infections in the Intensive Care Unit (Becker et al, 2014). *Staphylococcus haemolyticus* is the second most isolated CoNS from human blood cultures and has the highest level of anti-microbial resistance (Barros et al, 2012). *S. haemolyticus* is prevalent in healthcare environment, with a tendency to develop resistance to multiple antibiotics (Czekaj et al, 2015; Ruzauskas et al, 2014).

*Staphylococcus warneri* is also one of the CoNS pathogens that is mostly found on the skin (Ivić et al, 2013; Legius et al, 2012). A case of *S. warneri* sepsis with multiple abscesses in an immunocompetent patient free from risk factors for coagulase-negative staphylococcal infection has been
documented (Ivić et al, 2013). This indicates that S. warneri with other Staphylococcus species can cause nosocomial infections in individuals free from predisposing factors (Kahl et al, 2016; Tande et al, 2014).

Little is known about S. lentus and only one report of human infection exists (Rivera et al, 2014; Koller et al, 2011).

*Bacillus pumilus* is a Gram-positive, aerobic, spore-forming bacillus commonly found in soil. *B. pumilus* spores generally show high resistance to environmental stresses, including light exposure, desiccation, and the presence of oxidizers such as hydrogen peroxide (Clemente et al, 2016; Shivamurthy et al, 2016).

*Sphingomonas paucimobilis* is the only Gram-negative (Nandy et al, 2013) bacillus that was identified from the analyzed samples. Cases of severe infections and septic shock with this organism have been reported, particularly in immunocompromised patients, but only one case of death have been reported (Pascale et al, 2013). Infection include bacteraemia/septicaemia caused by contaminated solutions. *S. paucimobilis* can be isolated from a wide range of clinical specimens; it can survive in low nutrient environment and may then remain viable for longer period of time. *S. paucimobilis* is usually susceptible to most antibiotics and resistant to penicillins. A lethal case of an immunocompromised adult patient with *S. paucimobilis* bacteraemia that was resistant to prescribed antibiotics has been reported (Al-Halawani et al, 2015).

*Kocuria* organisms are generally of low virulence and considered harmless commensals of skin and oropharynx but may cause opportunistic infections in immunocompromised as well as immunocompetent individual with some underlying problems (Sohn et al, 2015). The most common co-morbid conditions associated with *Kocuria* infection are cancers, metabolic disorders, end stage renal diseases, diabetes and short bowel syndrome (Domont et al, 2014). As of now, 23 cases of infection due to *Kocuria* species have been reported in the literature, with *K. kristinae* as the most
common pathogen followed by *K. rosea*, *K. marina*, *K. rhizophila* and *K. varians* (Kandi *et al.*, 2016; Chen *et al.*, 2015). All of these have been recovered from the surfaces in the HHTC.

Other frequent colonizers include, *Granulicatella adiacens* (Schwebke *et al.*, 2014; Horau *et al.*, 2012) and *Gardnerella vaginalis*, which are often found on the skin and vagina, respectively. Recovery of *Gardnerella vaginalis* shows poor personal hygiene practices (Muzny and Schwebke, 2013).

b. Fungi

The result of the furniture sampled in the HHTC demonstrated the presence of myriad fungal species. Among the identified isolates the genus *Cladosporium, Aspergillus, Trichoderma, Penicillium* and *Fusarium* represented the bulk of the identified fungi. *Cladosporium* is one of the most frequently isolated airborne fungi, are ubiquitous and are infrequently associated with human opportunistic infections (Sandoval-Denis *et al.*, 2015). When *Cladosporium* causes infection it is usually associated with allergic rhinitis or localized superficial or deep lesions (Katotomichelakis *et al.*, 2015).

*Aspergillus niger* is commonly recovered from bird nests (Dagenais and Keller, 2009); its presences in the clinic may indicate the presence of birds near the clinic rooms. *Aspergillus niger* is more prevalent in warmer climates. There is no air conditioning system in the clinic, making the environment favourable for *Aspergillus* growth. The mold will be suspended in the air where they can be inhaled by immunocompromised patient or infant and cause diseases (Ergene *et al.*, 2013).

*Trichoderma* are filamentous fungi that are normally ubiquitous; however, they have shown be opportunistic particularly in immunocompromised patients (Sautour *et al.*, 2018). *Penicillium*, currently known as *Talaromycosis* is a fungus that has been known to only infect people living
in Southeast Asia. It only causes infection in people with HIV/AIDS (CDC, 2017b).

Construction is one activity that is known to produce fungal aerosols (Luo et al, 2018). The neighbouring lecture hall of the clinic underwent renovation during the sampling period. This may have potentially raised dust-rich Aspergillus spores, which may have entered via the clinic windows. Spores can also be brought into the building on the surfaces of shoes and clothing or spoiled plants and foods (Biranjia-Hurdoyal and Latouche, 2016).

5.3 Surface Hygiene in the Homeopathic Health training Centre

Many factors can influence the survival of microbes in the UJ HHTC. These factors can be stipulated as the number of foot traffic, occupant activities, inadequate surface cleaning and maintenance, poor building design, and the ventilation (Dramowski et al, 2017; Biranjia and Latouche, 2016; Boyce, 2014). Our findings recognize the potential for the surfaces in the HHTC to facilitate horizontal transmission and nosocomial infection. This again would be consistent with the conclusion that the main source of the bacteria is from the patients’ skin and mucus membrane (Chen et al, 2015; Russotto, 2015).

In the consultation rooms at the HHTC there are no washing basins (in each room) to facilitate hand washing before and after treating patients. This may be an obstacle as student might find it inconvenient and time consuming to go wash their hands at the dedicated hand washing facility. Poor compliance of hand hygiene will ultimately lead to spread of infections (Salah et al, 2017). The beds (26.83 cfu/cm²; 69.99 cfu/cm²) and tables (21.56 cfu/cm²; 16.94 cfu/cm²) had the highest bacterial and fungal load, respectively. The beds are covered with linen/sheets that are replaced every week. These sheets are then sent for washing at the laundry facility where they can further become contaminated with other pathogens or transfer the pathogens onto the people that are washing the
items. For this particular reason, the sheets should be removed from beds and students and practitioners should be required to wipe the beds with a disinfectant before consulting with a patient. Alternatively, they can be washed separately. With the tables (21.56 cfu/cm²) being one of the high-touched surfaces (Doll et al., 2018; Donskey, 2013; Dancer, 2004), they require routine cleaning and sanitization.

Contamination of surfaces plays an important role in the transmission of pathogens in healthcare environment (Donskey, 2013). In order to prevent nosocomial infections, attention must be directed toward the importance of environmental cleaning and disinfection. Routine microbial monitoring of surfaces in healthcare settings is not recommended, unless there is an outbreak or for the purpose of research. The reason being that there is no accepted and proven standard correlating environmental contamination and the risk of infection. Furthermore, there is no agreed methodology for surface sampling, and some approaches yield higher counts than others (Claro et al., 2015). Regardless, the irradiated count-Tact™ 3P pack+ method used in this study has been proven to assess the efficacy of cleaning and sanitation procedure. The contact plate can detect the presence or absence of living organisms (Daneau et al., 2016).

Despite evidence of transmission of pathogens from the environment to patient and healthcare workers, the role of a clean environment in the prevention of infections remains disputable. The degree to which environmental contamination contributes to nosocomial infections is still unpredictable (Dancer, 2014). This is because cleaning has never been regarded, let alone investigated, as an evidence-based science (Dancer, 2004; Han et al., 2015). Despite the lack of evidence, the healthcare environment may well act as a significant reservoir for potential pathogens. Surface cleaning in conjunction with other preventable practices like hand hygiene contributes to the fight of infections. A surface that is free from infectious organisms contributes to clean hands of healthcare worker (Bolon, 2016; Donskey, 2013; WHO, 2013).
Findings of this study confirm the need for improved overall cleaning of surfaces that frequently come into contact with patients and practitioners. It would be wise for the students and practitioner to pay closer attention to the possibility of the surfaces in the HHTC to serve as a potential source of nosocomial infections. The HHTC needs to implement a proper hand-hygiene and table-disinfecting protocol and a sequential sanitizing routine with strict adherence and compliance by all staff and students to reduce the risk of infection.

5.4 Limitations of the Study

Possible limitations of this study include:

- Small sample size could be considered a potential limitation; however, the researcher's goal was not to generalize to a large population but to get an insight on the bacterial and fungal loads on the surfaces at the HHTC.
CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Introduction

Measuring microbial contamination on the healthcare surfaces is essential to understand the current hygiene situation, facilitate change and to implement the necessary interventions to reduce the potential for horizontal transfer and nosocomial infection from the HHTC. Beds (26.83 cfu/cm² for bacteria; 69.90 cfu/cm² for fungi) and tables (21.56 cfu/cm² for bacteria; 16.94 cfu/cm² for fungi) had the highest bacterial and fungal counts. Kruskal-Wallis test showed a statistical significance (p= 0.000) for both fungi and bacteria across all groups. Mann-Whitney U test showed four significant statistical observations between table and locker (p= 0.000), bed and cupboard (p= 0.043), table and cupboard (p= 0.044), and bed and locker (p= 0.001) for bacteria. For fungi, Mann-Whitney U test showed three statistical significance between table and locker (p= 0.035), locker and cupboard (p=0.005) and bed and locker (p= 0.003).

This study suggests that there is possible transmission of microbial pathogens between patients/practitioners and the surfaces at the HHTC. The majority of surfaces sampled carried microorganisms. Most of the bacteria identified were harmless and are normally part of the human flora; however, they tend to cause infections in patients with compromised immune system. Among the identified harmful bacteria, *M. luteus* was the common pathogen found on beds and tables. Followed by *S. epidermis*, which was found on beds. *Cladosporium* and *Aspergillus* fungi were discovered on beds.

Hand hygiene and surface disinfection should become an educational priority. Educational interventions should also provide clear evidence that the surfaces can be eradicated of microbes. Further investigation will be needed to determine long-term compliance and if these efforts do control risk of microbes through behaviour change.
With the increased burden of nosocomial infections and antimicrobial resistance, it has become difficult for healthcare administrations and infection control committees to reach the goal for elimination of microbes. However, by practicing sound and healthy ways for care delivery designed by infection control committees, controlling transmission of these infections using appropriate methods for antimicrobial use, the resistance in emerging pathogens against antimicrobials can be reduced.

Overall, the information collected in this study supports and emphasizes the need for an effective disinfection protocol for the prevention of bacterial and fungal accumulation on the HHTC surfaces.

6.2 Recommendation

- Examining the microbial contamination on other “high touched surfaces” such as door-handles, toilet surfaces and medical instruments.
- Finding other possible sources of contamination- water sources, patients’ and practitioners’ clothing and air entrainment.
- Examining the effect of hand antiseptics or decontamination of surfaces in order to determine whether cleaning these potential sources of infection are associated with a reduced incidence of contamination in the HHTC.
- Examining other possible substrates for pathogen contamination inside the clinic building such as drywalls, ceiling tiles, floor coverings etc.
- Examining the potential of horizontal transmission of pathogens from patients to doctors, patients to patients, or doctors to patients.
- Active and passive methods of infection control should be investigated to enhance safety in the HHTC and other teaching institutions.
• Surveillance to measure the incidence of infection at the HHTC and identify problem areas, measure progress of prevention effort, and ultimately the potential of acquiring infection.
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Detection and Enumeration of Bacteria, Yeasts and Molds in surface samples of healthcare facilities

Quantitative Method: Sampling by contact agar on plane surfaces

**PREPARATION**
Surface sampling by contact application with Count-Tact™ range

FOR UNPROTECTED AREAS
- For Total Plate: Count-Tact Agar (ref. 43501 - 20 x 65 mm plates)
- For Yeasts & Molds: Count-Tact Sabouraud chloramphenicol (ref. 43500 - 20 x 65 mm plates)

Count-Tact Applicator (ref. 96300 - 1 unit) Standardization of sampling: 10 sec under 4 pressure of 500 g

Bi-Box for Count-Tact plates (ref. 96501 - 1250 units)

FOR PROTECTED AREAS (e.g. ISOLATOR)
- For Total Plating: Irradiated Count-Tact 3P Agar (ref. 43691 - 20 x 65 mm plates)
- For Yeasts & Molds: Irradiated Count-Tact Sabouraud dextrose 3P™ (ref. 43812 - 20 x 65 mm plates)

**INCUBATION**
- For Total Count: Incubate Count-Tact plates up to 3 days at (30 ± 1°C)
- For Yeasts & Molds: Incubate Count-Tact plates up to 5 days at (22.5 ± 2.5°C)

**ENUMERATION**
Count the colonies per plate (25 cm²) and report the result in CFU per cm²

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APPENDIX B
FACULTY OF HEALTH SCIENCES
HIGHER DEGREES COMMITTEE

HDC-01-16- 2018
20 April 2018

TO WHOM IT MAY CONCERN:

STUDENT: KALUBI, A
STUDENT NUMBER: 201300226

TITLE OF RESEARCH PROJECT: Evaluating Surface Hygiene in the Doornfontein Homeopathic Health Training Centre, University of Johannesburg

DEPARTMENT OR PROGRAMME: HOMOEOPATHY

SUPERVISOR: Dr R Razlog
CO-SUPERVISOR: Prof TG Barnard
CO-SUPERVISOR: Dr A Singh

The Faculty Higher Degrees Committee has scrutinised your research proposal and concluded that it complies with the approved research standards of the Faculty of Health Sciences; University of Johannesburg.

The HDC would like to extend their best wishes to you with your postgraduate studies.

Yours sincerely

Prof Y Coopoo.
Chair: Faculty of Health Sciences HDC
Tel: 011 559 6944
Email: yogac@uj.ac.za
FACULTY OF HEALTH SCIENCES

RESEARCH ETHICS COMMITTEE
NHREC Registration no. REC-241112-035

REC-01-32- 2018
17 May 2018

TO WHOM IT MAY CONCERN:

STUDENT: KALUBI, A
STUDENT NUMBER: 201300226

TITLE OF RESEARCH PROJECT: Evaluating Surface Hygiene in the Doornfontein Homoeopathy Health Training Centre, University of Johannesburg

DEPARTMENT OR PROGRAMME: HOMOEOPATHY

SUPERVISOR: Dr R Razlog
CO-SUPERVISOR: Prof TG Barnard
CO-SUPERVISOR: Dr A Singh

The Faculty Research Ethics Committee has scrutinised your research proposal and confirm that it complies with the approved ethical standards of the Faculty of Health Sciences, University of Johannesburg.

The REC would like to extend their best wishes to you with your postgraduate studies.

Yours sincerely

Prof C Stein
Chair: Faculty of Health Sciences REC
Tel: 011 559 6564
Email: cstein@uj.ac.za
APPENDIX C
REQUEST LETTER

DEPARTMENT OF HOMOEOPATHY

To: Dr Els

Re: Request to conduct study in DFC Homeopathy Training Clinic

Good day, my name is AGNES KALUBI. I am currently a Homeopathic student, completing my Masters Degree at the University of Johannesburg. I’m conducting a study titled “Evaluating Surface Hygiene in The Doornfontein Homeopathic Health Training Centre” under the supervision of Dr. R. Razlog, Prof. T.G. Barnard and Dr. A. Singh.

The aim of this study is to assess the surface hygiene of furniture in the Homeopathic Health Training Centre by determining the presence of the total culturable bacterial and fungal populations’ in the Homeopathic training rooms at the University of Johannesburg, Doornfontein Campus. We also request permission that the Water and Health Research Centre be allowed to use any bacteria and fungi isolated for further studies in the future.

This clinical study will be submitted to the University of Johannesburg, Faculty of Health Sciences Higher Degrees Committee (HDC) and Research Ethics Committee (REC) to obtain written approval.

I request your permission to access the facilities once I have received HDC and REC approval.
Yours sincerely,
Agnes Kalubi
RE: PERMISSION TO USE CLINIC CONSULTATION ROOMS

To whom it may concern:

I hereby give permission to Agnes Kalubi (201300226) to utilise the Homoeopathy consultation rooms to conduct her research and gather her data.

Regards,

Dr Tebogo Tsiele-Tebakang
Head Clinician
011 559 6701
tsiele-tebakang@uj.ac.za

Dr R Radog
HOD: Homoeopathy
011 559 6233
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