MONITORING TREATMENT TABLE HYGIENE IN A CHIROPRACTIC TRAINING CLINIC

A dissertation submitted to the Faculty of Health Sciences, University of Johannesburg, Johannesburg, in fulfilment of the requirements for the Degree of Masters of Technology: Chiropractic by

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DECLARATION

I, Mark Chris Kingham, declare that this dissertation is my own, unaided work, except where otherwise indicated in the text. It is being submitted for the Masters Degree in Technology: Chiropractic 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 31 day of July 2019.

DEDICATION

To my friends and family, I am grateful to have shared this journey with you. I cannot imagine what doing this would be like without all of you.

To my parents, Mark and Yvonne Kingham, thank you for your endless love, support, encouragement and assistance. Thank you for always believing in me and supporting me in achieving my dreams. For this, I am eternally grateful.

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ABSTRACT

OBJECTIVE

The purpose of the study was to monitor the bacterial and fungal loads on the Chiropractic treatment tables used within the DFC Chiropractic Training Clinic at the University of Johannesburg, as well as to develop a hygiene protocol guideline.

METHODOLOGY

Surface samples were taken from the head piece and thoraco-abdominal sections of the chiropractic treatment tables at University of Johannesburg chiropractic-training clinic. Samples were taken using the RODAC (Replicate Organism Detection and Counting) agar contact plates with Tryptone Soya Agar (growth nutrients for bacteria and fungi) and two commonly used disinfectant neutralisers; Polysorbate 80 (inactivates phenols, hexachlorophene, and formalin) and Lecithin (neutralises quaternary ammonium compounds).

Two of the chiropractic treatment tables were selected as control room tables, the surfaces of these tables were sampled before disinfection, and then sampled after disinfection to monitor the effectiveness of the disinfectant.

The samples were collected over an 8 week period, on Mondays before the clinic opened and on Thursdays after the clinics' normal hours of operation, in order to ensure none of the patients, students, or clinicians were aware of the study and thus change their normal habits.

Samples were then counted to determine the bacterial and fungal counts on each plate and some organisms were isolated and identified via the VITEK® 2 instrument.

All data from the samples collected on the chiropractic treatment tables were sent to STATKON and entered into an IBM SPSS 23.0 database.

Before statistical analysis, the data set was reviewed and aligned by Ms. Juliana Van Staden, the project biostatistician, for ease of interpretation.

RESULTS

During the eight weeks of monitoring surface hygiene of experimental chiropractic treatment tables, the results demonstrated that the treatment tables are not adequately disinfected when compared to the control beds. Surface sampling results before and after disinfection of the control rooms (G13 and G35) chiropractic treatment tables demonstrated a 96% (1.4 log reduction) and 92% (1.1 log reduction) reduction was achievable, resulting in results comparable to proposed *Levels of Hygiene* (Adequate, Inadequate and Inadequate) as described by Wirtanen, Nurmi, Kalliohaka, Mattila, Heinonen, Enbom, Salo, and Salmela, (2012). Based on the control data these levels were adapted for the chiropractic clinic environment. Only 33% of the samples taken of the experimental chiropractic treatment tables had microbial loads below 10 CFU/25cm^2 (which is below the Adequate level of hygiene (0 – 10 CFU/25cm^2) as proposed in this research). 67% of the samples had Fair (11 - 25 CFU/25cm^2) to Inadequate (>25 CFU/25cm^2) Levels of Hygiene.

When comparing the treatment table surfaces there were significant statistical differences (p-value = 0.025) in bacterial microbial loads (CFU/25cm^2) on these surfaces. Bacterial microbial loads were greater on the head piece (Md = 16, IQR = 33) than on the thoraco-abdominal section (Md = 14, IQR = 26). Another significant statistical difference is noted on microbial loads (CFU/25cm^2) between bacteria and fungi on the thoracoabdominal section of the treatment table (p-value = 0.005), there seems to be higher counts of fungi (Md = 20, IQR = 23) than bacteria (Md = 14, IQR = 26) on this surface.

When comparing outside (peripheral) – (Md = 20, IQR = 23) – and inside (central) – (Md = 17.5, IQR = 19) – treatment rooms, a significant difference

(p-value = 0.041) between the total fungal counts on the chiropractic treatment table surfaces was demonstrated.

Another observation identified when studying the data between fungi and bacteria is the significant statistical difference (p-value = 0.000) in fungal counts from samples that were taken on Mondays (Md = 24, IQR = 20) and Thursdays (Md = 15, IQR = 21).

DISCUSSION

The results from the control rooms demonstrate that the disinfectant and disinfection procedure used by the researcher was effective enough to make a considerable reduction in bacterial and fungal contamination on the chiropractic treatment table surfaces. Results from the experimental treatment rooms demonstrated that there was poor hygiene practices amongst the chiropractic interns because of the high microbial counts. This may also be due to a number of other variables such as environmental factors, number of patients treated and the presence of resistant strains of bacteria or fungi microorganisms. The results did demonstrate that environmental factors do play a role in the growth and survival of the microorganisms and thus, should be considered as a variable when monitoring surface hygiene.

CONCLUSION

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 chiropractic treatment tables at the UJ chiropractic-training clinic. This disinfection protocol was developed and is recommended for implementation within the clinic. It is important to also implement hygiene monitoring systems to monitor both the hygiene practices of the clinic staff and also identify possible pathogenic microorganisms on the treatment table surfaces or within the clinic environment.

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Chapter 1 – Introduction

1.1. Introduction

The National Infection Control Policy and Strategy of South Africa rate the various medical and health disciplines in terms of risk to patients and staff and recommend regular sampling to determine the surface cleanliness (Mseleku, 2007). Over the last few years, Chiropractic students have been studying various aspects related to Chiropractic treatment tables' cleanliness knowledge attitudes and practices in the University of Johannesburg Chiropractic clinic. Based on these activities, and results obtained from the individual studies, it can be assumed that proper surface hygiene practices should be in place in the clinic. This creates the opportunity to monitor the treatment table hygiene in the Chiropractic clinic to determine if adequate surface hygiene is maintained.

1.2. Aims of the Study

The aim of the study is to monitor the bacterial and fungal loads on the Chiropractic treatment tables used within the DFC Chiropractic Training Clinic at the University of Johannesburg.

1.3. Possible Outcomes and benefits of the study

Upon completion, this study will report and comment on the status of cleaning practises within the DFC Chiropractic Clinic and it will recommend regular sampling activities to monitor the cleaning practises of the treatment tables in the future. It is anticipated that at least one (1) manuscript will be submitted for possible publication in a national or international journal. At least one (1) abstract will be submitted to a national or international conference.

Chapter Two – Literature Review

5.1. Introduction

Over the last few years students at the University of Johannesburg (UJ) Chiropractic clinic at the Doornfontein campus (DFC) have been studying the occurrence of bacteria (Gram-negative and Gram-positive bacteria) and fungi on the chiropractic treatment tables (Perdijk, Yelverton and Barnard, 2017). These studies showed how the tables should be cleaned and disinfected (Kruger, Yelverton, Barnard and van der Loo, 2017) and how a simple education intervention could change the hygiene practises of students in their clinical training years (Bowes, Yelverton, Barnard and Singh, 2018). Based on these studies, it is evident that there is a need for proper surface hygiene practices in the clinic.

In a review of Nosocomial Infections (NI) by Dr. Shanil Naidoo (2017), it concluded that Healthcare-associated infections are no longer confined within hospitals and clinics. Instead, NI are spread across all health-care facilities exposing patients, health-care workers, and other public to pathogens with increasing levels of virulence and resistance (Naidoo, 2017). The National Infection Control Policy and Strategy of South Africa (2007) set minimum national standards for the effective prevention and management of health-care-associated infections so that hazards associated with microorganisms are minimized for patients, visitors and health care personnel in health care establishments. This policy highlights the risks to patients and staff and recommends regular sampling to determine the surface cleanliness. There are several interventions that mitigate these risks to patients; however, the implementation or utilisation of these interventions lies with educating all health-care professionals on their importance and their benefits (Naidoo, 2017).

Substandard/suboptimal hygiene and sanitation knowledge and practices, or non-compliance thereof, has significant implications for patients, visitors,

and health care providers (Mohapatra and Sarangi, 2018). Infection control researchers need to consider the reasons for substandard practices or non-compliance thereof and provide a supportive environment. It is beneficial to the routine and long-term application of hygiene and sanitation practices within a Chiropractic clinic (Gammon, Morgan-Samuel and Gould, 2008). This creates the opportunity to implement a monitoring system that can be adapted for continuous use in the DFC Chiropractic Training Clinic at the University of Johannesburg. Surveillance and monitoring practices can be interpreted as; "the ongoing, systematic collection, analysis, and interpretation of health data that is essential to the planning, implementation and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know" (Khan, Ahmad and Mehboob, 2015).

5.2. Nosocomial Infections

Nosocomial Infections, otherwise known as 'health-care-facility associated infections' (HCAI), appear in a patient under medical care in the hospital or other health care facilities which were absent at the time of admission (Khan, Baig and Mehboob, 2017). It is essential to highlight that this study will discuss the difference between nosocomial infections and infections that may be acquired when visiting a chiropractic clinic. Nosocomial infections are mainly associated with the use of invasive medical instruments or devices (Reed and Kemmerly, 2009). These medical devices associated infections include catheter-urinary tract infections, vascular catheter-associated infections, ventilator-associated infections, or infections caused by prosthesis implants (Haque, Sartelli, McKimm and Abu Bakar, 2018). HCAI acquisition occurs up to 48 hours after admission within a health care facility, up to 3 days after discharge or up to 30 days post-surgery (Mohapatra and Sarangi, 2018). It is estimated that annually, approximately 1.7 million hospital-associated infections caused or contributed to the deaths of 99 000 Americans per year (Haque, Sartelli, McKimm & Abu Bakar, 2018). In another American study published in the New England

Journal of Medicine in 2014 showed that one in 25 patients developed at least one hospital-acquired infection (Magill, Edwards, Bamberg, et al. 2014). In South Africa, approximately one in seven patients entering a healthcare facility are at high risk of acquiring nosocomial infections (Naidoo, 2017)

5.3. Community-acquired Infections

Community-acquired infections are infections acquired within the community, are present before and detected within 48 hours of hospital admission in patients without previous contact with healthcare services (Cardoso, Almeida, Carratalà, et al. 2015). Although these 'healthcare services' mentioned in the previous sentence are not specified, and that chiropractic healthcare does not use invasive medical devices and is considered a form of conservative treatment (Legorreta, Metz, Nelson, Ray, Chernicoff and DiNubile, 2004). It is assumed that possible infections acquired after Chiropractic treatment are community-acquired infections. They are most likely acquired from contaminated surfaces within a chiropractic treatment office, such as the chiropractic treatment tables. However, a significant acquisition factor of an infection within the community or in a hospital is poor hygiene practices (Mohapatra and Sarangi, 2018).

5.4. Chiropractic treatment tables as a possible source of pathogenic microbes

The chiropractic treatment table comprised of a headrest, armrests, thoracic, and pelvic sections and covered with non-porous vinyl upholstery making it easy to clean. However, more expensive chiropractic treatment tables are covered in leather, which is considered a porous material, which allows for more bacterial growth (Katsikogianni and Missirlis, 2004). The chiropractic treatment tables are inanimate objects, or otherwise known as a fomite, which are potential reservoirs in the transmission of pathogens. A recent research study done at the DFC Chiropractic Training Clinic at the University of Johannesburg has identified the Chiropractic treatment tables

to be potential reservoirs for microbial pathogens (Perdijk et al. 2017). Perdijk, (2017) and identified potentially pathogenic bacteria such as; *Pseudomonas spp., Klebsiella spp., Escherichia coli, Enterobacter spp.,* and other significant pathogens on surfaces in the clinic (Table 2.1, Table 2.2 and Table 2.6). Specific pathogens are capable of surviving from hours to days to weeks and even months on fomites. This survival depends on the numbers deposited, the type of microorganism, and the variable environmental conditions (Lopez, Vlamakis and Kolter, 2010).

5.5. Medical Microbiology

Understanding the importance of monitoring surface hygiene in health care facilities – including Chiropractic healthcare facilities – it is important to understand some of the fundamental aspects of medical microbiology and infectious diseases. Knowledge of the detrimental effects that pathological microbes have on the human body should motivate healthcare workers to have good hygiene, and cleaning practices, and this knowledge should also be extended to the patient.

Microbiology is the study of microorganisms and the microbiome of humans, animals, and environments. Medical microbiology investigates the roles that the microbiome has in human health and illness, it includes the study of microbial pathogenesis and epidemiology and is interrelated to the study of human pathology and immunology (Yamaoka and Matsumoto, 2019). It is recognised that the microbiome can change our genetic material and health status. The causes and pathogenesis of diseases are only somewhat understood; however; nutrients, metabolites, and microbes identified as critical players. The field of medical microbiology has spread out in many directions, with microbes and microbiomes being studied from various perspectives with different specificities (standard hygiene practices and monitoring systems) being developed (Hadrich, 2018) in clinical settings/environments at the forefront of preventative measures of diseases caused by pathological microbes. Medical Microbiology can be divided into

four categories; Medical Virology (the study of viruses), Medical Bacteriology (the study of bacteria), Medical Mycology (the study of fungi) and Medical Parasitology (the study of parasites) (Murray, Pfaller & Rosenthal, 2015). However, this study will only look at the monitoring systems for the presence of bacteria and fungi on the chiropractic treatment tables in the Chiropractic Clinic at the DFC campus of the University of Johannesburg of South Africa.

5.5.1. Medical Bacteriology

Medical Bacteriology is the "science and study of bacteria and their relationship to medicine, industry, and agriculture" (Shiel, 2018). Medical Bacteriology research has resulted in the development and advancement of many vaccines and antibiotics. These antimicrobial substances are therapeutically effective but do not entirely eradicate pathologic bacteria. Antibiotic efficacy may be decreased due to bacteria becoming resilient against them, which is now a significant medical management dilemma. However, hygiene control and surveillance have a more substantial and more distinguished impact on the incidence of bacterial infections than does the availability of antibiotics or bacterial vaccines (Baron, 1996). Prevention is the primary goal.

5.5.1.1. Bacteria

Bacteria are prokaryotes – unicellular microorganisms that lack a nucleus. Bacteria are either planktonic (floating or drifting bacterial cells) or sessile (attaching to surfaces within a biofilm) (Marshall, 2013). Bacteria reproduce asexually by binary fission. They have a mesh-like peptidoglycan cell-wall, a cell membrane, a chromosome, and ribosomes. Some bacteria also have pili and a flagellum (Salton and Kim, 1996).

Bacteria can be classified into two groups dependant on their structure of the microorganisms peptidoglycan cell wall:

5.5.1.1.1. Gram-negative Bacteria

Gram-negative bacteria (GNB) (Table 2.1) have a small peptidoglycan layer but have an additional membrane, known as the outer cytoplasmic membrane. A significant component of the cytoplasmic membrane that is unique to GNB is endotoxins - also known as lipopolysaccharides (Silhavy, Kahne & Walker, 2010). Endotoxins possess a range of powerful biologic activities and play an essential role in the pathogenesis of many GNB infections (Salton and Kim, 1996) including pneumonia, bloodstream, urinary tract, surgical site infections, and meningitis (Weinstein, Gaynes, Edwards & National Nosocomial Infections Surveillance System, 2005). The outer cytoplasmic membrane protects the microorganism from its hostile environments and additionally provides a stabilising layer for its relatively thin peptidoglycan cell wall layer (Silhavy et al. 2010).

5.5.1.1.2. Gram-positive Bacteria

Gram-positive bacteria (GPB) (Table 2.2) have a larger peptidoglycan structure cell wall than Gram-negative bacteria (Salton and Kim, 1996). GPB lack this outer cytoplasmic membrane found in GNB. Because GPB lives in similarly hostile environments that GNB survive in, the question becomes how do GPB survive if they lack this outer cytoplasmic stabilising protective layer? GPB have long anionic polymers, called teichoic acids that thread through and are covalently attached to this thicker peptidoglycan cell wall. Another class of polymers is lipoteichoic acids that are also attached to the membrane lipids. Collectively, these polymers make a large portion of the cell wall making them valuable contributors to the structure and function of the cell wall (Silhavy et al. 2010).

Table 2.1 Colonisation, transmission and infections of Gram-negative bacteria previously found on Chiropractic Treatment Tables.

Name	Sites of Colonisation in humans	Modes of Transmission / (Source of exposure)	Types of Infections caused	References
Acinetobacter lwoffii (Perdijk et al. 2017)	Human skin (also recognised to be part of the normal flora of the oropharynx)	**Indirect Contact** (Fomites (ie: catheters)/Enivromental Surfaces) / **Direct contact** (transient colonisation of the hands of health-care workers) / **Vehicle** (food-borne, nosocomial spread by aerosolized bacteria from infected or colonized patient)	Nosocomial Infections; Bacteremia, gram-negative peritonitis, pneumonia, acute gastroenteritis, liver abscess, septicaemia, and endocarditis	Wong, Nielsen, Bonomo, Pantapalangkoor, Luna & Spellberg, (2017) / Tas, Oguz & Ceri, (2017)
Brucella Melitensis (Perdijk et al. 2017)	Colonised mainly in goats and sheep, other less common animals are dogs, horses and pigs. Mucous membranes in humans	**Indirect Contact** (Fomite/Enivromental Surfaces (ie:contaminated environmental devices while assisting in birth delivery) / **Direct contact** (Vertical and horizontal- person-to-person (ie; blood transfusions, bone marrow transplants, sexual intercourse), animal-to-person) / **Vehicle** (food (ie. Unpastuarised dairy products), aerosolized bacteria)	Nosocomial Infections and community-aquired infections; Brucellosis	Vigeant, Mendelson & Miller, (1995) / The Centre for Food Security and Public Health, (2018)
Methylobacterium (Perdijk et al. 2017)	Soil, sewage, water and Plants (leaf surfaces). Human colonisation sites include: blood, bone marrow, sputum, pleural effusion, peritoneal fluid, cerebrospinal fluid, synovium, and skin)	**Indirect Contact** (Fomites (ie: catheters and endoscopes - because methylobacterium are major inhabitants of aqueous environments, these devices usually get contaminated with contaminated tap water when being sterilised) / **Vehicle** (water)	Nosocomial and community-acquired Infections : Bacteramia and peritonitis	Lai, Cheng, Liu, Tan, Huang, Chung, Lee & Hsueh, (2011) / Kovaleva, Degener & van, (2014)

Table 2.1 continued Colonisation, transmission and infections of Gram-negative bacteria previously found on Chiropractic Treatment Tables.

Name	Sites of Colonisation in humans	Modes of Transmission / (Source of exposure)	Types of Infections caused	References
Escherichia coli (E. coli) (Perdijk et al. 2017)	Environments/fomites, foods, water and intestines of humans and animals	**Indirect Contact** (Fomite/Enivromental) / **Direct contact** (Vertical and horizontal - person-to-person (poor hand sanititaion practices), animal-to-person (ie: petting zoos) / **Vehicle** (contaminated food or water with animal/human feces (ie: unpastuarised diary products and apple cider, undercooked hamburgers or contaminated vegatables)	Nosocomial and Cumminity-aquired infections:Bacterial diarrheal illness due 6 different pathotypes: shiga toxin-producing E. coli (STEC) (most common), Enterotoxigenic E. coli (ETEC), Enteropathogenic E. coli (EPEC), Enteroaggregative E. coli (EAEC), Enteroinvasive E. coli (EIEC), Diffusely adherent E. coli (DAEC)	CDC (Centre for Disease Control and Prevention), (2014)
Sphingomonas Paucimobilis (Perdijk et al. 2017)	Soil, drinking water and plants. Hospital equipment such as ventilators	**Indirect Contact** (Fomites (ie: catheters, ventilators, intravenous medications and haemodialysis machines) / **Vehicle** (water)	Nosocomial Infections: Bacteramia	Göker, Aşık, Yılmaz, Çelik and Tekiner (2017)
Pseudomonas fluorescens (Perdijk et al. 2017)	Soil and rhizophere. Human colonisation sites include: mouth, gastrointestinal,respiratory and blood.	**Indirect Contact** (Fomites (ie: catheters, intravenous medications) / **Direct Contact** (ie: Blood transfusions) / **Vehicle** (water)	Rare Nosocomial Infections : Bacteramia	Scales, Dickson, LiPuma and Huffnagle, (2014)

Table 2.2 Colonisation, transmission and infections of gram-positive bacteria previously found on Chiropractic Treatment Tables.

Name	Sites of Colonisation	**Modes of Transmission -** (Source of Exposure)	Types of Infections	References
Aerococcus Viridans (Perdijk et al. 2017)	Hospital Environments and Airborne. Human skin, respiartory and urinary tract.	**Indirect Contact -** (Fomites/Enivromental Surfaces) / **Vehicle -** (nosocomial spread by aerosolized bacteria)	Naosocomial Infections: Urinary tract infection (UTI), Endocarditis,Osteomyelitis, pyomyositis and Bacteremia.	Parrey, Sofi, Ahmad and Kuchay, (2016)
Garderella vaginalis (Gram-variable) (Perdijk et al. 2017)	Colonised mainly in the female vagina and distal urethra of the males genital tract	**Indirect Contact -** (Fomite/ Hospital Enivromental Surfaces)/ **Direct contact -** (Vertical (ie:during birth) and horizontal (sexually transmitted)	Nosocomial and community aquired infection: Sexually Transmitted Infection, Septic-Articular infections (post surgical) and bacteremia	Catlin, (1992) / Muzny, Schwebke and Josey, (2014)
Kocuria Rosea (KR) (Perdijk et al. 2017)	Environments/fomites, skin and mucous membranes of humans and animals (growing in variable conditions as acidophiles, alkaliphiles, halophiles, and thermophiles)	**Indirect Contact -** (Fomitel-most commonly medical devices)	Nosocomial and cummunity-aquired Infections: Peritonitis,urinary tract infections, cholecystitis, catheter-associated bacteremia, dacryocystitis, canaliculitis, keratitis, native valve endocarditis, descending necrotizing mediastinitis, brain abscess and meningitis. *KR* is nonpathogenic however in immunocomprised individuals becomes pathogenic.	Dotis, Printza and Papachristou, (2012) / Paul, Gupta, Khush-waha, and Thakur, (2015)

Table 2.2 continued Colonisation, transmission and infections of Gram-positive bacteria previously found on Chiropractic Treatment Tables (Continued).

Name	Sites of Colonisation	Modes of Transmission - (Source of Exposure)	Types of Infections	References
Stapylococcus hominis (Perdijk et al. 2017)	Fomites (Hospital equipment)	**Indirect Contact -** (Fomites (ie: catheters, ventilators, intravenous devices)	Nosocomial and cummunity-aquired Infections: bacteremia, septicemia, and endocarditis, becomes pathogenic in immunocomprised individuals.	Mendoza-Olazarán, Morfin-Otero, Rodríguez-Noriega, Llaca-Díaz, Flores-Treviño, González-González, Villarreal-Treviño and Garza-González, (2013)
Stapylococcus Aureus (Perdijk et al. 2017)	Environments/fomites, skin and mucous membranes of humans (most commopn site of colonisation is the nasal mucousa)	**Indirect Contact -** (Fomites)/ **Direct Contact -** (person-to-person)	Nosocomial and cummunity-acquired Infections: bacteremia, infective endocarditis, skin and soft tissue infections (ie: mpetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (ie: pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections.	Taylor and Unakal, (2019)

5.5.1.2. Bacterial Pathogenicity and Virulence

The capacity of bacteria to cause disease despite the hosts' immune defenses reflects its relative pathogenicity (Peterson, 1996). The correct use of terminology to describe pathogenicity and virulence of invertebrate pathology is by this definition; “Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease, whereas virulence is the disease producing power of an organism, the degree of pathogenicity within a group or species.” (Shapiro-Ilan, Fuxa, Lacey, Onstad & Kaya, 2005). New research evidence indicates that microbial pathogens that have different characteristics, use common mechanisms – ability to grow, adhere, invade, and cause damage to host cells and tissues, as well as to survive host defence mechanisms and initiate infection – to cause pathology (Wilson, Schurr, LeBlanc, Ramamurthy, Buchanan & Nickerson, 2002). These mechanisms, as well as the microorganisms cell structure, are recognised as bacterial virulence factors (**Table 2.3**).

Table 2.3 Bacterial Virulence Factors.

Virulence Factor	Reference
Membrane Associate Virulence Factors	
Adherence, Invasion and Evasion Factors	Foster, Geoghegan, Ganesh, and Höök, (2013)
Capsules	Boyce *and Adler,* (2000)
Cell Wall	Bhat, Rather, Maqbool, Lah, Yousuf, and Ahmad, (2017)
Secretory Associated Virulence Factors	
Endotoxins	Kahler and Stephens, (1998)
Exotoxins	Blackwood, Stone, Iglewski, and Pennington, (1983)
Other Associated Virulence Factors	
Antibiotic Resistance	Mundy, Sahm and Gilmore, (2000)
Host Immune Susceptibility	Alegado, Campbell, Chen, Slutz and Tan, (2003)

5.5.1.3. Bacterial Transmission

It is known that bacteria microbes can be spread by different modes of transmission as described by the Centres for Disease Control (**Table 2.4**). As mentioned before, a Chiropractic treatment table is classified as a fomite and is a potential reservoir in the transmission of pathogens.

Table 2.4 Spread of Bacterial Pathogens within a health-care facility. Modified from CDC, (2016).

Mode of Transmission	Example
Contact	Hands of healthcare workers or patients become contaminated by touching microbial colonised medical equipment or common touch surfaces (this occurs when there is poor surface hygiene sanitation practices), they then transfer the microorganisms from their hands to a susceptible person (this may occur due to poor hand hygiene practices).
Sprays	Sprays and splashes occur when an infected person coughs, sneezes and talks. Droplets with bacteria form which may travel short distances (approximately two meters). These droplets can land on fomites and on susceptible person's eyes, nose, or mouth.
Inhalation/Aerosolised	Inhalation occurs when bacteria are aerosolized. These bacteria microbes can survive on air currents and travel over greater distances to reach a susceptible person whom inhales the tiny particles.
Sharps Injuries	Sharps injuries can lead to infections (ie: HIV, HBV, HCV) when bloodborne pathogens enter a person through a skin puncture by a used needle or sharp instrument.

Fomites serve as routes for both enteric and respiratory pathogen transmissions (Lopez, Gerba, Tamimi, Kitajima, Maxwell, and Rose, 2013). Saliva, mucus, nasal secretions, blood, urine, and feces – all which are considered bodily fluids that potentially contain bacterial pathogens transmitted by means of fomites (e.g., Chiropractic treatment table).

Fomites become contaminated with bacteria with direct contact of body fluids, contact with soiled hands or indirect contact with aerosolized bacteria – generated from sneezing, talking, or coughing (Boone and Gerba, 2007). A person will then come in contact with the contaminated fomite and fomite-to-human transmissions then occur either directly, by surface-to-mouth - this is usually the case when a patient places their head on the headrest of the chiropractic table without a protective covering layer when laying prone or when the chiropractic treatment table is not adequately disinfected) - or transmitted indirectly, by contamination of fingers with subsequent hand-to-mouth, hand-to-eye and hand-to-nose transfer (Lopez, et al., 2013).

5.5.1.4. Bacterial Colonisation

Bacterial colonisation is the presence of bacteria on a surface without causing disease. However, with the right conditions, virulence factors, and appropriate entry portal, colonisation may be identified as the first step of microbial infection (Dani, 2014) (**Figure 2.1**). There is a close relationship between colonisation and the development of HCAI (Bonten and Weinstein, 1996), and understanding colonisation will help with strategies that can be used either to prevent colonisation from occurring, to eradicate colonising microorganisms, or to prevent the progression from colonization to infection (Boyce, 1996). These strategies should be the implementation of effective and strict infection control and surveillance measures (Jeyakumari, Nagajothi, Kumar, Ilayaperumal and Vigneshwaran, 2017).

Colonisation primarily involves the process of surface adhesion and biofilm formation. A biofilm is an architectural colony of microorganisms, within a matrix of extracellular polymeric substance that bacteria produce. Bacterial biofilms are usually pathogenic and known to possibly cause hospital-acquired infections (Jamal, Ahmad, Andleeb, Jalil, Imran, Nawaz, Hussain, Ali, Rafiq and Kamil, 2018). The biofilm also enhances bacterial survival on fomite surfaces (Marks, Reddinger and Hakansson, 2014).

Bacterial attachments to a surface (both innate or living) consists of a couple of phases. The initial phase is the primary reversible attachment phase – which is characterised by non-specific interactions of cells and when bacteria are considered easily removed by a gentle rinse, an irreversible secondary attachment, and biofilm formation phase and finally the detachment phase (Dunne, 2002).

5.5.1.5. Bacterial Attachment

The solid-liquid boundary between a surface and an aqueous medium (e.g., water, blood, body fluids, etc.) provides an ideal environment for the attachment and growth of planktonic bacterial microorganisms. Further understanding of the process of attachment of bacteria and biofilm formation, it is crucial to look at the properties of the substratum, the properties of the bulk fluid and the properties of the bacterial cell (Donlan, 2002).

5.5.1.5.1. The Substratum

The substratum is a base or solid surface to which living organisms adhere to while they grow. Several properties (**Table 2.5**) are important in the attachment process of bacteria to the substratum (Donlan, 2002). Substrata either have very hydrophobic materials – such as Teflon, various plastics, latex, and silicone – to highly charged hydrophilic materials – such as glass and various metals. Certain materials are rough or textured – such as water pipes or environmental surfaces – while others are much smoother – such as medical silicone or Teflon catheters (Donlan, 2001). Porous surfaces that are irregular and rough favour and promote bacterial adhesion and colonisation (Katsikogianni and Missirlis, 2004). Thus, it is highly recommended that chiropractic treatment tables covered in leather or any other porous materials, should be regularly disinfected and monitored for pathogenic bacterial growth and colonisation, or otherwise should be covered with a non-porous material cover. Non-porous materials are more

smooth and therefore, do not favour the growth or colonisation of bacteria (Lorite, Rodrigues, de Souza, Kranz, Mizaikoff and Cotta, 2011).

Table 2.5 Properties of the substratum, bulk fluids and bacterial cells. Modified from Donlan, 2002.

Substrutum	Environment and Fluids	Bacterial Cells
Texture/Roughness	Flow Velocity	Cell surface Hydrophobicity
Hydrophobicity	pH	Fimbriae
Conditioning Film	Temperature	Flagella
	Nutrient/electrolyte Solution	Extracellular Polymeric Substances
	Charge (cations)	
	Presence of antimicrobial agents	
	Time of exposure	

Another characteristic of the substratum is it's hydrophobicity – In Greek, hydro means water, and phobicity means lack of affinity (Law, 2014). Surface hydrophobicity is regarded as a contributing factor for microbial cell adhesion (Lorite, Rodrigues, de Souza, Kranz, Mizaikoff and Cotta, 2011). Hydrophobic interactions promote protein folding and aggregation, cell membrane fusion, and cell adhesion. There is a relationship between the hydrophobicity and the number of adhered bacterial cells to a surface. It was found that a decrease in the hydrophobicity of a metal surface decreased the number of adhered bacterial cells to the surface (Oliveira, Azeredo, Teixeira and Fonseca, 2001). It was demonstrated that the substratum/fomite surface hydrophobicity plays a more critical role in bacterial adhesion than the bacterial cell surface hydrophobicity (Katsikogianni and Missirlis, 2004).

The substratum that is exposed to an aqueous medium (also known as the conditioning film) will become conditioned or covered by polymers from that conditioning film. The resulting chemical modification will affect the rate and extent of attachment of the microbe to the surfaces (Donlan, 2002). Examples of conditioning films are blood, tears, urine, saliva, and respiratory secretions (Mittelman, 1996). Gubner and Beech showed that

conditioning films on the surface play a more critical role in cell adhesion than the surface hydrophobicity or texture/roughness (Gubner and Beech, 2000). In general, a substratum with the appropriate conditioning film, with a rougher and more hydrophobic surface, will allow for more effective bacterial cell adhesion to the surface. Once this attachment occurs, biofilm formation will begin.

In addition to the importance of the substratum, the characteristics of the bacterial cell wall (such as the flagella, fimbriae, pili and the glycocalyx) which all enable the cell to maintain attachment until more permanent attachments take place is also considered important (Donlan, 2001).

5.5.1.5.2. Bacterial Biofilm Formation on Dry Surfaces

Biofilm formation is the process involving bacterial microorganisms irreversibly attaching to the surface (i.e., those that are not removed by gentle rinsing), begin cell division to grow and colonise on living or inanimate environmental surfaces. They produce extracellular polymers that promote attachment and matrix formation (Donlan, 2001). The extracellular polymeric substances (EPS) are primarily polysaccharides, secreted proteins, and cell-surface adhesins that provide the structural integrity of the biofilm (López, Vlamakis & Kolter, 2010). Bacteria living in biofilms are protected from hostile and deleterious conditions (Bogino, Oliva, María de las Mercedes, Sorroche and Giordano, 2013). Biofilms are found in medical, industrial, and natural environments.

Biofilms on medical devices such as catheters are known to cause hospital-acquired infections due to the high resistance and tolerance against antimicrobial treatments and the body's immune response (Srivastava and Bhargava, 2016). This is associated with a high morbidity and mortality rate (Donlan, 2008). Moreover, it is known that bacterial biofilm formations can occur on almost any surface, including the skin and mucosal surfaces such as oral and genitourinary tract mucosa (Hatt and Rather, 2008). This is of concern in a Chiropractic clinic as most patients will have direct or indirect

contact with a chiropractic treatment table, which may be the source of pathogenic microbes (Perdijk et al. 2017). Many scientific studies have focused on biofilm formations in wet environments; however, new research shows that bacterial biofilms can grow on dry surfaces (Ledwoch, Dancer, Otter, Kerr, Roposte, Rushton, Weiser, Mahenthiralingam, Muir and Maillard, 2018). The resistance of bacterial microorganisms to disinfectants on dry surfaces is frequently associated with the presence of biofilms (Bressler, Balzer, Dannehl, Flemming and Wingender, 2009). Biofilms prolong the survival of bacterial microorganisms and render them tolerant to disinfectants on dry surfaces (Almatroudi, Hu, Deva, Gosbell, Jacombs, Jensen, Whiteley, Glasbey and Vickery, 2015). It has been suggested that bacterial biofilms on dry surfaces can be transferred from one fomite to other fomites by hands (Chowdhury, Tahir, Legge, Hu, Prvan, Johani, Whiteley, Glasbey, Deva and Vickery, 2018). Thus, highlighting the importance of handwashing in infection control (Griffith, Malik, Cooper, Looker and Michaels, 2003).

5.5.1.5.3. Bacterial Detachment and Dispersal

Biofilms must release and disperse bacterial microorganisms into the environments to colonise new cites. Biofilm detachment is essential to bacterial dispersal, survival, and disease transmission. Biofilm dispersal plays a role that enhances fomite-to-human transmissions and a function that exacerbates infections in the host (Kaplan, 2010). Research on biofilm dispersal has identifying antibiofilm-agents that may promote biofilm detachment and inhibit subsequent biofilm formation preventing dispersal and possible infections. These agents are nontoxic and are believed to ward off future drug resistance (Rabin, Zheng, Opoku-Temeng, Du, Bonsu & Sintim, 2015).

5.5.2. Medical Mycology

Mycology is the scientific study of fungi. Fungi are eukaryotic microorganisms – unicellular or multicellular microorganisms containing a nucleus – as compared to the prokaryotic bacterial microorganisms (McGinnis and Tyring, 1996). Fungi have a solid rigid cell wall made of chitin, mannan, glucan, and a cell membrane consisting of ergosterol, which is an essential target for antifungal agents (Berkowitz and Jerris, 2016). Fungi can be morphologically grouped as either a yeast or a mold. Yeasts are usually unicellular and are identified as round, pasty or mucoid colonies on agar plates. Molds are typically identified as filamentous, hairy, or woolly colonies on agar plates (Murray, Pfaller and Rosenthal, 2013). Medically important fungi are termed dimorphic as they can appear to exist in both a mold or yeast form (Murray, Rosenthal and Pfaller, 2015).

5.5.2.1. Yeasts

A Yeast is a fungus that reproduces by either fission or budding (Murray, Rosenthal & Pfaller, 2015). The buds that form are known as blastoconidia, which remain attached to form a long chain called pseudohypha. Medically important yeasts belong to the *Candida*, *Cryptococcus*, *Malassezia* and *Trichosporon* genera (Berkowitz and Jerris, 2016).

5.5.2.2. Molds

Molds reproduce both asexually and sexually. When reproducing asexually, they produce spores, called conidia. These conidia are easily airborne and serve to spread the fungus. Molds are filamentous fungi that appear to be cylindrical cells that branch, these branches are known as hyphae (Berkowitz and Jerris, 2016). Many hyphae form together to produce a matt-like structure known as mycelium (Murray, Rosenthal & Pfaller, 2015).

5.5.2.3. Classification of Human Mycoses

Depending on which human tissue fungi infect, the infections and fungi can be classified either as Superficial, Cutaneous, Subcutaneous, Endemic or Opportunistic Mycoses (Murray, Rosenthal and Pfaller, 2015).

5.5.2.3.1. Superficial Mycoses

In Superficial Mycoses, the skin and its appendages are predominantly involved (Bitar, 1973). They are non-destructive and have only cosmetic importance (Murray, Rosenthal and Pfaller, 2015). They are usually non-inflammatory infections such as Pityriasis versicolor and Tinea nigra (Dias, Maria Fernanda Reis Gavazzoni, Quaresma-Santos, Bernardes-Filho, Amorim, Adriana Gutstein da Fonseca, Schechtman and Azulay, 2013).

5.5.2.3.2. Cutaneous Mycoses

These are fungal infections of the keratinised layer of skin, hair, and nails (Murray, Rosenthal and Pfaller, 2015). These are common infections worldwide, which is mainly caused by dermatophytes, yeasts, and non-dermatophytes (Khadka, Sherchand, Pokharel, Pokhrel, Mishra, Dhital and Rijal, 2016). Growth of fungi causing superficial infections is directly related to heat and humidity, which are ideal conditions for this growth (Dias, Maria Fernanda Reis Gavazzoni, Quaresma-Santos, Bernardes-Filho, Amorim, Adriana Gutstein da Fonseca, Schechtman and Azulay, 2013). Transmission of infectious microbes can occur from direct contact, either from a contaminated surface (Fomite-to-human) or host (human-to-human) (Dias, Quaresma-Santos, Bernardes-Filho, Amorim, Adriana Gutstein da Fonseca, Schechtman and Azulay, 2013). Therefore, it is possible for Cutaneous Mycoses to occur in a chiropractic healthcare facility.

5.5.2.3.3. Subcutaneous Mycoses

These are fungal infections of deeper tissue layers of the human body – deep skin layer, cornea, muscle, and connective tissue (Murray et al. 2015).

These are much less common than Cutaneous Mycoses. Usually, traumatic injury caused by direct penetration serves as a route of direct and indirect transmission of these infectious fungi (Koga, Matsuda, Matsumoto and Furue, 2003). These fungi will cause abscess formation, sinus tracts, and ulcers (Murray et al. 2015). Chiropractic care makes use of a soft tissue treatment called Dry Needling, which involves the insertion of thin monofilament needles deep into the skin and muscle tissue. These needles are inserted for 10 to 30 minutes to treat different injuries (Unverzagt, Berglund and Thomas, 2015; Dunning, Butts, Mourad, Young, Flannagan and Perreault, 2014). Adverse events from Dry Needling include bleeding, pneumothorax, organ and nerve trauma, and infection. According to the Swiss Guidelines for safe Dry Needling, overall hygiene control is of paramount importance (Bachmann, Colla, Gröbli, Mungo, Gröbli, Reilich and Weissmann, 2014). It is, therefore, possible for Subcutaneous Mycoses occur in a Chiropractic Healthcare facility if there are poor sanitation and hygiene control.

5.5.2.3.4. Endemic Mycoses

Endemic Mycoses are fungi infections caused by a diverse group of fungi that share common characteristics – they are able to cause infection to immunocompetent hosts, each occupy a specific environmental niche, and demonstrate temperature dimorphism, existing as molds at temperatures in the environment between 25°C to 30°C and exist as yeasts at body temperatures (Kauffman, 2006). Community-acquired pneumonia is commonly overlooked and should be classified as important endemic mycoses (Hage, Knox and Wheat, 2012).

5.5.2.3.5. Opportunistic Mycoses

Opportunistic mycoses are infections caused fungi that are found in healthy human flora and commensals or the environments.

Table 2.6 continued Transmission, Classification of human mycoses and infections of Fungi previously found on Chiropractic Treatment Tables.

Name	Modes of Transmission - (Sources of exposure)	Classification of Human Mycoses	Types of Infections	References
Aspergillus flavus (Perdijk et al. 2017)	**Direct/Indirect Contact** - (Fomites/Enivromental Surfaces/other infected humans) **Vehicle** - (spread by aerosolized fungi or from a food-borne source)	Cutaneous Mycoses	Onychomycosis	Gianni and Romano, (2004)
Aspergillus fumigatus (Perdijk et al. 2017)		Subcutaneous Mycoses	Mycotic kertitis	Thomas and Kaliamurthy, (2013)
Aspergillus niger (Perdijk et al. 2017)		Opportunistic Mycoses	Aspergillosis	CDC, (2019)
Aspergillus ochaceus (Perdijk et al. 2017)				
Cladosporium cladosporioides (Perdijk et al. 2017)		Subcutaneous Mycoses	Chromoblastomycosis	Nath, Barua, Barman, Swargiary, Borgohain and Saikia, (2015)
Cryptococcus neoformans (Perdijk et al. 2017)		Opportunistic Mycoses	Cryptococcis	Perfect and Casadevall, (2002)
Curvularia lunata (Perdijk et al. 2017)		Subcutaneous Mycoses	Eumyectoma / chromoblastomycosis / Fatal Cerebral Phaeohyphomycosis	Garg, Sujatha. Garg, Parija, Thappa, (2008) / Bordoloi, Nath, Borgohain, Huda, Barua, Dutta and Saikia, (2015) / Carter and Boudreaux, (2004)
Fusarium Oxyporum (Perdijk et al. 2017)		Subcutaneous Mycosis	Mycotic Keratitis	Dóczi, Gyetvai, Kredics and Nagy, (2004)
Fusarium proliferatum (Perdijk et al. 2017)		Opportunistic Mycoses	Hyalohyphoycosis	Tortorano et al., (2014)

Table 2.6 continued Transmission, Classification of human mycoses and infections of Fungi previously found on Chiropractic Treatment Tables.

Name	Modes of Transmission - (Sources of exposure)	Classification of Human Mycoses	Types of Infections	References
Mucor plumeus (Perdijk et al. 2017)	**Direct/Indirect Contact** - (Fomites/Enivroment al Surfaces/other infected humans) **Vehicle** - (spread by aerosolized fungi or from a food-borne source)	Opportunistic Mycoses	Mucormycosis	Gomes, Lewis and Kontoyiannis, (2011)
Mucor racemosus (Perdijk et al. 2017)				
Rhizopus stolnifer (Perdijk et al. 2017)				
Rhizopus Oryzae (Perdijk et al. 2017)				
Ulocldium botrytis (Perdijk et al. 2017)		Cutaneouis Mycoses / Subcutaneous Mycoses	Onychomycosis / Ulocladium atrum keratitis	Romano, Maritati, Paccagnini and Massai, (2004) / Badenoch, Halliday, Ellis, Billing and Mills, (2006)
Moniliella suaveoens (Perdijk et al. 2017)		Subcutaneous Mycoses	Phaeohyphomycosis	A McKenzie, D Connole, R McGinnis and Lepelaar, (1984)

Except for *Cryptococcus neoformans* (which have been fungi identified to be present on Chiropractic Treatment Tables (Perdijk et al. 2017), all other fungi causing opportunistic mycoses have low virulence and pathogenicity, occurs in immunosuppressed individuals and are nosocomial infections (Murray, Rosenthal and Pfaller, 2015).

5.5.2.4. Fungal Pathogenicity and Virulence Factors

Fungal pathogens possess virulence factors that increase their pathogenicity in humans. A variety of combinations of these virulence factors (play a role in growth and colonisation of fungi tolerance to temperature and humidity, evasion of the hosts' immune system defenses, dimorphism, and enzymatic activities) play a role in growth and colonisation (Rhodes, 1988). These virulence factors play a role in the two processes that determine the pathogenicity of fungi: (a) the survival and growth of the pathogenic fungi microorganisms and (b) damage caused to the host (Brunke, Mogavero, Kasper and Hube, 2016).

The presence of fungi on chiropractic treatment tables was demonstrated by Perdijk et al. 2017 (**Table 2.6**). These pathogenic fungi increase the risk of fungal infections for immunosuppressed or immunocompromised patients (Neely and Orloff, 2001). With this in mind, Chiropractic Clinics serves the public, who may have conditions that weaken the immune system – e.g., tuberculosis; HIV/AIDS; Autoimmune conditions (Rheumatoid arthritis, multiple sclerosis, polymyositis, etc.) (Neely and Orloff, 2001). These conditions may be present in patients who seek health care from chiropractors, therefore increasing risk of infection, highlighting the importance of hygiene control within a Chiropractic clinic. Moreover, fungi and their metabolites found in indoor environments can be allergenic, triggering or aggravating allergic conditions, e.g., allergic rhinitis, asthma, airborne dermatitis, or allergic conjunctivitis (Zukiewicz-Sobczak, 2013).

5.5.2.5. Fungal Transmission

Fungi can be transmitted in many ways as seen with the transmission of any living infectious microorganism. However, the discussion focuses mainly on two routes of fungi transmission: (a) Direct or indirect contact – usually spread when people contact the skin of an infected/contaminated person, animal, object/fomite, or even soil (Al-Shorbaji, Gozlan, Roche, Britton and Andreou, 2015; Dworecka-Kaszak, 2008). (b) Vehicle/airborne

transmission - Inhalation of the spores of fungi from the indoor or outdoor environments (Sabiiti and May, 2012). Fungal spores can survive for several days in the environment, increasing the probability of transmission to the host (Al-Shorbaji, Gozlan, Roche, Britton and Andreou, 2015).

5.5.2.6. Fungal Colonisation

The colonisation of fungi occurs in similar ways that bacteria colonise – following similar phases in attachment, biofilm formation and maturation, and dispersal. There is a small difference in the formation of biofilms associated with the two morphologically different fungi: yeasts and molds. Yeasts follow similar steps in colonisation that bacteria do except that it features pseudohyphal or hyphal growth (**Figure 2.2**). Whereas, filamentous molds have similar yet distinct differences in the steps of colonisation as compared to that of bacteria and yeasts (**Figure 2.3**) (Costa-Orlandi et al., 2017). This is due to filamentous fungi lacking the growth characteristics that result from binary fission or budding commonly seen in both bacteria and yeasts (Harding, Marques, Howard & Olson, 2009).

For fungal colonisation to occur, the initial phase of attachment must occur. Attachment of cells to each other and fomite surfaces is crucial for multicellular development, colonisation and pathogenesis (Brückner and Mösch, 2012). Tronchin et al. 2008 identified several factors that affect fungal attachment that is similar to bacteria with regards to the substratum, environment, and fluids (aqueous medium) (**Table 2.3**).

The primary issue relating to colonisation and biofilm formation of pathogenic microbes on Chiropractic treatment tables, is that the surface is usually dry, whereas many studies focus on the colonisation and biofilm formation in wet environments, related to indwelling medical devices.

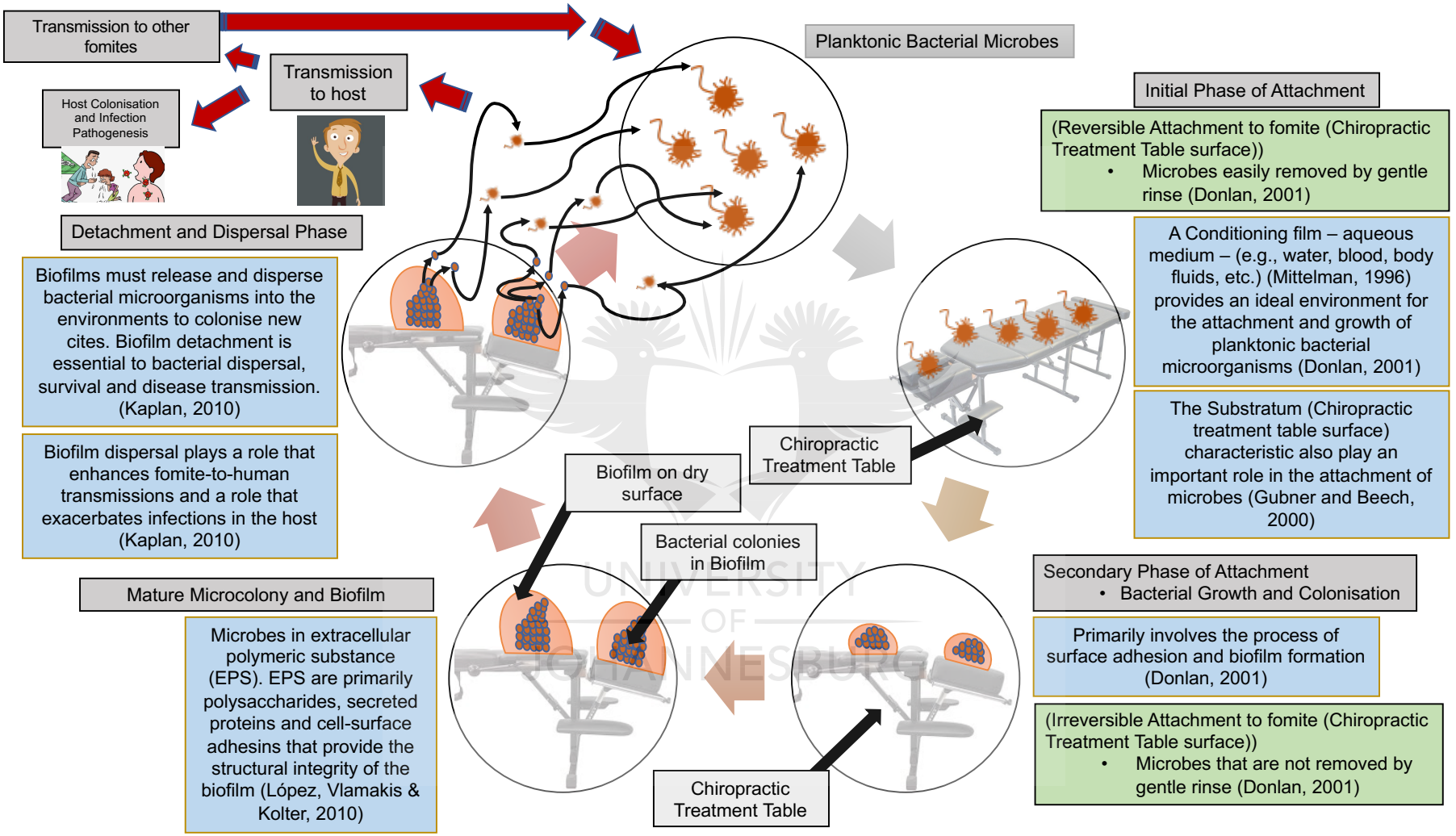

Figure 2.1 Life-cycle of Bacteria on the Chiropractic Treatment Table (Transmission and Colonisation)

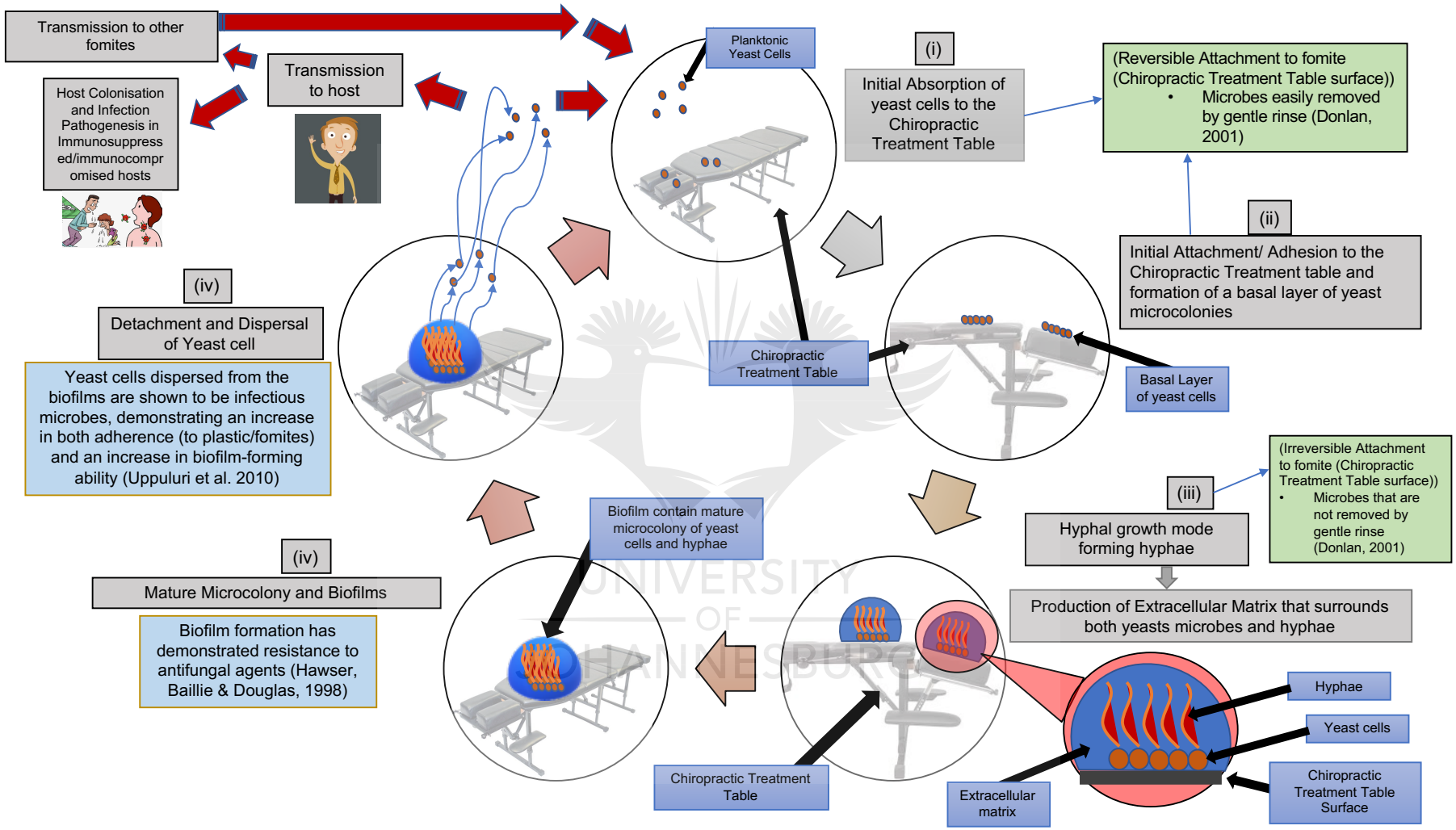

Figure 2.2 Life-cycle of Yeast Fungi on the Chiropractic Treatment Table (Transmission and Colonisation) – modified from Harding et al, 2009.

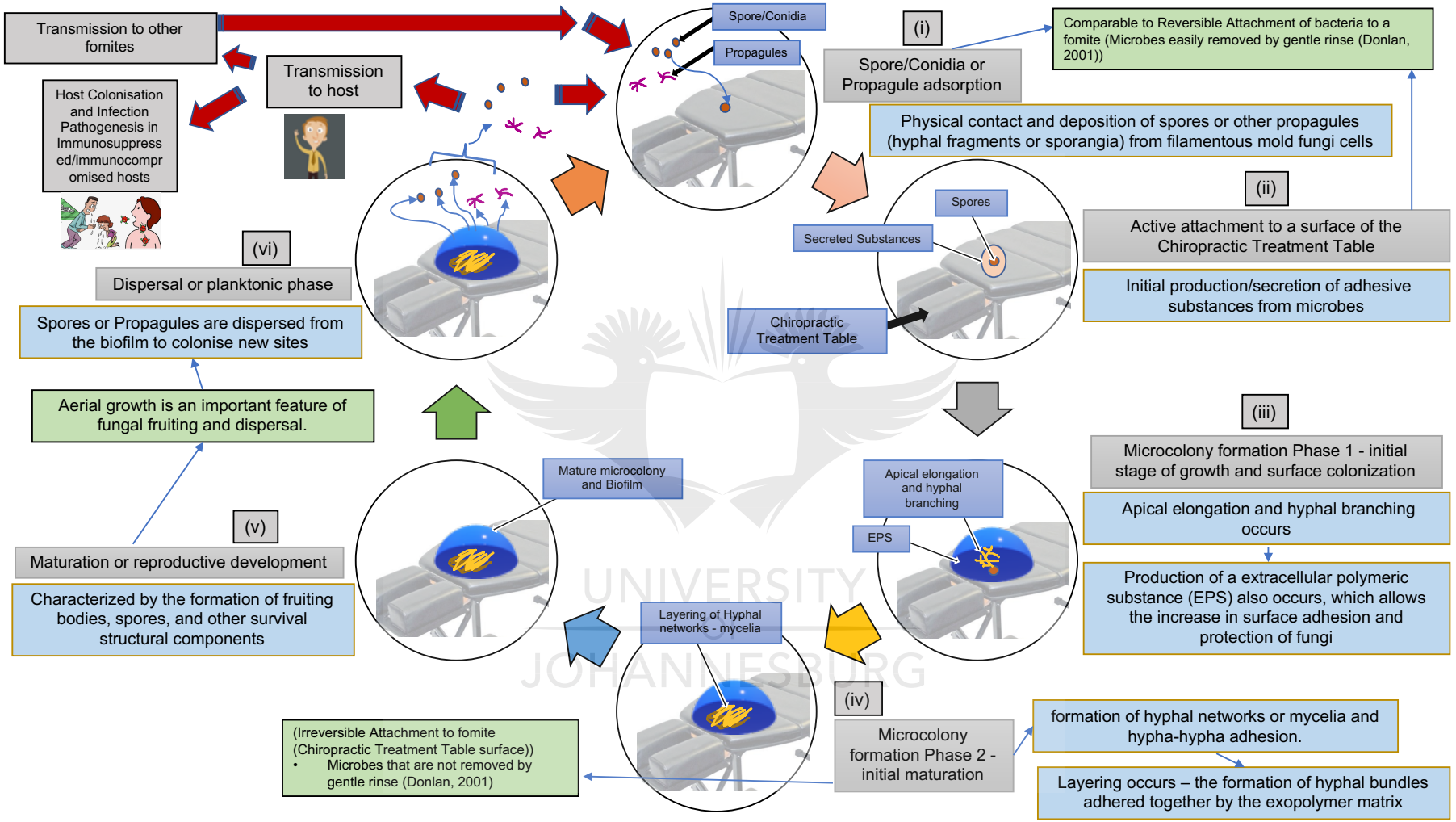

Figure 2.3 Life-cycle of Filamentous mold Fungi on the Chiropractic Treatment Table (Transmission and Colonisation) – modified from Harding et al, 2009.

However, it is now known that these pathogenic microbes are cable of attaching, adhering, forming biofilms and surviving for extending durations in a dehydrated state on the hospital bed and other dry surfaces (Garvey, Andrade Fernandes & Rowan, 2015). Biofilm formation has demonstrated resistance to antifungal agents (Hawser, Baillie & Douglas, 1998).

5.6. Hygiene Control and Infection Prevention

The increasing number of patients who are now being cared for in ambulatory-care (outpatient facilities) and home settings might have infectious diseases, immunocompromising conditions, or are treated with invasive devices. Therefore, the Centre of Disease Control (CDC) suggests that there should be adequate disinfection in these settings as it is necessary to provide a safe patient environment (Ling, Ching, Widitaputra, Stewart, Sirijindadirat and Thu, 2018). However, due to possible suboptimal cleaning practices performed amongst Chiropractors and the fact that it is human nature to forget key procedural steps, or when hurried, to take short cuts that break procedure protocols (Mohapatra and Sarangi, 2018). This causes an increased cross-contamination from specific equipment (chiropractic treatment tables), leading to an increased risk of infection transferral. Therefore, the CDC suggests that routine monitoring of sterilization procedures should be performed and thus, this study serves to educate students about and assess cleaning and disinfection practices of Chiropractic interns.

The CDC provides the methodology for surveillance of nosocomial infections along with the investigation of significant outbreaks (Khan, Baig, and Mehboob, 2017). Surveillance/monitoring procedures allow hospitals and other health-care facilities devise a strategy comprising of infection control practices. These gaps in knowledge and practice amongst health care providers and patients in controlling infection indicate a policy for strict implementation in the health care settings (Mohapatra and Sarangi, 2018). More research studies are required to determine the extent to which

microbially contaminated surfaces in the community, including those of alternative health care – Chiropractic, physiotherapist, biokineticist, etc. – facilities contribute to the transmission and infection of patients, health-care workers and anyone who make use of these alternative therapies (Gebel, Exner, French, Chartier, Christiansen, Gemein, Goroncy-Bermes, Hartemann, Heudorf, Kramer, Maillard, Oltmanns, Rotter and Sonntag, 2013).

Prevention of microbial colonization by disinfectants still must rely heavily on necessary infection control measures and monitoring systems to prevent contact between patient and pathogen (Bonten and Weinstein, 1996).

5.6.1. Cleaning and Disinfection

Disinfection is defined as "the antimicrobial reduction of microorganisms to a level previously specified as appropriate" (McDonnell, 2011). With the understanding of colonisation, transmission, and infection of pathogenic microbes on environmental surfaces, the importance of surface hygiene and disinfection should be essential. The fact that worldwide there is an increase in the occurrence of pathogenic microorganism resistance to antimicrobial treatments with high-rates of both hospital and community-acquired infections and evidence for the transmission of these microorganisms between surfaces and patients (Gebel et al. 2013), hygiene and disinfection control should be the first step towards overcoming this medical management dilemma. Hygiene and disinfection control has a greater and more distinguished impact on the incidence of infections than does the availability of antimicrobial treatments (Baron, 1996).

5.6.1.1. Spaulding Classification

Classification systems (**Table 2.7**) are used to aid healthcare workers to choose suitable Disinfection methods to reduce patient infection risk safely. Disinfectants can be classified as either Chemical Disinfectants or Miscellaneous Inactivating Agents (Rutala and Weber, 2008) (**Table 2.8**).

Table 2.7 Spaulding Classification – modified from Biosafety in Microbiological and Biomedical Laboratories - 5th Edition (2009).

Body part	Surface Classification (Examples)	Disinfection activity-level Classification Requirements	Disinfection activity-level Classification Description
Sterile human tissue or the vascular system (Rutala and Weber, 2008)	**Critical Surfaces** (surgical instruments, cardiac and urinary catheters, implants, and ultrasound probes)	High-level Disinfection	These Disinfectants kill vegetative microorganisms and inactivates viruses, but does not kill many bacterial spores. These disinfectants are classified as FDA Disinfectants and should not be used on Semi-critical or non-critical surfaces.
Mucous membranes or non-intact skin (Rutala and Weber, 2008)	**Semi-critical Surfaces** (respiratory therapy and anesthesia equipment, some endoscopes, laryngoscope blades, esophageal manometry probes, cystoscopes, anorectal manometry catheters, and diaphragm fitting rings)	Intermediate-level Disinfection	These Disinfectants kill vegetative bacterial microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. These disinfectants are Environmental Protection Agency (EPA) approved can be used on both Semi-critical and Non-critical Surfaces.
Intact skin (Rutala and Weber, 2008)	**Non-critical Surfaces** (bedpans, blood pressure cuffs, crutches, computers, bed rails, some food utensils, bedside tables, patient furniture and floors)	Low-level Disinfection	These disinfectants kill most vegetative bacteria except M. tuberculosis, some fungi, and inactivates some viruses.

Spaulding Classification characterises disinfectants to their activity level (High-level, Intermediate-level or Low-level Disinfection) as well as surfaces according to which body part is in contact with that surface (Critical, semi-critical, or non-critical surfaces) (McDonnell, 2011).

Table 2.8 Disinfectant Classification modified from CDC Guideline for Disinfection and Sterilization in Healthcare Facilities (CDC: Rutala and Weber, 2008).

Chemical Disinfectants - Biocides	
Disinfectant	**Disinfection activity-level Classification**
Alcohols	Intermediate-level Disinfection
Formaldehyde	High-level Disinfection
Glutaraldehyde	High-level Disinfection
Chlorine compounds	Intermediate-level Disinfection
Hydrogen Peroxide	Intermediate-level Disinfection
Iodophor	Intermediate-level to Low-level Disinfection
Ortho-phthalaldehyde	High-level Disinfection
Peracetic Acid	High-level Disinfection
Oxidising gents	High-level to Intermediate-level Disinfection
Phenolic compounds	Intermediate-level to Low-level Disinfection
Quaternary ammonium compounds	Low-level Disinfection
Miscellaneous Inactivating Agents	
Other Germicides (mercurials, sodium hydroxide, β-propiolactone, chlorhexidine gluconate, cetrimide-chlorhexidine, glycols (triethylene and propylene), and the Tego disinfectants)	
Metals as Microbicides	
Ultraviolet Radiation (UV)	
Pasteurization	
Flushing- and Washer-Disinfectors	

5.6.1.2. Classification of the Chiropractic Treatment Table Surface

According to **Table 2.7**, the Chiropractic Treatment Table can be classified as a Non-critical Surface requiring Intermediate-level to Low-level Disinfection (McDonnell, 2011) (**Table 2.9**). Non-critical surfaces have not been implicated directly in disease transmission due to little or no research (Rutala and Weber, 2004). However, as mention before, fomite-to-human transmissions can occur, either directly by surface-to-mouth, or indirectly, by contamination of fingers with subsequent hand-to-mouth, hand-to-eye and hand-to-nose transfer (Lopez et al. 2013). Cross-transmission can also occur by transient hand carriage by health care personnel due to contact with a contaminated surface or patient (Rutala and Weber, 2004).

Table 2.9 Justification for use of Intermediate-level Disinfection method on the Chiropractic Treatment Table (CTT) – modified from Rutala and Weber, 2004.

Justification	Examples
CTT surfaces may contribute to transmission of epidemiologically important bacteria and fungi.	Refer to **Table 2.1**, **Table 2.2**, **Table 2.6**
Detergents become contaminated and result in seeding the patients' environment with bacteria and fungi.	Water becomes increasingly contaminated during cleaning – therefore surface-to-surface transmission of bacteria and fungi can occur via mop-heads and cleaning cloths (Dharan et al. 1999).
Disinfectants are an established component of hospital infection control.	(Dettenkofer, Wenzler, Amthor, Antes, Motschall, and Daschner, 2004)

5.6.1.3. Factors Affecting Efficacy of Disinfectants

The effectiveness of disinfectants against bacterial and fungal microorganisms depends on a number of factors (**Table 2.10**). Knowledge and awareness of these factors listed in **Table 2.10** should lead to better

use of the disinfectant, and help with the development of an effective disinfection procedural guidelines (Rutala and Weber, 2008).

Table 2.10 Factors Affecting Efficacy of Disinfectants – modified from Rutala and Weber, 2004.

Factor	Description/Example	Reference
Microbial Load	An increase in growth and colonisation of microbes will lead to an increase in time that a biocide needs to destroy them all	Rutala and Weber, (2004)
Resistance of Microorganisms to Disinfectants	Microbes vary in their susceptibility to biocides. Bacterial spores being the most resistant, followed by mycobacteria, then Gram-negative bacteria – this is usually due to intrinsic resistance. Other Microorganism factors are degradative enzymes, cellular impermeability, as well as cell wall and membrane structures.	Russell, (1999) / Rutala and Weber, (2004)
Concentration and Potency of Disinfectants	The higher the concentrated the disinfectant, the greater the efficacy of the disinfectant and the shorter the time needed to kill microbes	Rutala and Weber,(2004)
Physical and Chemical Environmental Factors	• **Temperature** – most disinfectant efficacy increases with increased temperatures. • **pH levels** - pH influences the antimicrobial activity by altering the disinfectant molecule or the microbe cell surface. It may either increase or decrease disinfectant efficacy. • **Relative Humidity** - influences the activity of gaseous disinfectants	Rutala and Weber, (2004)
Organic/Inorganic Matter	Organic/Inorganic matter may either reduce the biocide activity or may protect microbes from the biocide.	Rutala and Weber, (2004)
Duration of Disinfectant Exposure	Items must be exposed to the disinfectant for the appropriate minimum contact time specified by the label on the Disinfectant labels of EPA-registered products. If this is not followed and results in subsequent injury/infection to patient, by law, the user assumes liability.	Rutala and Weber, (2004)
Biofilms	The resistance of bacterial microorganisms to disinfectants is frequently associated with the presence of biofilms on surfaces	Hawser, Baillie and Douglas, (1998) / Bridier, Briandet, Thomas & Dubois-Brissonnet, (2011) / Bressler, Balzer, Dannehl, Flemming and Wingender, (2009)

5.6.1.4. Cleaning and Disinfection Procedures

Environmental cleaning and disinfection practices are essential for reducing the transmission of pathogenic microorganisms and the risk of patient or occupational injury, infection, or disease (Hayden, 2006). These practices cultivate values of safety amongst health-care workers by providing an atmosphere of cleanliness and order (Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control, (PIDAC-IPC), 2018). A patients basic expectation in a health-care facility is a clean environment (Jha, Orav, Zheng and Epstein, 2008). Cleaning and disinfection in a health-care facility (especially on high-touch surfaces) should be performed on a routine basis; this allows for a safe and sanitary environment (Dharan et al. 1999).

Therefore, cleaning and disinfection procedures must be applied regularly, consistently, and appropriately to remove soil, dust, and debris to prevent accumulation, growth, and transmission of microorganisms. Adequate hygiene control procedures increase the efficacy of infection prevention. It is highly recommended that health-care facilities should have written policies and procedures for the appropriate cleaning and disinfection of equipment and environmental surfaces. These written policies and procedures should clearly define the frequency and level of cleaning (Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control, (PIDAC-IPC), 2018).

The first step to hygiene control in a health-care facility is to understand that surfaces, equipment or any other items that are difficult or impossible to clean and disinfect should not be purchased or used in the health-care facility (Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control, (PIDAC-IPC), 2018). Surfaces that are not easily cleaned or disinfected may increase the risk of infection transmission. It is also recommended that if equipment or any surfaces are damaged and

cannot be adequately cleaned, must be repaired, replaced or removed from the health-care facility. Thus, making maintenance of equipment essential.

The second step to hygiene control is the implementation of hand-hygiene procedures within the clinic. It involves five simple and effective steps (Wet, Lather, Scrub, Rinse and Dry). Health-care professionals should educate their patients about the importance of hand-hygiene; this helps them, and their communities stay healthy (Boyce and Pittet, 2002). Effective and regular (usually before and after a particular activity) hand-washing and sanitation has shown to be essential to the prevention of microbial transmission and subsequent infection (CDC, 2018).

The Third step to hygiene control is surface-hygiene; it begins with cleaning methods before using a disinfectant. Cleaning surfaces should be done with hot water and detergent – this process removes soil, dust, and debris that decrease substantial amounts of microorganisms and increase the efficacy of the disinfectant (Rutala and Weber, 2008). Once adequate cleaning has been performed, surface disinfection should be done with the supplied disinfectant that is approved by the EPA. It is important to follow the instructions on the label of the EPA approved disinfectant. Low-touch surfaces should always be cleaned and disinfected before high-touch surfaces, as this prevents transmission of microbes from high-touch surfaces to low-touch surfaces Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control (PIDAC-IPC), 2018).

5.6.2. Hygiene Monitoring and Surveillance

Knowing the microbiota on chiropractic treatment tables or any other surface within the treatment rooms is the basis for ensuring hygiene control quality by assessing any changes (Tršan, Seme and Srčič, 2019). Substandard/suboptimal hygiene knowledge and practices, or non-compliance thereof, has significant implications for patients, visitors, and health-care providers (Mohapatra and Sarangi, 2018). This creates the problem that if the Chiropractic Treatment Tables are not routinely cleaned

and disinfected, a fungal and bacterial build-up on the Chiropractic Treatment table can become a source of contamination in the Chiropractic profession. Therefore, it is required to establish an environmental monitoring and surveillance system to be routinely used at Chiropractic Clinics.

5.6.2.1. Monitoring/Surveillance Methods - Microbiologic Sampling of the Environment.

5.6.2.1.1. Bioaerosol Sampling

The term "Bioaerosol" is used to refer to airborne microbial particles such as bacterial cells or fungal spores and their by-products (Grinshpun, Buttner, Mainelis and Willeke, 2016). The presence of these particles in the air is the result of dispersal from a site of colonization or growth, usually from a site on a surface (Srikanth, Sudharsanam and Steinberg, 2008). Most Bioaerosol sampling methods (**Table 2.12**) involve techniques that separate particles from the air stream and collect them in or on a preselected medium (Jensen and Schafer, 1996).

Table 2.11 Most commonly used Bioaerosol Sampling Methods.

Methods	Description	Reference
Impaction on Solid Surfaces	Impaction collects micro-organisms directly on soft or hard surfaces – agar plates that requires incubation and colony growing for enumeration – or on adhesive or non-coated glass slides for immediate microscopic analysis.	Clauß, Springorum & Hartung, (2010)
Impingement in liquid	In high velocity liquid impingers, air is drawn through a small jet and directed against a liquid surface – the particles are collected in this liquid.	Mouilleseaux, (1990)
Sedimentation	This technique uses culture settling plates, and is based on the deposition of microorganism particles on the surface of a solid	Salustiano, Andrade, Brandão,

	culture medium per a given exposure time.	Azeredo, and Lima, (2003)
Air Filtration	Filter media are available in both fibrous (typically glass) and membranous forms. The air is allowed to pass through these mediums that serve as sieves for the microorganism particles. Therefore, microorganisms smaller than the pore size of the filter media may be efficiently collected by this method	Hinds, (1999)

5.6.2.1.2. Water Sampling

Routine sampling of water in health-care facilities are not usually indicated. However, sampling of water during infection outbreaks should be performed to investigate the possible causes and help determine appropriate infection-control methods (Sehulster and Chinn, 2003). This method, as mentioned, should not be considered as part of a routine Monitoring or Surveillance System for the DFC Chiropractic Training Clinic, but part of a separate microbiological testing system.

5.6.2.1.3. Environmental Surface Sampling

Previous studies at chiropractic clinics have shown that: (1) The Chiropractic Treatment Tables serve as potential reservoirs for microbial pathogens and sources of contamination and (2) has demonstrated microbial survival on environmental surfaces (Perdijk et al. 2017). This quantitative study is designed to assess hygiene practices amongst Chiropractic student interns as part of a comprehensive approach for specific quality assurance purposes and as an educational tool. Therefore, meaningful results depend on the selection of appropriate sampling methods (Sehulster and Chinn, 2003).

RODAC (Replicate Organism Direct Agar Contact) Sampling is the environmental sampling method of choice for this research study, as it provides a simple, selective and quick sampling procedure to assess all kinds of surfaces for microbial contamination and thus evaluating the hygienic status of the surface. RODAC plates have an advantage for being non-destructive to surfaces (Sehulster and Chinn, 2003; Clemons, 2010) and a disadvantage or limitation of this method is that it does not detect a variety of unwanted potentially pathogenic microorganisms but merely detects easily cultivatable bacteria and fungi. Thus, there may be several other undetected microorganisms present on the surfaces. The use of RODAC plates is limited further by long incubation periods required for the growth of bacteria and especially fungi and therefore not suitable for immediate assessment, another limitation is the inability to identify parasites, non-bacterial or non-fungal pathogens (Turner, Daugherity, Altier and Maurer, 2010).

RODAC plate method is a quantifiable method because, after the contact between the plate and the surface of the Chiropractic Treatment Table, it provides information relating to the number of microbial colonies. The quantification is derived from recording the number of colony forming units (CFU) per square centimetre (Sandle, 2016). The RODAC sampling method will be, therefore, an effective method for Monitoring microbial loads on the Chiropractic Treatment Tables and correlating that with the hygiene practices of the Chiropractic Student Interns.

However, new technology is allowing for alternative rapid microbiological sampling with results that are obtained within a few minutes after sampling. An example of this type of method that measures the cellular components is the bioluminescent measurement of adenosine triphosphate (ATP). Advantages of the ATP method is that it does not require or is not limited to trained laboratory personnel and due to its quick accessibility of the results, any suboptimal cleaning by any health-care facility staff can be reported with the implementation of corrective measures immediately (Tršan, Seme

and Srčič, 2019). This method should, therefore, be recommended for further studies on effective methods for monitoring surface hygiene at the DFC Chiropractic Training Clinic at the University of Johannesburg, South Africa.

5.7. Conclusion

Literature shows the need for appropriate monitoring systems for environmental surface hygiene, and this will be described in the next chapters.

Chapter Three – Methodology

5.1. Introduction

This chapter describes the methods used, statistical analysis, and data evaluation used in the study.

5.1.1. Study Design

This study is an exploratory quantitative study utilising surface sampling to monitor the presence and numbers of bacteria and fungi.

5.1.2. Sample population

All treatment tables in use by Chiropractic Interns in the DFC Chiropractic Training Clinic at the University of Johannesburg (n=23) were included in the study. Samples were collected from the headrest and central section of the thoraco-abdominal part of the Chiropractic treatment table (**Figure 3.1**). Therefore, the total number of surfaces sampled is n=46 per day.

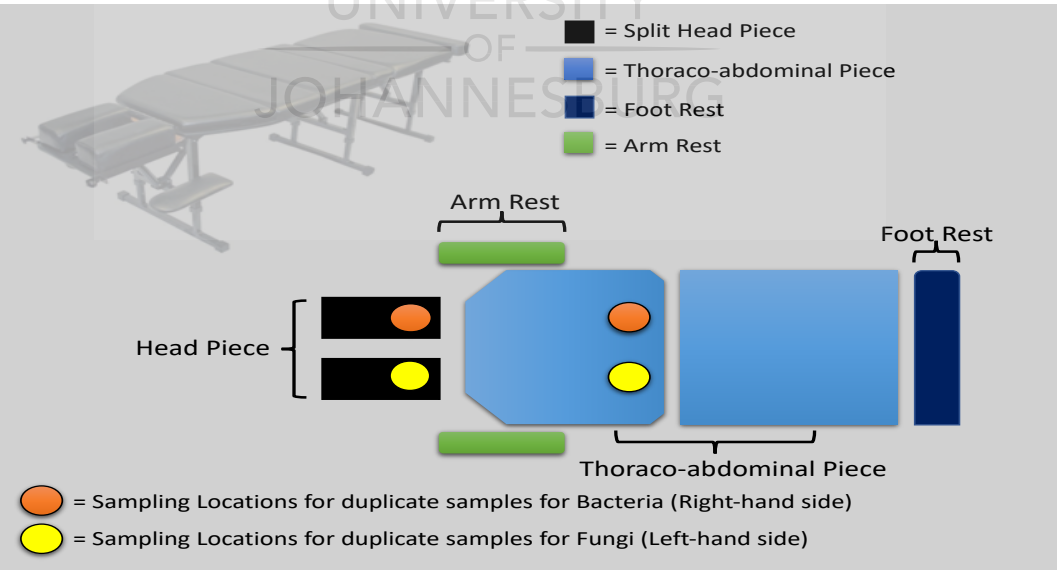

Figure 3.1 Areas Sampled on the Chiropractic Treatment Table.

5.1.2.1. Inclusion Criteria

Inclusion criteria required Chiropractic treatment tables included to be used for assessments in the clinic for the treatment of patients and be covered in vinyl or leather surfaces.

5.1.2.2. Exclusion Criteria

No other surfaces in the rooms (plinths, tables, chairs, or doorknobs) were sampled as part of this study. No additional materials, such as the contents of the students' diagnostic kits or patient charts, were considered in this sampling.

5.2. Sampling Equipment

The RODAC (Replicate Organism Detection and Counting) agar contact plates with Tryptone Soya Agar (growth nutrients for bacteria and fungi) and two commonly used disinfectant neutralisers; Polysorbate 80 (inactivates phenols, hexachlorophene, and formalin) and Lecithin (neutralises quaternary ammonium compounds) was used for the surface sampling (**Appendix A**). Samples was taken using the Count-Tact® Applicator (**Figure 3.2**) (**Appendix B**) to ensure equal pressure (roughly 500g) and required time (10 seconds) is applied to the plate during the sampling (Perdijk, Yelverton and Barnard, 2017).

Figure 3.2 Count- Tact® Applicator (UFAG Laboratorien, 2016).

5.3. Sample Approach

Figure 3.3 is a flow diagram to provide a clear understanding of the methodology used in this study.

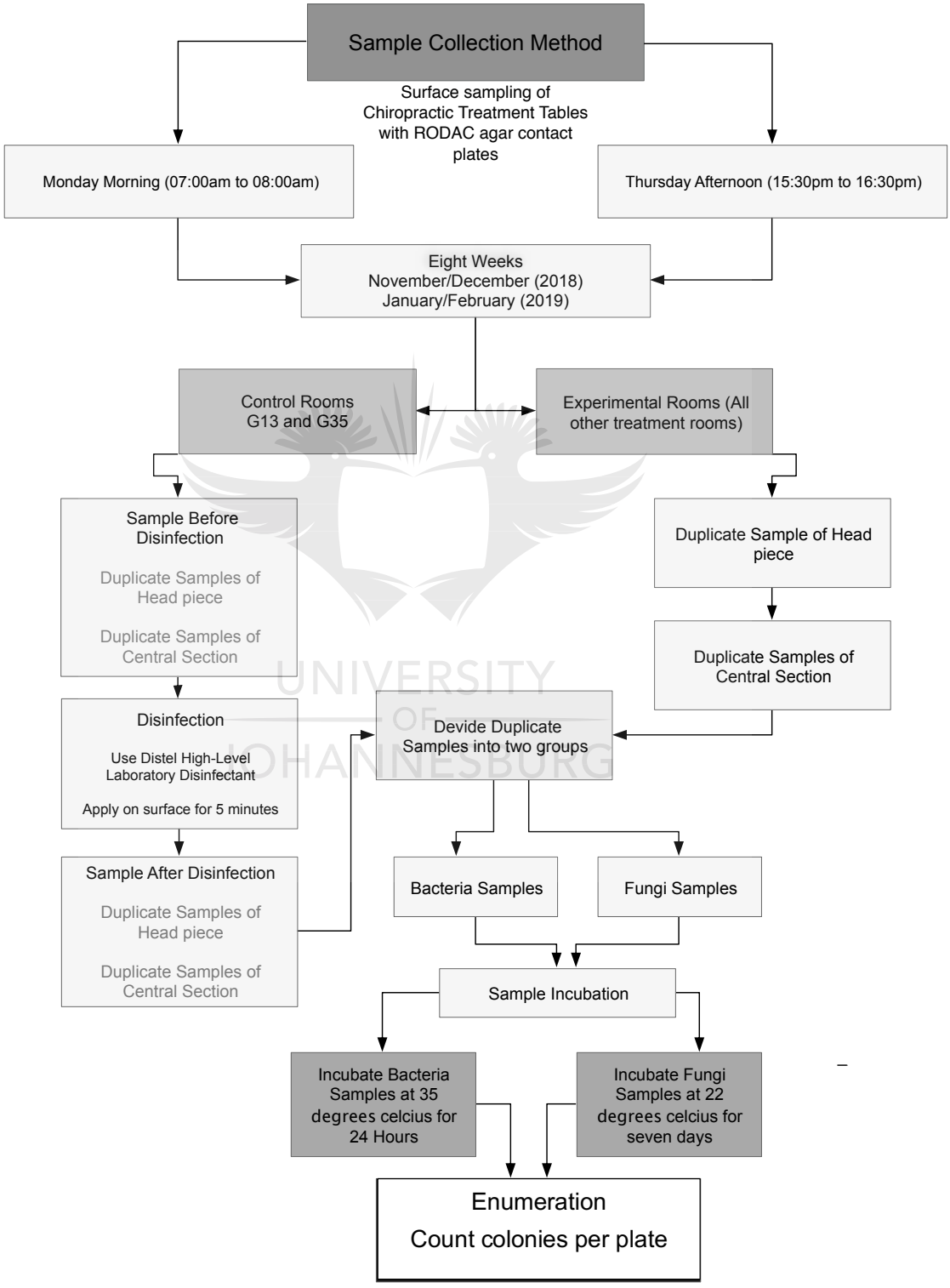

Figure 3.3 Flow Diagram of sampling methodology.

5.3.1. Surface Sample Collection Method

Duplicate samples were collected (Figure 3.4) from the headrest and central sections of the treatment tables twice a week (Monday and Thursday), for a total of eight weeks (November / December 2018 and January / February 2019), to monitor the cleaning and disinfection of the treatment tables and potential build-up of bacteria and fungi. The headrest and central section of the thoraco-abdominal part of the chiropractic treatment table were selected as our primary sampling sites due to recent research demonstrating these areas to have high numbers of colony forming units, therefore indicative of greater contaminated areas (Perdijk et al. 2017).

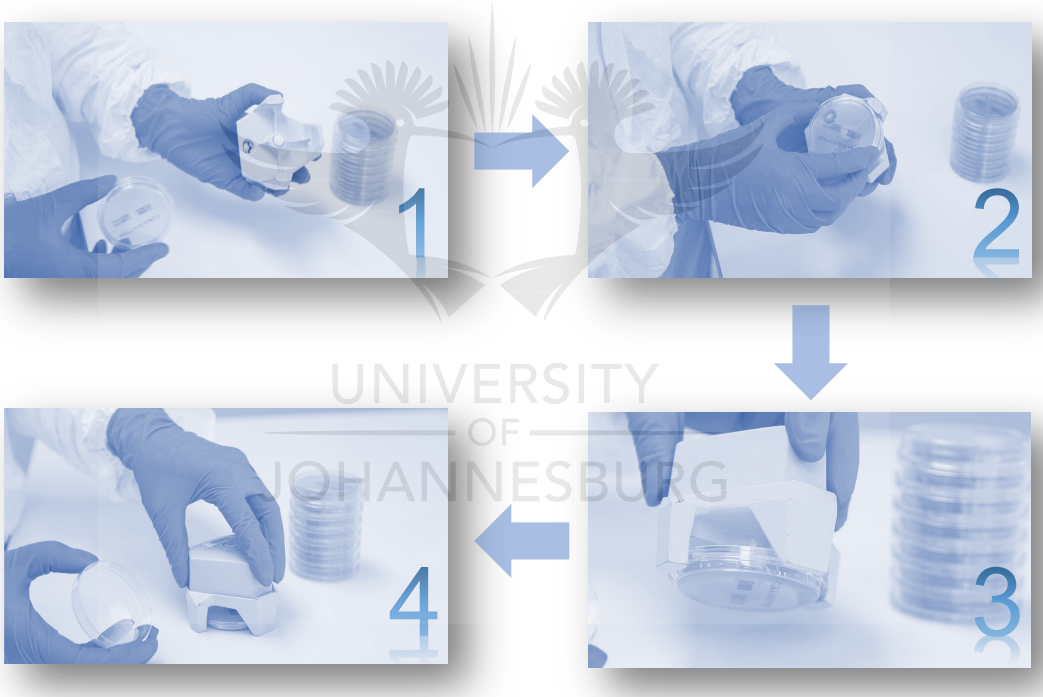

Figure 3.4 Sample collection with a single RODAC agar contact plate that slides onto the Count- Tact® Applicator which is then depressed directly onto the sample surface (UFAG Laboratorien, 2016).

Samples were taken on Monday mornings before the clinic opened (this allowed for the build-up of naturally occurring bacteria and fungi that may settle onto the tables) and on Thursday afternoons after the clinic closed

and all Chiropractic student Interns vacated the clinic. The sampling period selected pre- and post- the December 2018 holiday to assist in monitoring the initial cleaning and disinfection practices of the treatment beds after the holidays as well.

Two Chiropractic treatment tables (G35 and G13) were used as the study control rooms where the chiropractic treatment table was sampled before and after cleaning and disinfection by the researcher, with the current disinfectant (Distel High-Level Laboratory Disinfectant) provided by the UJ Chiropractic training clinic. G35 was our initial Control room; however, after initial results in week one, it was recommended to include G13 as a second control room.

Figure 3.5 Sampling of Chiropractic Treatment Tables done by researcher and trained laboratory staff member at the DFC Chiropractic Training Clinic.

5.3.2. Sample Incubation

One of the duplicate samples was incubated for 24 hours at 35°C and the second duplicate sample for seven days at 22°C for the isolation of the

bacteria and fungi respectively. This study monitored for the presence and numbers of bacteria and fungi present and only identified the bacteria or fungi present when the trained lab assistant needed to identify the organism that may be potentially pathogenic.

5.3.3. Sample Analysis

Interpretation and colony counting occurred after 24 hours for bacteria and after 7 days for fungal cultures were made using the Promega Colony Counter application, the numbers were then confirmed by identifying the different colony morphological characteristics of size, form, colour, elevation, margin, surface, and density. These were then checked and confirmed by a trained and qualified microbiology laboratory staff member from the Water and Health Research Department of the University of Johannesburg, South Africa.

5.3.4. Primary Organism Isolation and Identification

Bacterial isolates that were of concern were plated onto sheep blood agar plates for characterization. They were then further characterized using the VITEK® 2 Compact (bioMérieux, Inc.), using the methods and consumables specified by the manufacturer. Fungal isolates where sent to Inqaba for identification using sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region and sequences compared to other know sequences using a BLAST search.

5.4. Reliability and Validity

Various controls were included in the study to ensure the reliability and validity of the results. This includes disinfectant control rooms (sampling chiropractic treatment tables cleaned by the researcher with the disinfectant). Further measures include thermometers fitted to the incubators to monitor the incubation temperature, control of humidity in the incubators to ensure that the plates did not dry out and control of the room

temperature to ensure the proper functioning of the equipment. All experiments were done under the supervision of qualified laboratory staff.

5.5. Data Management

All data from the samples collected on the chiropractic treatment tables were sent to STATKON and entered into an IBM SPSS 23.0 database. Before statistical analysis, the data set was reviewed and cleaned by Ms. Juliana Van Staden, the project biostatistician.

5.6. Data Analysis

The microbiology data (bacterial and fungal counts) from the sample analysis were entered into Microsoft Excel sheets. Statistical analysis was conducted in IBM SPSS Statistics v 25 by STATKON and the hypothesis was tested. With descriptive statistics, non-parametric testing such as the Mann-Whitney Test was used to describe the data and test the hypothesis. This method was used due to the relative skewness by the influence of outliers amongst the data. For this reason, the mean and interquartile range is used as the measure of central tendency and variability respectively. Data was used to describe possible changes that occur; before and after disinfection, over days, weeks, months, as well as the sampling locations. Data was considered statistically significant when (p-value < 0.05).

5.6.1. Variables

Variables for the sampling of the chiropractic treatment tables are as follows:

- Continuous variables are the fungi and bacteria.
- Categorical variables are the rooms (peripheral, central and control rooms), sampling locations (Head piece and central section of the thoraco-abdominal part of the Chiropractic treatment table), days and time that sampling is performed (Monday Mornings and Thursday Afternoons)

5.7. Ethical Considerations

Approval to conduct this study was requested from the UJ Faculty of Health Science Higher Degrees Committee (HDC) and Research Ethics Committee (REC).

All aspects of the study were conducted in accordance to the Declaration of Helsinki and conformed to international ethical standards. Ethical approval (**Appendix C**) was obtained from the Research Ethics Committee (REC) and Higher Degrees Committee (HDC) of the Faculty of Health Sciences, University of Johannesburg; while the Director of the UJ Doornfontein campus Health Training Centre, Dr. Pieter Els, and the clinic coordinator, Dr. Caroline Hay, issued the administrative clearance.

This dissertation was submitted via anti-plagiarism software, Turnitin, and found to be within acceptable required levels (**Appendix D**).

Chapter Four – Results and Discussion

5.1. Introduction

This chapter describes the objective measurements, the statistical analysis, and data evaluation used in the study. The objective data includes the total microbial load on the treatment tables, as well as some pathogens isolated and classified using the VITEK® 2 instrument.

5.2. Objective Data Analysis

5.2.1. Clinical setting and demographics

The Doornfontein campus (DFC) Chiropractic training clinic is part of the Health Training Centre at the University of Johannesburg (UJ). The training clinic is a three story building, with the chiropractic department situated on the ground floor. The chiropractic training clinic consists of a total of 20 treatment rooms [13 rooms located on the outer perimeter (outside rooms) and 7 on the inner perimeter (inside rooms)] (**Figure 4.1**). A further two sets of 2 rooms are located on the second and third floor respectively.

Control rooms included were treatment room G13 and G35. The experimental rooms included were; G07, G09, G12, G14, G15, G17, G19, G21, G23, G25, G26, G27, G29, G30, G31, G34, G36, C3, C5, 155, 157 treatment rooms (**Figure 4.1)**.

There is an estimated total number of 70 chiropractic interns (1st and 2nd year) currently practicing in the UJ chiropractic training clinic. These interns work within a fixed two-shift system, with the morning shift allocated to the 2nd year interns and the afternoon shift allocated to the 1st year interns. Interns from both years are divided into two groups, each group working on alternating days.

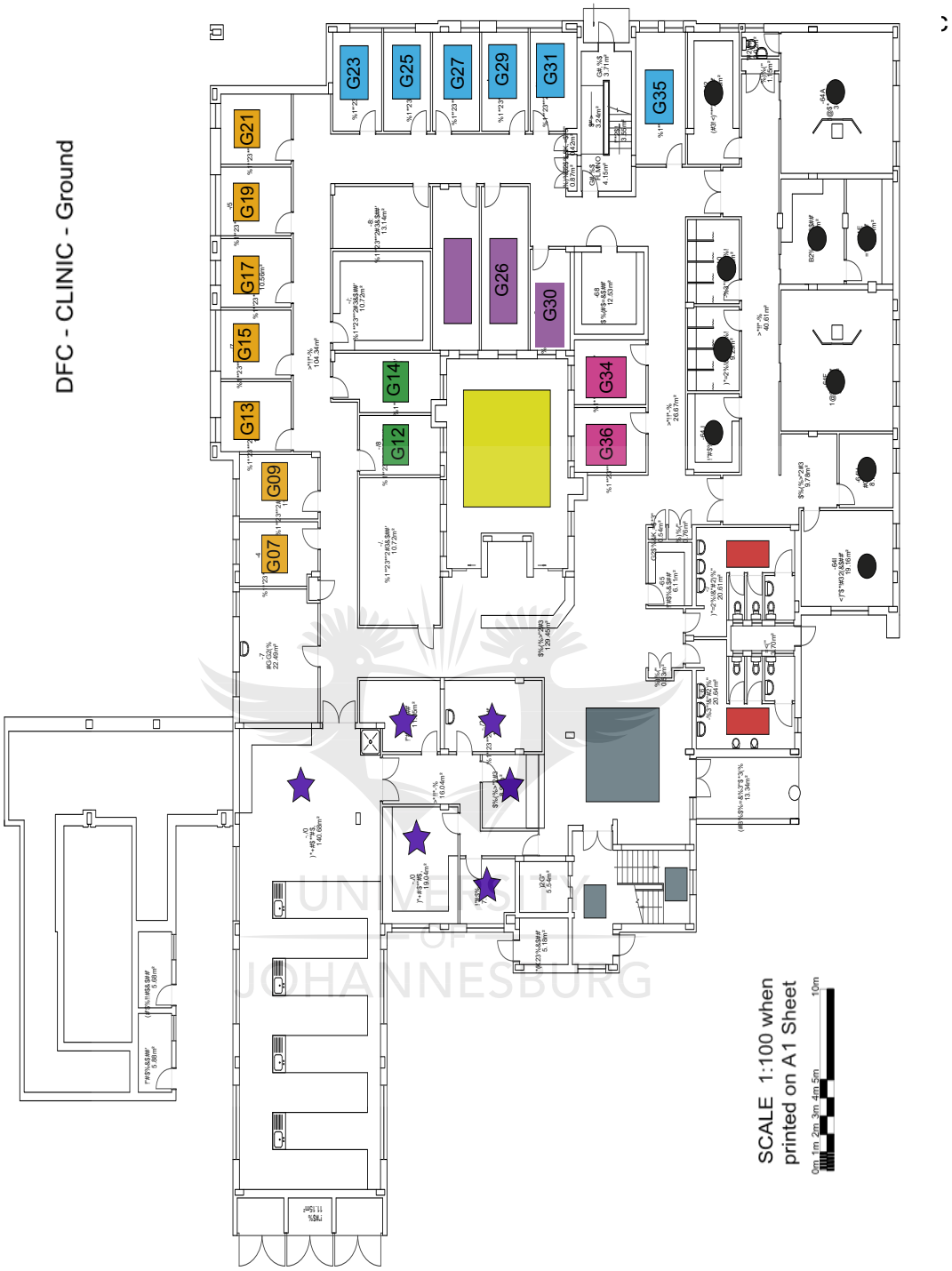

Figure 4.1 The University of Johannesburg chiropractic training clinic on the first floor (Perdijk, Yelverton and Barnard, 2017).

5.2.2. Control Room Data for disinfection of Chiropractic Treatment Tables

a) Current Disinfectant Agent used at the DFC Chiropractic Training Clinic for Chiropractic Treatment Table Disinfection.

Distel High-Level Laboratory Disinfectant is currently the disinfectant in use at the DFC Chiropractic Training clinic to disinfect the Chiropractic Treatment Tables. It is an EPA approved disinfectant and approved under the Biocidal Products Regulation (EU) No. 538/2012.

Distel High-Level Laboratory Disinfectant contains the following chemicals:

ii) Polymeric biguanide hydrochloride (PHBM)
iii) Didecyl dimethyl ammonium chloride (DDAC)
iiii) Alkyldimethyl benzyl ammonium chloride (ADBAC)
ivi) Stabalisers, Chelating agents and demineralised water balance.

Figure 4.2 Distel High-Level Laboratory Disinfectant

Table 4.1 Research Studies proving effectiveness and safety of the chemical ingredients of Distel High-Level Laboratory Disinfectant

Ingredient (Chemical Agent)	Description	Reference for Research on effectiveness and saftety
Polymeric biguanide hydrochloride (PHBM)	PHBM is an antiseptic with antiviral and antibacterial properties.	Mashat, (2016)
Didecyl dimethyl ammonium chloride (DDAC)	DDAC and ADBAC are both Quaternary ammonium compounds – they both cause autolysis and have bactericidal activity, contributing to cell death	Ioannou, Hanlon and Denyer, (2007)
Alkyldimethyl benzyl ammonium chloride (ADBAC)		

b) Control room results

Control rooms G13 and G35 were sampled before and after disinfection procedures were performed. It is hypothesised that there would be a significant decrease ($p < 0.05$) in bacterial and fungal contamination after chiropractic treatment tables were disinfected by the researcher.

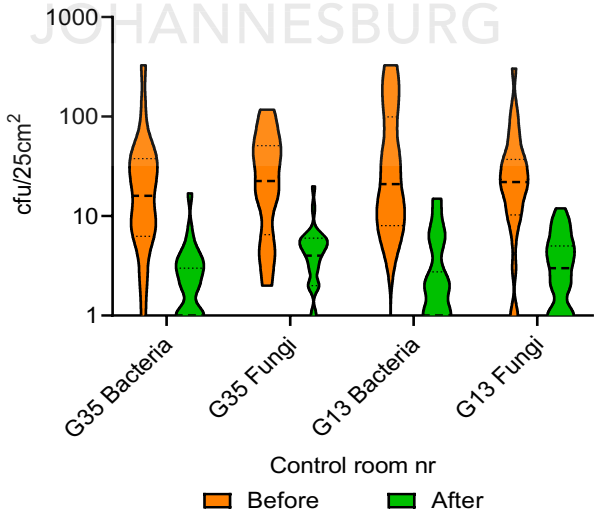

Figure 4.2 Control Rooms – Total Bacteria vs Total Fungi (Before and After Disinfection)

Results from surface sampling before and after disinfection of the control rooms (G13 and G35) chiropractic treatment tables (**Table 4.2**) showed that there was a significant difference (**p ≤ 0.000**) after disinfection was performed. The data in **Table 4.2** is represented in the linear scale. There is a substantial decrease in both fungal and bacterial colony-forming units (CFU/cm^2) after the disinfection procedures were performed on all the control rooms treatment tables. This is confirmed in the violin graph, which demonstrates the data in a log scale (**Figure 4.2**). Room G35 had a 92% reduction (1.1 log reduction), whereas room G13 had a 96% reduction (1.4 log reduction) (**Figure 4.2**). Both the data represented in both the linear and log scales prove that the disinfectant and disinfection procedure used by the researcher was effective enough to make a statistical difference and a considerable reduction in bacterial and fungal contamination on the Chiropractic treatment table surfaces.

Table 4.2 Evidence for statistical difference of microbial (bacterial and fungal) contamination on the control rooms Chiropractic treatment tables after disinfection procedures were performed.

	G35 Bacteria		G35 Fungi		G13 Bacteria		G13 Fungi	
	B*	A*	B*	A*	B*	A*	B*	A*
Median (CFU/25cm^2)	16	1	22.5	4	21	1	22	3
Minimum	0	0	2	0	0	0	0	0
25th Percentile	6,25	0	6,5	2	8	0	10,25	1
75th Percentile	37,75	3	51	6	99,5	2,75	37	5
Maximum	330	17	118	20	330	15	305	12
Interquartile Range	31,5	3	44,5	4	91,5	2,75	6,75	4
P-value	**0.000**		**0.000**		**0.000**		**0.000**	

B* - Before disinfection / A* - After disinfection

Currently to the knowledge of the researcher, there are no benchmarks for microbial counts that can be used for determining effective disinfection procedures and hygiene control on chiropractic treatment tables. Research that studied cleanliness in operating theatre environments (Wirtanen et al. 2012), proposed three benchmark categories and values for levels of hygiene (**Table 4.3**), namely; Adequate, Inadequate and Poor. However, because Inadequate and Poor are synonyms of each other and there is no distinct difference between the words. It is suggested that a separation between Inadequate and Poor should be made. Therefore, for this study, the following categories for Levels of Hygiene are proposed: Adequate, Fair and Inadequate (**Table 4.4**) with regards to colony forming units (CFU/25cm^2) found on the RODAC plates for both bacteria and fungi on chiropractic treatment tables.

Table 4.3 The microbial levels of high hygiene surfaces in operating theatres – modified from Wirtanen et al. 2012.

Microbial Level CFU/cm^2	Adequate	Inadequate	Poor
Environmental surface	<20	20-50	>50
Indirect patient contact surface	<10	10-25	>25
Possible patient contact surface	<3	3-10	>10

Operating theatre room surfaces require high-level disinfection as compared to chiropractic treatment tables only requiring intermediate to low-level disinfection (**Table 2.7**). Therefore, it proposed that there should be a broader range for levels of hygiene (CFU/25cm^2) when monitoring surface hygiene on chiropractic treatment tables (**Table 4.5**) as compared to the operating theatre room surfaces (**Table 4.3**). The above results from control room treatment tables (**Figure 4.1**) demonstrates that adequate levels are achievable and should be the set as a benchmark.

Table 4.4 Proposed categories, description and recommended values for *Levels of Hygiene* when monitoring surface microbial contamination with RODAC plates.

Category for Levels of Hygiene	Proposed Definition	Level of Hygiene CFU/25cm^2
Adequate	Satisfactory or acceptable levels of microbial counts with minimal risk of infection transmission.	0-10
Fair	Reasonable levels of microbial counts with moderate risk of infection transmission.	11-25
Inadequate	Dissatisfactory or unacceptable levels of microbial counts with high risk of infection transmission.	>25

It must be noted that to be able to make mention of infection transmission risk relating to the *Levels of Hygiene* in **Table 4.4**, is because of the relationship between colonisation and infection transmission. A study from the *Journal of Hospital Infection* highlights a significant correlation between surface contamination and incidence of infection in a hospital facility (Alberti, Bouakline, Ribaud, Lacroix, Rousselot, Leblanc & Derouin, 2001). Another study from the *Journal of Intensive Care* also makes mention that higher environmental contamination has been reported around infected patients more so than around patients who are only colonised by the microbes (Russotto, Cortegiani, Raineri & Giarratano, 2015). This is critical to understand, as high microbial counts on surfaces may not be directly implicated in infection transmission because not all bacteria or fungi are pathogenic and because some may be beneficial to humans. However, for the reason that pathogenic bacteria (**Table 2.1**) and fungi (**Table 2.6**) have been found on the chiropractic treatment tables, infection transmission is possible. Infection transmission risk should, therefore, depend on what microorganisms are located on the surfaces and on their related pathogenicity and virulence, as well as on the state of the hosts' immune system.

The question remains how an increased microbial load relate to an increased risk of infection transmission? It is related, as increased microbial counts mean that there are poor hygiene practices, therefore allowing both non-pathogenic and pathogenic microorganisms to survive, grow, and colonise on surfaces, and when the conditions are right, may enable pathogenic microorganisms to cause infection transmission.

Infection transmission prevention is the main objective and to be able to prevent infection transmission, it is essential to apply strict preventative measures with the implementation of hygiene control guidelines and making use of monitoring systems (Alberti, *et al* 2001).

5.2.3. Monitoring Experimental Rooms Microbial Loads

There were a total number of 21 experimental rooms – there were a total of 23 treatment tables sampled of which 2 were selected as control room tables and 21 were selected as experimental room tables – monitored twice a week for eight weeks. Both the bacterial and fungal microbial loads were monitored on two different locations on the chiropractic treatment tables. As previous research studies have focused on the presence of microbes on Chiropractic treatment tables at the UJ Chiropractic Training clinic, a disinfectant was introduced to the clinic to help with hygiene control and removal of these microbes. With general knowledge and standard hygiene practices that students should have developed and learned, these Chiropractic treatment tables should be disinfected with a resultant decrease in microbial contamination in both bacteria and fungi and subsequent reduced risk of infection transmission.

During the eight weeks of monitoring surface hygiene of Chiropractic treatment tables, the results (**Figure 4.3**) demonstrated that the treatment tables are not adequately disinfected.

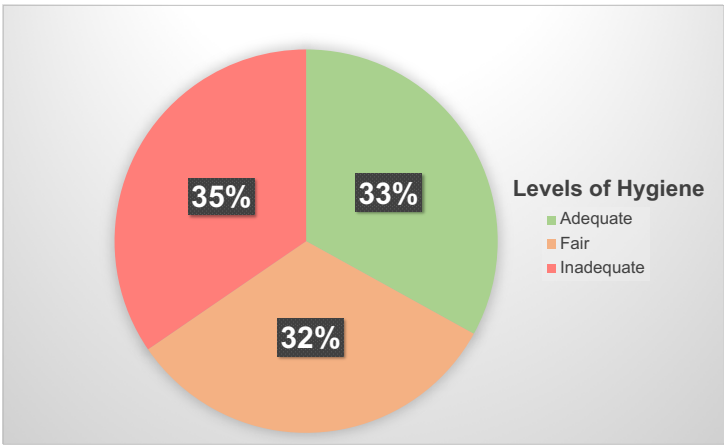

Figure 4.3 A pie chart representing the frequency (percentage) of samples taken from chiropractic treatment table surfaces that had microbial counts that were of Adequate, Fair and Inadequate levels.

Only 33% of the samples taken of the treatment tables had microbial loads below the proposed 10 CFU/cm^2 which are *Adequate Levels of Hygiene*.

This statistical frequency method of determining the levels of hygiene together with other parametric and nonparametric statistical tests can be applied to monitor each room individually to determine the levels of hygiene of the treatment table in each room. This will allow the researcher to identify the chiropractic student with the poorest standard of hygiene practices. However, because students work rotational shifts and do not have an assigned room, it would be very hard to monitor the cleaning and disinfection standards of individual chiropractic students. Therefore, for the purpose of this research, the monitoring and reporting of the standards of hygiene practices reflects the group of chiropractic intern students as a whole.

The above results reflects that the chiropractic intern students as a group have poor hygiene practice with 67% of the samples having *Fair* to *Inadequate* Levels of Hygiene and moderate to high risk of infection transmission. These high microbial counts may be because there are no

hygiene guidelines or protocols in place for chiropractic students to follow as yet.

However, while comparing results (**Table 4.6**) with the current study and a previous study done in the clinic (Perdijk et al. 2017) – which also recorded microbial counts on the chiropractic treatment tables at the UJ chiropractic training clinic, there is a reduction in the mean (average) of both bacterial and fungal microbial loads. So even though the chiropractic treatments tables are still inadequately disinfected, there is obviously an improvement because of measures that have being introduced to improve Hygiene control within the UJ Chiropractic training clinic since the onset of similar research studies.

Table 4.6 Comparison between the bacteria and fungi means (averages) CFU/cm^2 on the head pieces and thoraco-abdominal sections of the chiropractic treatment tables in Perdijk et al. (2017) study and the current study.

	Perdijk et al. (2017) study	**Current Study**
	Bacteria (CFU/25cm^2)	
Head Piece	92.59	42.67
Thoraco-abdominal section	86.32	29.96
	Fungi (CFU/25cm^2)	
Head Piece	92.58	25.58
Thoraco-abdominal section	86.35	24.75

5.2.4. Comparison between Total Bacteria and Total Fungi data from experimental rooms

Bacteria and fungi thrive at different environments and have variety of different growth requirements (**Table 2.5**). Due to the following reasons: (1) bacteria's growth rates been faster (only taking 24 hours to see results on RODAC plates) than that of fungi (taking 5-7 days to identify results on

RODAC plates); (2) the direct contact and transmission of microbes from patients to the treatment tables; (3) and the effects that inside and outside environmental factors have on survival, colonisation, growth and transmission of microbes, it is hypothesised that bacteria is more abundant than fungi on the treatment tables.

The data is skewed with many extreme outliers in the bacterial and fungal data sets (**Figure 4.4** and **Figure 4.5**). Considering all the data where bacteria colonies were counted, there was more variability in the bacterial data set (IQR = 27) when considering the middle fifty percent (IQR) than in the fungi (IQR = 22), where the middle fifty percent is smaller (**Table 4.7**). The median values for fungi were higher (M = 20) than that of bacteria (M = 15). However, a Mann-Whitney U Test revealed no significant difference between the total bacterial counts (n = 662) and total fungal counts (n = 658), with a p-value = 0.0505.

Table 4.7 Non-parametric analysis comparing data between Total Bacteria and Total Fungi.

	Total Bacteria	**Total Fungi**
Median (CFU/25cm^2)	15	20
Minimum	0	0
25th Percentile	7	10
75th Percentile	34	32
Maximum	330	330
Interquartile Range (IQR)	27	22
P-value	0.0505	

Bacteria and fungi microbial loads on the chiropractic treatment table surfaces are of equal magnitude with no real difference in microbial loads between them. Both bacteria and fungi contamination does occur on the Chiropractic treatment tables, these two microorganisms seem to co-exist, and each may play a role to the survival, growth, colonisation and transmission of each other (Frey-Klett, Burlinson, Deveau, Barret, Tarkka & Sarniguet, 2011). To what extent is unknown.

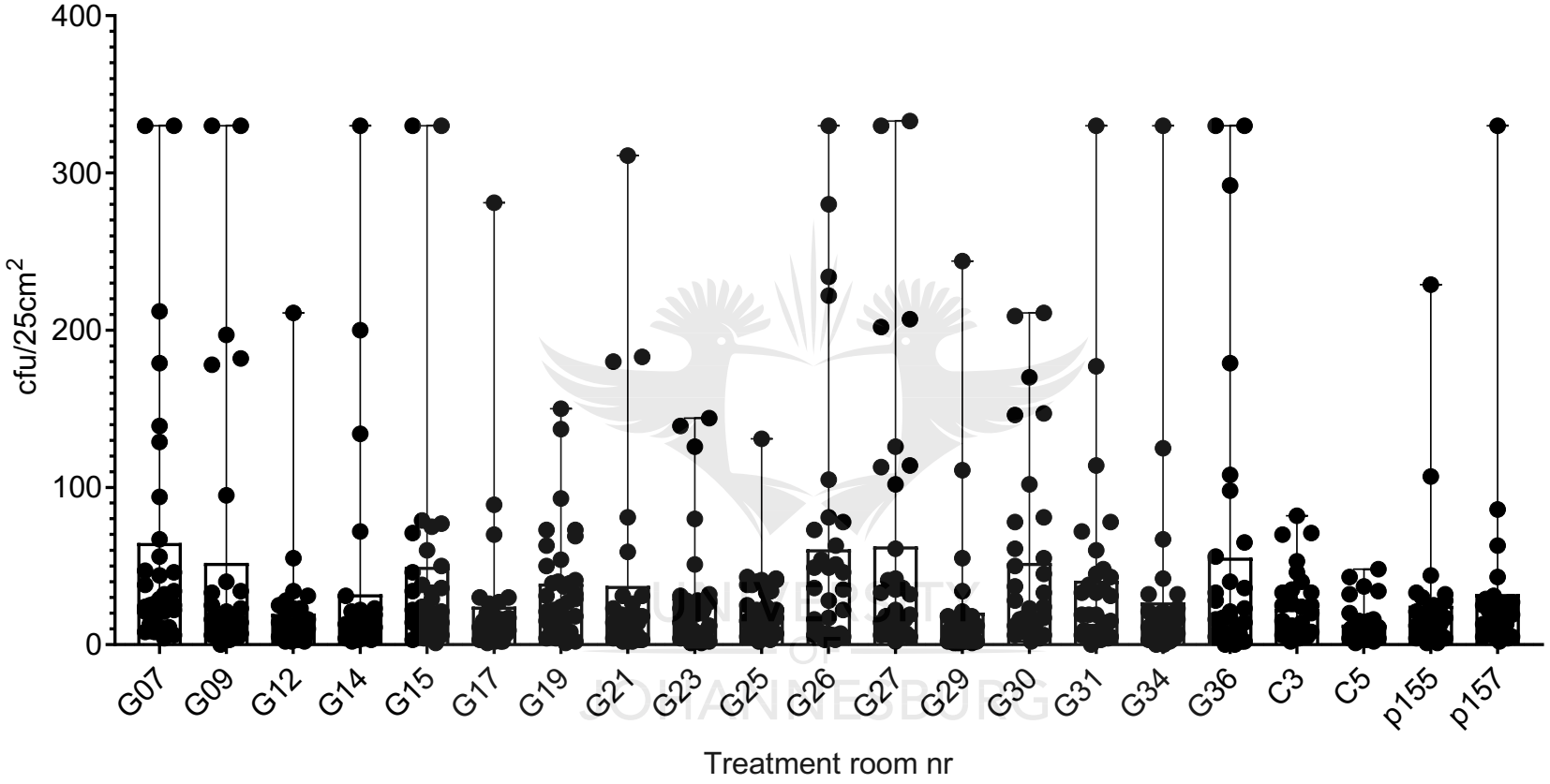

Figure 4.4 Graph of treatment rooms showing mean of the bacterial data with the data ranges drawn on linear scale

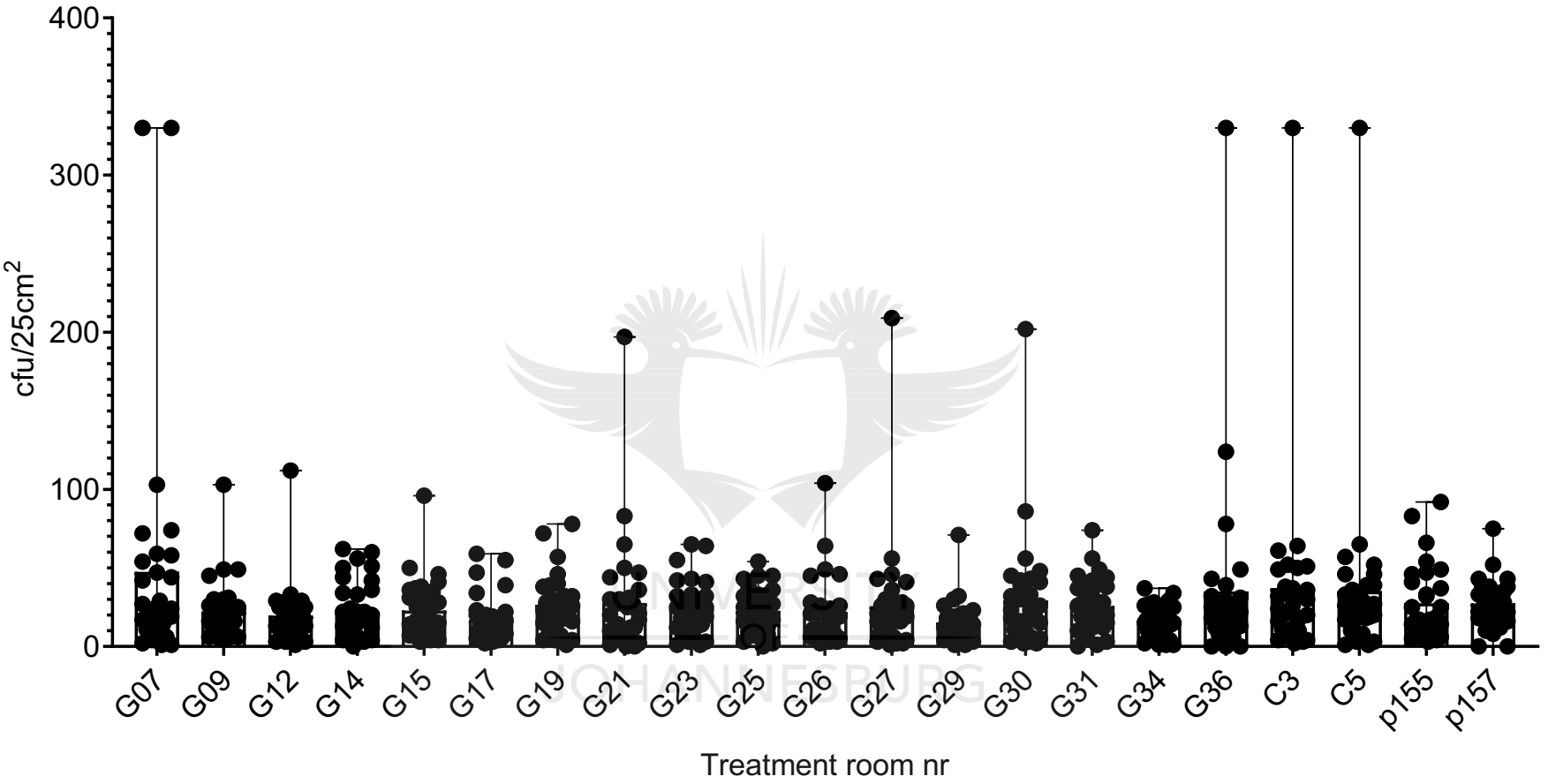

Figure 4.5 Graph of treatment rooms showing mean of the fungi data with the data ranges drawn on linear scale

5.2.5. Bacterial and fungal loads on the head piece vs the thoraco-abdominal section

During the eight weeks of sampling, duplicate samples were taken to compare bacteria and fungi loads on the chiropractic treatment tables. These duplicate samples were taken from the head piece and the thoraco-abdominal sections of the treatment tables. As mentioned before, there is no significant difference between the total bacteria and total fungi microbial loads on the treatment tables. However, when monitoring the microbial loads on the chiropractic treatment tables, it is important to monitor the head piece and thoraco-abdominal sections separately to determine where any likely source of infection may be transmitted and what sort of transmission may occur.

Table 4.8 Comparison between bacteria and fungi data on the head piece and thoraco-abdominal section of the chiropractic treatment tables.

			p-value (HP vs TAS)	
			Bacteria	Fungi
Head Piece (HP)			**0.025**	**0.389**
	Bacteria (B)	**Fungi (F)**		
Median	16	19		
Interquartile Range (IQR)	33	23		
p-value (B vs F)	**0.866**			
Thoraco-abdominal section (TAS)				
	Bacteria (B)	**Fungi (F)**		
Median	14	20		
Interquartile Range (IQR)	26	23		
p-value (B vs F)	**0.005**			

When comparing the treatment table surfaces (**Table 4.8**), there were significant statistical differences in bacterial microbial loads (CFU/25cm^2) on these surfaces. Bacterial microbial loads were greater on the head piece

(Md = 16, IQR = 33) than on the thoraco-abdominal section (Md = 14, IQR = 26), with a **p-value = 0.025**. Another significant statistical difference is noted on microbial loads (CFU/25cm^2) between bacteria and fungi on the thoracoabdominal section of the treatment table (**p-value = 0.005**), there seems to be higher counts of fungi (Md = 20, IQR = 23) than bacteria (Md = 14, IQR = 26) on this surface. These results suggest that bacteria transmission may occur directly from the head piece to the patients face integument and mucous membranes more so than indirect contact from contamination of hands with subsequent hand-to-mouth, hand-to-eye and hand-to-nose transfer.

Fungi transmission to the patients face integument and mucous membranes may occur from indirect contact from contamination of hands – from the treatment table surface of the thoraco-abdominal section where patients use their hands to lift themselves off of the table, or from patients clothes due to contamination of fungi when patient is laying on the thoraco-abdominal section – with subsequent hand-to-mouth, hand-to-eye and hand-to-nose transfer more so than contact with the head piece. However, if the patient is not wearing clothing over the torso or no protective barrier is used on the thoraco-abdominal section, fungi will be transmitted directly from this treatment table surface to the skin of the patient's torso. Fungal spores in the treatment room may also be directly inhaled. These conclusions demonstrate the importance of good hygiene practices between both the chiropractic intern and patient. The treatment table should be disinfected, protective barriers should be used, and hand hygiene from both the patient and chiropractic intern should be of utmost importance.

5.2.6. Possible Factors affecting increased microbial loads of bacteria and fungi on chiropractic treatment tables.

a) Hygiene Control

It is important to remember that one of the major contributing factors to increased microbial loads is poor cleaning and disinfectant control practices

of the UJ chiropractic interns. Other factors that should not be overlooked are the factors affecting the efficacy of the disinfectant (**Table 2.1**). Studying the *Levels of Hygiene* for bacteria and fungi separately, there is a difference in the frequency of samples with microbial counts that are within adequate, fair and inadequate levels between bacteria and fungi (**Figure 4.6**). Bacteria had more samples with microbial counts below 10 CFU/25cm^2 (39% within adequate levels of hygiene). Whereas, fungi had fewer samples with microbial counts below 10 CFU/25cm^2 (27% within adequate levels of hygiene).

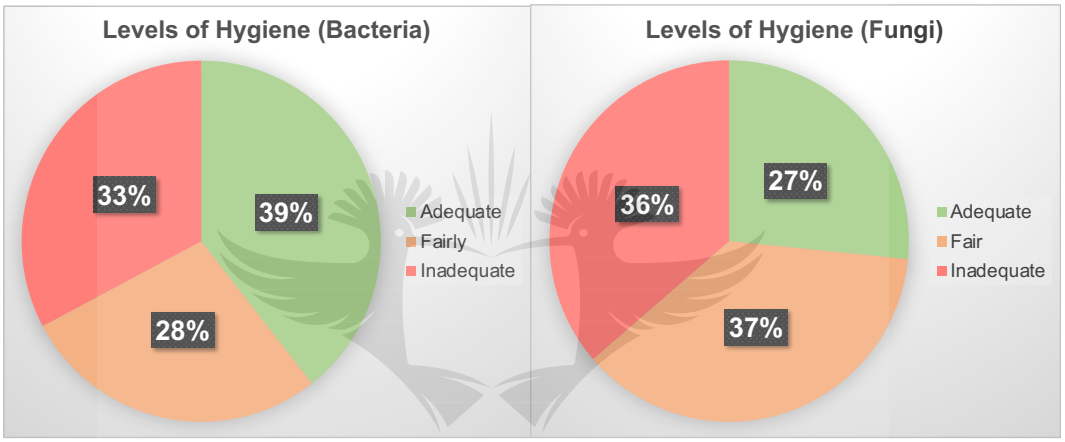

Figure 4.6 Comparison between bacterial and fungal levels of hygiene

However, when disinfection procedures are performed on the chiropractic treatment tables, and the disinfectant is effective, other factors are affecting the recontamination of bacteria and fungi on the treatment tables. Identifying these factors and implementing strategies to control them is important and will help with overall hygiene control within the UJ chiropractic training clinic.

Two crucial factors that are considered in this study are environmental factors and direct or indirect contact on treatment tables from patients or chiropractic students who may be colonised or infected.

b) Environmental factors

Environmental factors play a role in both bacterial and fungal survival, growth, colonisation and transmission. However, it is hypothesised that fungi are influenced greater by the environmental factors than that of bacteria. The eight weeks of sampling occurred in the warmest and most wet and humid summer months (December to February) in South Africa (Zijlma, 2019). Fungi that are abundant outdoors may vary considerably from one climate to another. In tropical and subtropical places where both heat and moisture are present, some fungi tend to be more abundant, and the incidence of fungal infections (especially sinus infections) tend to be higher in these areas (Burge, 2016). Therefore, during these months of sampling of the chiropractic treatment tables, there were higher levels of outdoor fungi. Furthermore, it is important to mention that there is no central airflow or air-conditioning systems in the chiropractic training clinic and chiropractic interns have to open windows to try to allow for airflow and subsequent cooling of indoor temperatures. However, this allows for the inflow of outdoor airborne fungi inside through the open windows with subsequent increases in indoor surface fungi microbial loads.

To study the effects that the environmental conditions have of microbial loads on chiropractic treatment tables, a comparison was made between the inside (central) and outside (peripheral) treatment rooms (**Figure 4.1**). The outside treatment rooms are located on the outer perimeters of the clinic building, where there are open areas with the movement of vehicles, people, and the presence of vegetative gardens. Natural airflow is also greater from these open areas. The inside treatments are located on the inner perimeters of the clinic building where is there is a courtyard. The inside rooms do have windows that get opened during summer seasons. However, the courtyard is non-vegetative, has little to no movement of people, and is with limited airflow. It is therefore hypothesised that the outside rooms have higher levels of fungal microbial contamination than the

indoor rooms. This will directly relate to the effect that the environment has on microbial loads on chiropractic treatment tables.

A Mann-Whitney U Test revealed a significant difference between the total fungal counts on the chiropractic treatment table surfaces between the outside (peripheral) treatment rooms (Md = 20, IQR = 23) and the inside (central) treatment rooms (Md = 17.5, IQR = 19), with a **p-value = 0.041**. Therefore, the environment is a factor affecting the survival, growth, colonisation and transmission of fungi microorganisms, as the outside treatment rooms have higher levels of fungi than the inside treatment rooms.

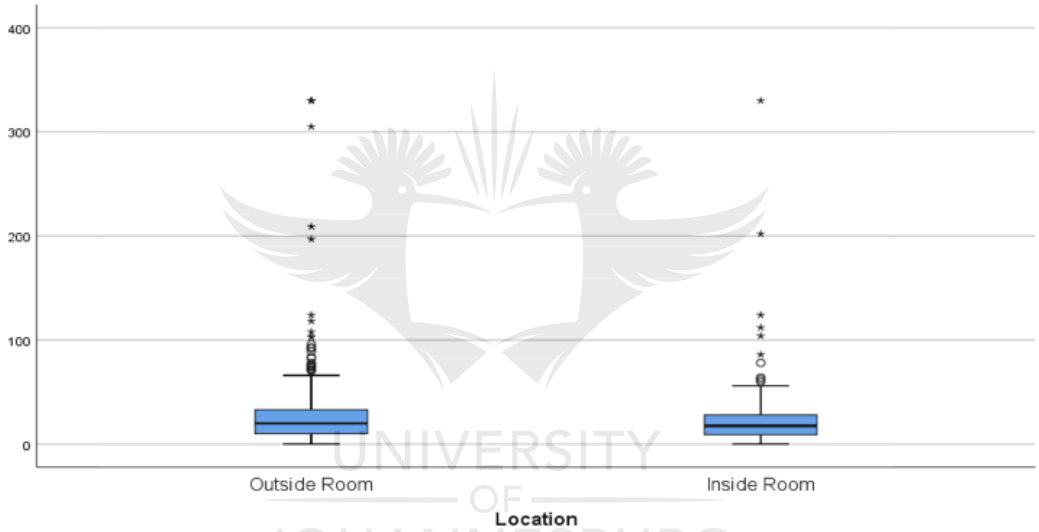

Figure 4.7 Comparison between Fungi counts on samples taken from chiropractic treatment tables in outside (peripheral) treatment rooms and inside (central) treatment rooms in the Uj Chiropractic training clinic to determine the environmental role as a factor for fungal survival, growth, colonisation and transmission.

A Mann-Whitney U Test revealed no significant difference between the total bacterial counts on the chiropractic treatment table surfaces between the outside (peripheral) treatment rooms (Md = 16, IQR = 30) and the inside (central) treatment rooms (Md = 14, IQR = 27), with a **p-value = 0.337**. It can be assumed, the environmental factors influence fungi survival, growth,

colonisation and transmission more so than bacteria. To what extent does the influence that environmental factors have on bacterial survival, growth, colonisation and transmission on chiropractic treatment table surfaces is unknown and further research is required to determine this.

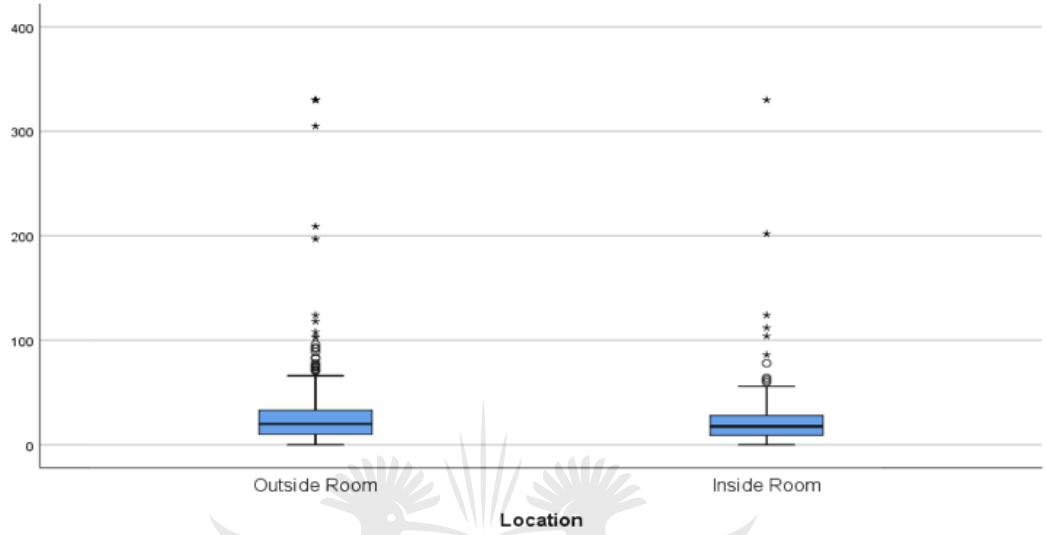

Figure 4.8 Comparison between bacterial counts on samples taken from chiropractic treatment tables in outside (peripheral) treatment rooms and inside (central) treatment rooms in the UJ Chiropractic training clinic to determine the environmental role as a factor for bacterial survival, growth, colonisation and transmission.

Another observation identified when studying the data between fungi and bacteria that confirms that the environment influences fungal loads more than bacterial loads on treatment tables, is the significant statistical difference in fungal counts from samples that were taken on Mondays and Thursdays (**Figure 4.9**), **p-value = 0.000**. There were no significant differences in bacterial microbial loads between the two days (**Figure 4.10**), with a **p-value = 0.356**. Fungi samples on Mondays had greater counts CFU/25cm^2 (Md = 24, IQR = 20) than fungal microbial samples taken on Thursdays (Md = 15, IQR = 21). This difference could be because over the weekend the clinic is closed and this extended time could allow for fungal spores to deposit onto the surfaces of the treatment tables and allow for the

growth and colonisation to occur without interruption of the treatment room environment.

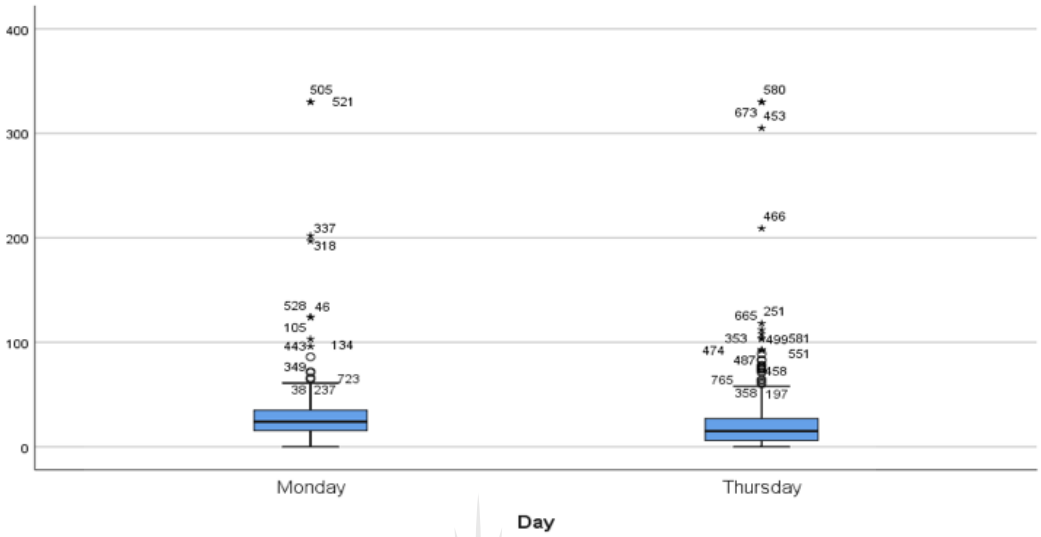

Figure 4.9 Comparison between fungi counts on samples taken from chiropractic treatment tables on Mondays and Thursdays.

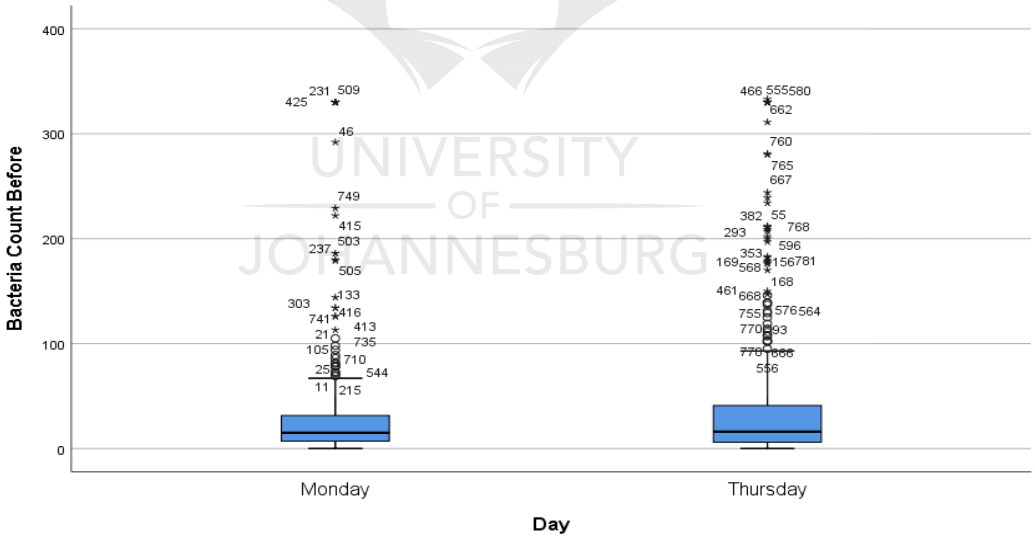

Figure 4.10 Comparison between bacterial counts on samples taken from chiropractic treatment tables on Mondays and Thursdays.

4.2.7. Types of pathogens identified on the chiropractic treatment tables that were not identified during Perdijk et al. (2017) study.

*Many bacterial and fungal pathogens have been identified on the surfaces of the chiropractic treatment tables (*Perdijk et al. 2017), these can also be reviewed in chapter two, in **Table 2.2** and **Table 2.6** in the current study. However, a few additional pathogens were identified using Vitek (bacteria) and sequencing (fungi) during this study.

Fusarium equiseti

Fusarium equiseti are typically soil-borne fungi species, common in warm temperate and subtropical areas (Palmero, de Cara, Iglesias, Gálvez & Tello, 2011). This coincides with the data that was studied to determine that fungi is influenced greater by the environment than bacteria. This is also a reason why this fungi species was not identified in Perdijk et al. (2017) study as it was done in the winter season of South Africa which is dry and cold. *Fusarium* species are reported as etiologic microbes of opportunistic infections in humans. These infections are usually superficial mycoses, deep tissues and disseminated infections, especially in patients with an underlying immunosuppressive condition. The characteristic signs of these infections are disseminated skin nodules, fungemia, multiorgan involvement and myalgia (Jain, Gupta, Misra, Gaur, Bajpai & Issar, 2011).

Bacillus spp.

Bacillus spp. are aerobic spore forming rods, they are gram positive or gram variable bacteria. Except for few species the large majority have no pathogenic potential. They are widely distributed in the environment and are usually found in soil, decaying organic matter, dust, vegetables, water, and

some species are part of the normal human flora. In hospitals, infection outbreaks from *Bacillus spp.* have been traced back to contaminated ventilator equipment and hospital linen. Infections caused by Bacillus spp. include food poisoning, localized infections related to trauma (usually ocular infections), deep tissue infections, and systemic infections such as meningitis, endocarditis, osteomyelitis, and bacteraemia (Tuazon, 2017).

Globicatella sanguinis

Globicatella sanguinis appears to cause sporadic disease occurring more often in older females, and it has been noted to colonise the skin and form part of the urogenital or lower gastrointestinal microbiome, with potential to cause disease in susceptible hosts. It is a Gram positive bacteria that is known to cause infections of the bloodstream (bacteraemia), CNS (meningitis), and urinary tract, and also known to cause osteoarticular infections in humans. It represents a rare and emerging pathogen worthy of careful attention and further examination (Miller, Buckwalter, Henry, Wu, Maloney, Abraham, Hartman, Brause, Whittier, Walsh & Schuetz, 2017).

Staphylococcus cohnii

Although *coagulase-negative staphylococcal* species are frequently isolated from blood cultures, *Staphylococcus cohnii* is rarely responsible for human systemic infections. It has a low pathogenic potential to cause severe illness (Basaglia, Moras, Bearz, Scalone & Paoli, 2003). *Staphylococcal cohnii* is known colonise on human skin and has been also isolated from opportunistic infections in patients with immunosuppressive disorders. At the same time, is a species that exists numerously in the hospital environment (Szewczyk, Nowak, Cieślikowski & Różalska, 2011).

Exiguobacterium sp.

Bacterial species belonging to the genus *Exiguobacterium* are Gram-positive bacilli, and rarely associated with human infections. It has been

distributed extensively and have been isolated from sources, including water, the rhizosphere of plants, and the environment of food processing plants. As documented, most infections due to *Exiguobacterium spp.* had underlying diseases and immunosuppression, such as liver cirrhosis, intravenous drug abuse and multiple myeloma. However, there is a case of a generally healthy patient with type II diabetes, who had severe community-acquired pneumonia and bacteraemia due to possible inhalation of the microorganism. Therefore, *Exiguobacterium sp.* may be a potential risk to patients who are healthy and not just immunocompromised patients visitng the UJ chiropractic training clinic (Chen, Wang, Zhou, Wu, Li, Cui & Lu, 2017).

4.3. Results Summation

It would be prudent for the chiropractic community to pay closer attention to the possibility that chiropractic treatment tables to serve as a potential source of community-acquired infections and to mitigate the risk of spread of these pathogens in the academic and clinical settings. It is recommended that the UJ chiropractic training clinic needs to implement a proper hygiene control protocol with strict adherence and compliance by all staff and students to reduce the probability of infection transmission. It is also important to implement hygiene monitoring systems, to monitor both the hygiene practices of the clinic staff and also identify possible pathogenic microbes on the treatment table surfaces or within the clinic environment.

Chapter Five – Conclusion and Recommendations

5.1. Introduction

Prevention of chiropractic treatment table contamination and infection transmission is a multifaceted task for a chiropractic intern and other cleaning staff. A number of essential protocols and guidelines are required for the purpose of setting standards for hygiene control in the DFC Chiropractic Clinic at the University of Johannesburg. The findings of this study suggests that although there is an improvement in the chiropractic treatment tables, there are not currently adequality cleaned or disinfected. This has lead to an increased chiropractic treatment table contamination and possible further risk for associated infection transmission. It is for this reason that these guidelines and protocols for hygiene control are therefore developed and proposed (**Figure 5.1**).

All sampled surfaces on the chiropractic treatment tables carried both bacterial and fungal microorganisms. Although, most of these were harmless skin bacteria and/or environmental fungi, to what extent they cause infection is unknown. However, with precise environmental condition factors and with immunosuppressed or immunocompromised patients, these microorganisms may pose a direct threat to the patient and indirectly to the community. It is therefore necessary to take all the precautionary hygiene control measures when working in the healthcare sector.

5.2. A Proposed Guideline of Hygiene Control Procedures for Chiropractic Practitioners and Clinics.

The following hygiene control procedures is proposed for implementation into the Chiropractic clinic and comprises five steps outline below.

1. **Assessment**

Before a shift begins, the Chiropractic Practitioner (CP) or Chiropractic Intern (CI) should assess the room to determine what equipment or items need replacing – these items include paper towels, hand sanitisers, bed covers and emptying of the sharps and waste bins. The CP or CI should also assess what equipment or surfaces require cleaning and disinfection – these surfaces should be the Chiropractic Treatment Table, desk, door handles and chairs (Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control (PIDAC-IPC), 2018).

2. **Preparation**

Gather all supplies and equipment required for room cleaning before starting. Put on additional personal protective equipment (PPE) such as latex gloves if required to avoid exposure to blood, body fluids or other hazardous cleaning chemicals.

3. **Cleaning and Disinfection**

The CP or CI should collect and remove waste and replace soiled bed-covers and sharps bins. This should then be followed by cleaning surfaces with hot water and detergent – this removes soil, dust, and debris that decreases substantial amounts of microorganisms on surfaces, and allow for an increase in the efficacy of the disinfectant (Rutala and Weber, 2008). The CSI should then apply disinfectant to surfaces listed in 1 – it is important to follow the instructions on the label of the EPA approved disinfectant.

The disinfectant should be left to settle on the surface for the stipulated time (usually 5 to 10 minutes) as indicated on the label of the disinfectant (PIDAC-IPC), 2018). The disinfectant should be spread evenly with a paper towel and left to air dry or wiped off with a tissue paper after the stipulated time (Evans et al. 2009). The tissue paper should then be placed in the waste bin. It is important to understand that some disinfectants are corrosive

to materials and with over exposure, may be a health hazard. Therefore, a less aggressive disinfectant such as Isopropyl alcohol 70% should be cycled with the recommended disinfectant (Eissa, Naby & Beshir, 2014). This will allow for effective reduction of microorganisms and maintenance of the surface of the chiropractic treatment table.

4. Routine Practices and Precautions

Hand hygiene should be performed routinely before entering, and after exiting the treatment room, including before and after treating the patient (Boyce and Pittet, 2002). It is highlighted that inadequate hand hygiene before and after entering or exiting a patient treatment room may result in cross-transmission of pathogens and patient colonization or infection (Russotto, Cortegiani, Raineri & Giarratano, 2015). The Chiropractic treatment table surfaces must be routinely cleaned and disinfected between treatments of patients (Rutala and Weber, 2008). The Chiropractic Treatment Table head-piece must be covered with a paper towel as this provides a barrier between the patient and the surface preventing the transmission of microorganisms (Perdijk et al. 2017).

5. Patient Education

It is recommended that the CI should educate their patients about personal hygiene, and especially the importance of hand hygiene. Hand hygiene is considered an essential practice for reducing the risk of transmission of infection among patients and health care personnel (Boyce and Pittet, 2002).

6. Conclusion

These guidelines, written for Chiropractic Interns and Chiropractic Practitioners contains evidence-based recommendations for the prevention of infection transmission and community-acquired infections.

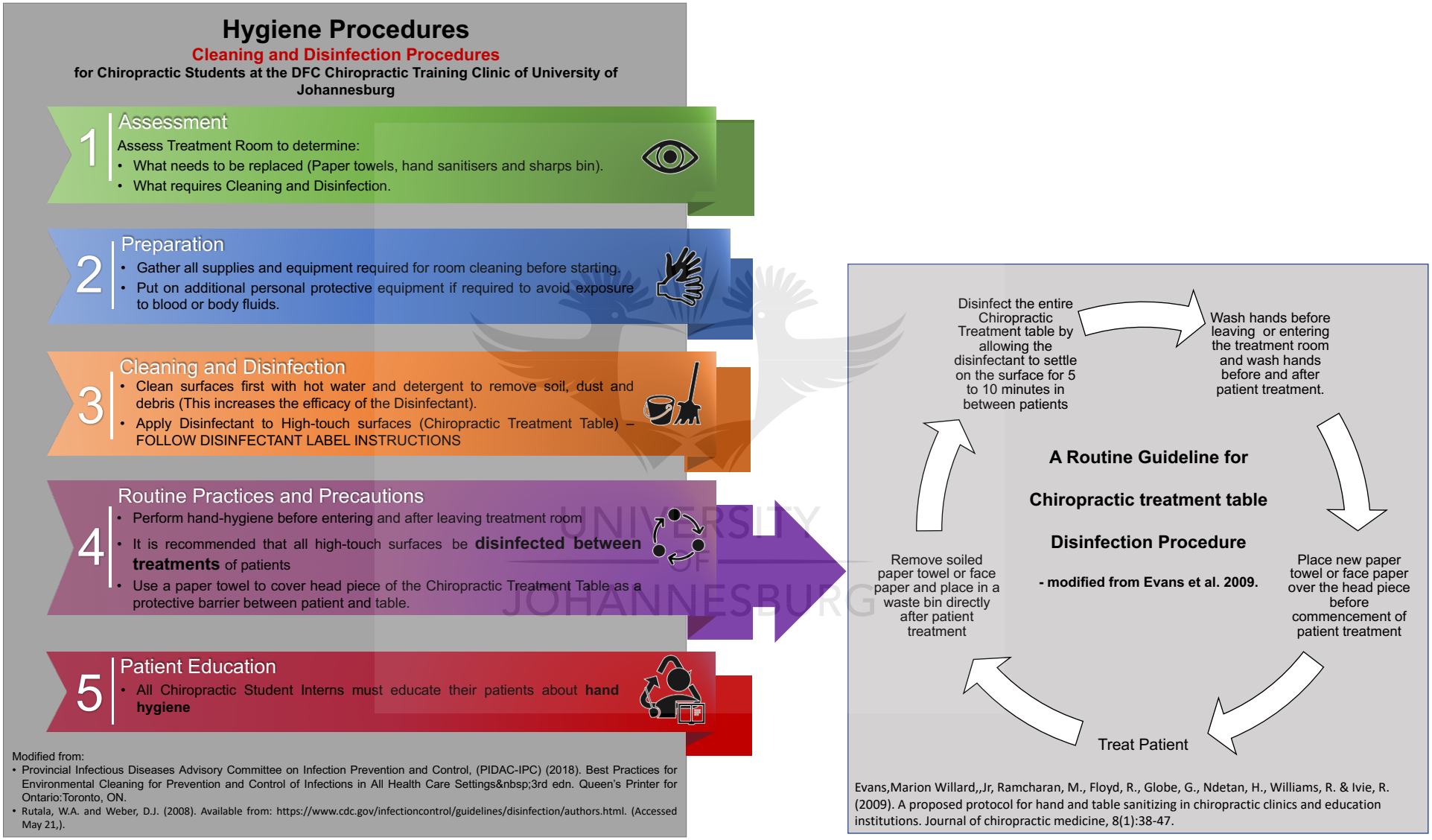

Figure 5.1 A Proposed Guideline of Hygiene Control Procedures for Chiropractic Practitioners and Clinics

5.3. Limitations

The limitations included the following:

1) The growth depends on the requirements of each individual bacterial and fungi. It is therefore possible that a few unidentified microorganisms sampled, may not have developed on the agar plates. This is due to the fact that some bacteria and fungi have different growth factor requirements (**Table 2.5**).

2) Because there is no assigned room per student, students therefore rotate rooms when consulting, making it difficult to locate the exact source of contamination.

5.4. Recommendations

Further possible monitoring investigations should focus on:

1. Faster and more cost effective methods for monitoring surface hygiene within the clinic environment. These faster methods will allow for quick feedback and help prevent further contamination and possible infection transmission.
2. Investigations should give feedback regarding the effectiveness of the guidelines that are recommended in this study (**Figure 5.1**). Input and feedback from other clinical training institutions and private healthcare facilities should also be investigated.
3. Studies that investigate if the guidelines and protocols are useful for chiropractors and patients.
4. To what extent does the influence that environmental factors have on bacterial survival, growth, colonisation and transmission on chiropractic treatment table surfaces is unknown and further research is required to determine this.

5.5. Conclusion

Overall, the information gathered by this study both supports and emphasizes the need for an effective disinfection protocol for the prevention of bacterial and fungal build-up on the chiropractic treatment tables at the UJ chiropractic-teaching clinic.

Chiropractic clinics and teaching or training facilities should consider adoption of these or similar measures or protocols that are proposed in this study, and disseminate them to other training or teaching institutions, and private practitioners.

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Appendices

Appendix A – TRYPTIC SOYA AGAR WITH POLYSORBATE 80 AND LECITHIN

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

51414 Tryptic Soya Agar with Polysorbate 80 and Lecithin (Microbial Content Test Agar; Tryptone Soya Agar with Polysorbate 80 and Lecithin; TSA with Polysorbate 80 and Lecithin; CASO Agar with Polysorbate 80 and Lecithin; Soybean Casein digest Agar with Polysorbate 80 and Lecithin)

Tryptone Soya Agar with Polysorbate 80 and Lecithin is used for determining efficiency of sanitization of containers, equipment surfaces, water miscible cosmetics etc.

Composition:

Ingredients	Grams/Litre
Peptone from casein	15.0
Papaic digest of soyabean meal	5.0
Sodium chloride	5.0
Polysorbate 80 (Tween 80)	5.0
Lecithin	0.7
Agar	15.0

Final pH 7.3 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance: Light yellow coloured, homogeneous, free flowing powder.
Colour and Clarity: Light to medium amber coloured, slightly opalescent gel forms in petri plates.

Directions:
Suspend 45.7 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 12 to 15 lbs pressure (118°C - 121°C) for 15 minutes. Mix well and pour in Petri dishes or RODAC (Replicate Organism Detection and Counting) plates (~ 17 ml).

Principle and Interpretation:
Peptone from casein and papaic digest of soyabean meal act as a source of nitrogen, carbon and amino acids. Sodium chloride maintains the osmotic balance. Lecithin and Polysorbate 80 neutralize many residual desinfectants. Polysorbate 80 inactivate phenols, hexachlorophene and formalin, while lecithin neutralize quaternary ammonium compounds (1-3). Agar is the solidifying agent.

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Growth with disinfectant*
Escherichia coli (25922)	+++	++
Pseudomonas aeruginosa (27853)	+++	++
Staphylococcus aureus (25923)	+++	++

* depends on concentration of quaternary ammonium compounds

References:

1. R.I. Quisno, W. Gibby, M.J. Foter, A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts, Am. J. Pharm., 118, 320-323 (1946)
2. A.L. Jr. Erlandson, C.A. Lawrence, Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants, Science, 118, 274-276 (1953)
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4. E.H. Lennette, E.H. Spaulding, J.P. Truant, Manual of Clinical Microbiology. 2nd ed. Washington D.C.: American Society for Microbiology (1974)

Appendix B – SURFACE SAMPLING WITH COUNT-TACT® RANG

SURFACE

Detection and Enumeration of Bacteria, Yeasts and Molds in surface samples of healthcare facilities

Quantitative Method: Sampling by contact agar on plane surfaces

PREPARATION

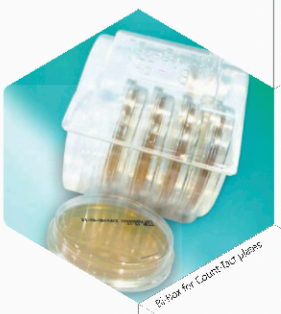
Surface sampling by contact application with Count-Tact® range

FOR UNPROTECTED AREAS

- For Total Flora: **Count-Tact Agar (ref. 43501 - 20 x 65 mm plates)**
- For Yeasts & Molds: **Count-Tact Sabouraud chloramphenicol (ref. 43580 - 20 x 65 mm plates)**

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

Bi-Box for Count-Tact plates (ref. 96301 - 1x10 units)



FOR PROTECTED AREAS (e.g. ISOLATOR)

- For Total Flora: **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

bioMérieux S.A.
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Appendix C – HDC AND REC ETHICS COMMITTEE LETTERS

FACULTY OF HEALTH SCIENCES
RESEARCH ETHICS COMMITTEE

NHREC Registration: REC 241112-035

ETHICAL CLEARANCE LETTER
(RECX 2.0)

Student/Researcher Name	Kingham, M	Student Number	201380218
Supervisor Name	Prof TG Barnard	Co-Supervisor Name	Dr C Yelverton; Dr A Singh
Department	Chiropractic		
Qualification	367		
Research Title	Monitoring Treatment Table Hygiene in a Chiropractic Training Clinic		
Date	25September 2018	Clearance Number	REC-01-120-2018

Approval of the research proposal with details given above is granted, subject to any conditions under 1 below, and is valid until 31 January 2019.

1. **Conditions:**
None

2. **Renewal:**
It is required that this ethical clearance is renewed annually, within two weeks of the date indicated above. Renewal must be done using the Ethical Clearance Renewal Form (REC 10.0), to be completed and submitted to the Faculty Administration office. *See Section 12 of the REC Standard Operating Procedures.*

3. **Amendments:**
Any envisaged amendments to the research proposal that has been granted ethical clearance must be submitted to the REC using the Research Proposal Amendment Application Form (REC 8.0) prior to the research being amended. Amendments to research may only be carried out once a new ethical clearance letter is issued. *See Section 13 of the REC Standard Operating Procedures.*

4. **Adverse Events, Deviations or Non-compliance:**
Adverse events, research proposal deviations or non-compliance must be reported within the stipulated time-frames using the Adverse Event Reporting Form (REC 9.0). *See Section 14 of the REC Standard Operating Procedures.*

The REC wishes you all the best for your studies.

Yours sincerely.

Prof. Christopher Stein
Chairperson: REC
Tel: 011 559 6564
Email: cstein@uj.ac.za

FACULTY OF HEALTH SCIENCES

HIGHER DEGREES COMMITTEE

HDC-01-108- 2018

30 October 2018

TO WHOM IT MAY CONCERN:

STUDENT: KINGHAM,MC
STUDENT NUMBER: 201380218

TITLE OF RESEARCH PROJECT: Monitoring Treatment Table Hygiene in a Chiropractic Training Clinic

DEPARTMENT OR PROGRAMME: CHIROPRACTIC

SUPERVISOR: Prof TG Barnard **CO-SUPERVISOR:** Dr C Yelverton
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 H Abrahamse
Acting Chair: Faculty of Health Sciences HDC
Tel: 011 559 6550
Email: habrahamsc@uj.ac.za

Appendix D – TURNITIN REPORT

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

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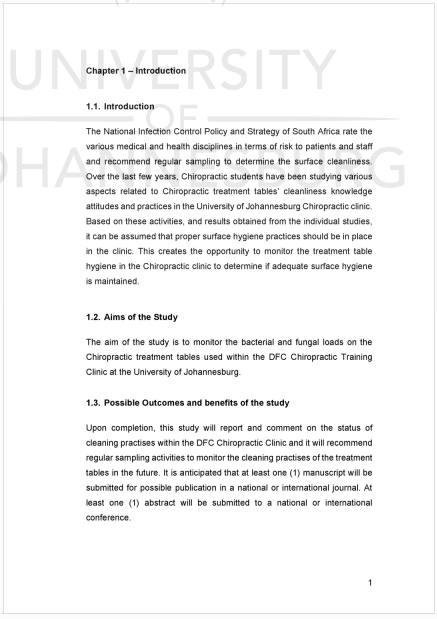

Chapter 1 – Introduction

1.1. Introduction

The National Infection Control Policy and Strategy of South Africa rate the various medical and health disciplines in terms of risk to patients and staff and recommend regular sampling to determine the surface cleanliness. Over the last few years, Chiropractic students have been studying various aspects related to Chiropractic treatment tables' cleanliness knowledge attitudes and practices in the University of Johannesburg Chiropractic clinic. Based on these activities, and results obtained from the individual studies, it can be assumed that proper surface hygiene practices should be in place in the clinic. This creates the opportunity to monitor the treatment table hygiene in the Chiropractic clinic to determine if adequate surface hygiene is maintained.

1.2. Aims of the Study

The aim of the study is to monitor the bacterial and fungal loads on the Chiropractic treatment tables used within the DFC Chiropractic Training Clinic at the University of Johannesburg.

1.3. Possible Outcomes and benefits of the study

Upon completion, this study will report and comment on the status of cleaning practises within the DFC Chiropractic Clinic and it will recommend regular sampling activities to monitor the cleaning practises of the treatment tables in the future. It is anticipated that at least one (1) manuscript will be submitted for possible publication in a national or international journal. At least one (1) abstract will be submitted to a national or international conference.

1

Monitoring Treatment Table Hygiene in a Chiropractic Training Clinic

ORIGINALITY REPORT

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