

Origins and control of bacterial contamination during spinal manipulation

By

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I, Fariya Amod, declare that this dissertation is a representation of my own work in both conception and execution, except where assistance was sought and indicated via references.

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DEDICATION

I would like to dedicate this dissertation to:

My parents, Khalil and Farhana Amod, I need not look to the world's greatest icons for inspiration; I found them in my own home. Your success drives me each day to aspire to my dreams. Thank you for your guidance, support and encouragement throughout my entire student life.

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ABSTRACT

Origins and control of bacterial contamination during spinal manipulation

Background: Research has revealed that healthcare workers' hands serve as a source and vehicle for the transmission of micro-organisms within the healthcare sector, thus resulting in nosocomial infections, better known as healthcare-associated infections. The chiropractic profession is traditionally known as a hands-on profession, where the fundamental treatment protocol includes manual manipulation of the spine. In order to perform these procedures hand-to-patient interaction is required, resulting in skin-to-skin contact. Poor hand hygiene practice has been attributed by the World Health Organization as the primary cause for the spread of micro-organisms within the healthcare environment. Unwashed hands harbour microbes, thus increasing contamination levels and subjecting patients to these potential pathogens. This study aimed to determine the presence and transfer of bacterial contamination occurring during spinal manipulation, as well as analysing the efficacy of decontaminants used by chiropractic practitioners against the isolated bacteria cultivated.

Research design: The study was located in the quantitative experimental paradigm and conducted as a cross-sectional investigation.

Method: Samples were obtained from chiropractors' hands before and after spinal manipulation. Samples were then serially diluted, plated in duplicate (using the spread plate technique) and incubated for 24-48 hours at 37°C. Viable counts of colony forming units (CFUs) were then enumerated in order to verify the presence of bacteria on the chiropractors' hands, as well as to establish the direction of transfer occurring during spinal manipulation. Macroscopic and microscopic characteristics of each bacterial isolate were used to identify the bacteria cultivated. A modified Kirby Bauer technique was used to ascertain the efficacy of decontaminants commonly used by chiropractors, against the isolated bacteria obtained from their hands.

Results: Bacterial flora were present on 100% of the chiropractors hands both pre- and post-spinal manipulation. A mean of 16,456 (27,718) cfu/ml⁻¹ were enumerated from the samples collected from the chiropractors' hands during manipulation. A paired *t*-test

indicated a significant difference noted in the viable count of bacteria found on the chiropractors' hands before and after manipulation ($p<0.001$). A significant difference was observed in the viable count of bacteria post-manipulation (70%), as opposed to the pre-manipulation readings (30%). This was indicative of a higher rate of bacteria being transferred from the patient to the chiropractor during spinal manipulation. The majority of the microorganisms identified were either primary or opportunistic pathogens. Staphylococci were most prevalent in the pre-spinal manipulation readings accounting for 53% of the colonies, followed by micrococci with 39%, bacilli with 4%, *Staphylococcus aureus* with 3% and streptococci with 1%. *Pseudomonas* spp. were present but uncommon. *Escherichia coli* were not present on the chiropractors' hands in any of the samples obtained pre-manipulation. The post-manipulation readings constituted a high prevalence of micrococci accounting for 57% of the colonies, followed by staphylococci with 32%, *Pseudomonas* spp. with 5%, *E. coli* with 3%, *Staphylococcus aureus* with 2% and bacilli with 1%. Streptococci were present but uncommon. The decontaminants tested were most effective against gram-positive bacteria such as *Bacillus*, *Micrococcus*, *Staphylococcus*, *Staphylococcus aureus* and *Streptococcus*. The bacteria isolated were most susceptible to the D-Germ hand disinfectant, while the Dis-Chem instant hand sanitizer was the least effective decontaminant tested. Ciprofloxacin was the antibiotic used as a positive control. A significant difference was noted between the performance of the positive control and the decontaminants on the bacteria isolated.

Conclusions and recommendations: The study proved the presence of primary and opportunistic pathogens found on the chiropractors' hands. These included *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *E. coli*, *Streptococcus* spp. and *Staphylococcus aureus*. A higher rate of bacteria was observed being transferred from the patient to the chiropractor during spinal manipulation. The majority of the microorganisms identified were either primary or opportunist pathogens, thus predisposing both the patient and the chiropractor to potential infection. A significant difference was noted between the performance of the positive control and the decontaminants on the bacteria isolated. None of the decontaminants were as effective against the bacteria isolated as the positive control. It can therefore be deduced that each bacterial flora was not removed by some of the decontaminants currently used by chiropractic practitioners in practice.

Key concepts: Chiropractic, Bacterial flora, Bacterial contamination, Spinal manipulation, Healthcare-associated infections, Hand hygiene, Decontaminants, Disinfection, Quantitative research.

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GLOSSARY

Acquired Immune Deficiency Syndrome (AIDS): A life-threatening viral disease caused by the human immunodeficiency virus (HIV), where the T-lymphocytes cells are destroyed and opportunistic infections occur in immunocompromised patients (Alcamo 1994).

Acute: In reference to infections; a short-term, dramatic onset of infection, with a rapid recovery rate (Madigan, Martinko and Parker 2000).

Agar: A derivative of marine seaweed used as a solidifying agent in microbiological media (Alcamo 1994).

Antibiotic: A chemical agent produced by one organism that is harmful to other organisms (Madigan, Martinko and Parker 2000).

Antibiotic resistance: The acquired ability of a micro-organism to grow in the presence of an antibiotic, to which the micro-organism is usually sensitive (Madigan, Martinko and Parker 2000).

Antimicrobial: Harmful to micro-organisms by either killing or inhibiting growth (Madigan, Martinko and Parker 2000).

Aseptic technique: a manipulation of sterile instruments or culture media in such a way as to maintain sterility (Madigan, Martinko and Parker 2000).

Autoclave: A steriliser that destroys micro-organisms by high temperature using steam under pressure (Madigan, Martinko and Parker 2000).

Bacteria: All prokaryotes that are not members of the domain Archaea (Madigan, Martinko and Parker 2000).

Chiropractic: For the purpose of this study, chiropractic will be defined as per the World Federation of Chiropractic (2001): “a health profession specializing in the diagnosis, treatment and prevention of disorders of the musculoskeletal system and the effects of these disorders on the function of the nervous system and general health”.

Colony: A macroscopically visible population of cells growing on solid medium, arising from a single cell (Madigan, Martinko and Parker 2000).

Commensal bacteria: Micro-organisms that are usually found associated with healthy body tissue (Madigan, Martinko and Parker 2000).

Complementary and Alternative Medicine (CAM): A spectrum of medical treatment that falls beyond the scope of conventional medicine but forms part of the treatment of disease, including conservative care. Complementary and alternative medicine is a term used to describe health care practices and professions that are not integrated into the primary health care system of a country (Meeker and Haldeman 2002; Menke 2003; Redwood and Cleveland 2003; Haldeman 2005; Morgan 2005; Logtenberg 2009).

Culture: A particular strain or kind of organism growing in a laboratory medium (Madigan, Martinko and Parker 2000).

Culture medium: An aqueous solution of various nutrients suitable for the micro-organisms (Madigan, Martinko and Parker 2000).

Decontamination: Treatment that renders an object or inanimate surface safe to handle (Madigan, Martinko and Parker 2000).

Diagnosis: The recognition and classification of illness through the examination of symptoms.

Disease: An abnormality present in a person's physical or mental state that negatively affects their overall wellbeing resulting in the presentation of signs and symptoms (Madigan, Martinko and Parker 2000).

Disinfectant: An agent that kills micro-organisms but may also be harmful to human tissue (Madigan, Martinko and Parker 2000).

Gram-negative cell: A prokaryotic cell whose cell wall contains relatively little peptidoglycan but has an outer membrane composed of lipopolysaccharide, lipoprotein, and other complex macromolecules (Madigan, Martinko and Parker 2000).

Gram-positive cell: A prokaryotic cell whose cell wall consists chiefly of peptidoglycan and lacks the outer membrane of gram-negative cells (Madigan, Martinko and Parker 2000).

Hand hygiene: A process whereby hands are rid of micro-organisms, both commensal and pathogenic, by using soap (antimicrobial or non-antimicrobial) together with water, in order to cleanse the surface of the hands (Busari *et al.* 2012; Oluwole *et al.* 2013).

Healthcare-associated infection: “A healthcare-associated infection has been defined as an infection that occurs in a patient, within a healthcare facility, in whom the infection is not present or incubating at the time of admission or that is acquired during the stay in the healthcare facility, but arises within 48 hours after discharge” (World Health Organisation 2002; Moor and Ferguson 2006).

Host: An organism capable of supporting the growth of a virus or other parasite (Madigan, Martinko and Parker 2000).

Immunodeficiency: Having a dysfunctional or completely non-functional immune system (Madigan, Martinko and Parker 2000).

Incidence: In reference to disease transmission, the number of cases of the disease in a specific subset of the population (Madigan, Martinko and Parker 2000).

Infection: Growth of an organism within the body (Madigan, Martinko and Parker 2000).

Inoculum: Material used to initiate a microbial culture (Madigan, Martinko and Parker 2000).

Kirby Bauer test: An agar diffusion test used to determine the antibiotic concentration effective against a test organism (Alcamo 1994).

Manipulation/Adjustment: A technique utilized by chiropractors that involves the deliverance of a controlled force to a joint that is of high velocity and low amplitude in order to achieve a neurophysiological, therapeutic effect (World Health Organisation 2005).

Micrometre: One-millionth of a meter, or 10^{-6} m (abbreviated μm); the unit used for measuring micro-organisms (Madigan, Martinko and Parker 2000).

Micro-organism: A microscopic organism consisting of a single cell or cell cluster, also including the viruses (Madigan, Martinko and Parker 2000).

Musculoskeletal System: A combination of muscles, tendons, ligaments and fascia (soft tissue structures) and bones and joints and related structures within the body.

Nervous System: A physiological system in the body that manages internal body functions and both receives, interprets, and responds to stimuli. The nervous system is constituted of nerves, brain and spinal cord and sensory organs.

Nosocomial infection: Hospital-acquired infection (Madigan, Martinko and Parker 2000).

Pathogen: An organism able to inflict damage on a host it infects (Madigan, Martinko and Parker 2000).

Pathogenicity: The ability of a parasite to inflict damage on to the host (Madigan, Martinko and Parker 2000).

Pure culture: A culture containing a single kind of micro-organism (Madigan, Martinko and Parker 2000).

Sterile: Free of living organisms and viruses (Madigan, Martinko and Parker 2000).

Treatment: Medical care or therapy given to a patient for the purpose of achieving therapeutic effects.

Vehicle: Non-living source of pathogens that infect large numbers of individuals; common vehicles are food and water (Madigan, Martinko and Parker 2000).

Vertebral Subluxation: In chiropractic practice a vertebral subluxation refers to a state of change within the body whereby there is an altered neurological afferent input, which over time may lead to signs and symptoms and inability of adaption (Taylor *et al.* 2010). A subluxation results in a change of kinesiology, myopathy, neurology and histology eventually resulting in pathophysiology. Also known as a restriction or immobilization of a joint in a certain position that may have an effect on physiological function (World Health Organisation 2005).

Viable count: The number of living (viable cells) (Baveja 2005).

Virulence: Degree of pathogenicity of a parasite (Madigan, Martinko and Parker 2000).

ABBREVIATIONS USED IN THIS DISSERTATION

AHPCSA: Allied Health Professions Council of South Africa

CAM: Complimentary and Alternate Medicine

CASA: Chiropractic Association of South Africa

CDC: Centers for Disease Control and Prevention

CFUs: Colony Forming Units

HAI: Healthcare-associated infection

HIV/AIDS: Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome

MRSA: Methicillin Resistant *Staphylococcus aureus*

TSA: Tryptic Soy agar

TSB: Tryptic Soy broth

WFC: World Federation of Chiropractic

WHO: World Health Organization

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

The chiropractic profession is traditionally known as a hands-on profession, treating a variety of musculoskeletal conditions. The fundamental treatment protocol used by chiropractic practitioners includes manual manipulation of the spine, as well as extremity joints. In order to perform these procedures hand-to-patient interaction is required, resulting in skin-to-skin contact (Evans *et al.* 2009).

Literature has shown that healthcare workers' hands serve as a source and vehicle of transport in the proliferation of micro-organisms within the healthcare sector, thus resulting in nosocomial infections, better known as healthcare-associated infections (Allegranzi and Pittet 2009). "A healthcare-associated infection has been defined as an infection that occurs in a patient, within a healthcare facility, in whom the infection is not present or incubating at the time of admission or that is acquired during the stay in the healthcare facility, but arises within 48 hours after discharge" (World Health Organisation 2002; Moor and Ferguson 2006). According to Banfield and Kerr (2005), minimal research exists on the patient care interactions that occur between healthcare workers and their patient, and the propagation of infections. Borkow and Gabbay (2007) found that direct interaction between the skin of healthcare workers and their patients leads to contamination, with the most prevalent incidence of transmission occurring between an infected individual and a susceptible host.

Escherichia coli and *Staphylococcus aureus*, which are normal microflora occurring within the body, are known to be micro-organisms frequently associated with the spread of healthcare-associated infections (Struelens *et al.* 2004). These micro-organisms protect the host from the colonisation of pathogenic flora under normal circumstances, however should the host's immune system become compromised due to either disease or the administration of antibiotics these micro-organisms may result in infection (World Health Organisation 2002).

Poor hand hygiene practice has been attributed by the World Health Organisation (WHO) as the primary cause in the spread of micro-organisms within the healthcare environment

(De Alwis *et al.* 2012). Unwashed hands harbour microbes, thus increasing contamination levels and subjecting patients to these potential pathogens (Banfield and Kerr 2005; Pittet *et al.* 2006). Improved hand hygiene is effective for reducing and preventing the spread of antimicrobial resistance, infection transmission, cross-contamination and reducing healthcare-associated infections in both private and public hospital settings (Kac *et al.* 2005; Allegranzi and Pittet 2009; Rotter *et al.* 2009; Mehmood *et al.* 2014). There is a lack of literature available on healthcare-associated infections and hand hygiene practices within the chiropractic profession, and little or no research has been conducted within a chiropractic setting. This study therefore aimed to determine the origin of bacterial flora on the hands of chiropractic practitioners' in private practice pre- and post-spinal manipulation, as well as analysing the efficacy of decontaminants used by the practitioners against the isolated bacteria cultivated.

1.2 AIM

The aim of the study was to quantitatively determine the presence and transmission of bacterial contamination during spinal manipulation as well as the efficacy of control measures applied to hands among chiropractors in private practice within a South African setting.

1.3 OBJECTIVES AND HYPOTHESES

Objective One: To determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post- spinal manipulation of patients, by means of hand swabs, serial dilution and plate counts.

Hypothesis One: Micro-organisms will be present on the chiropractic practitioners' hands pre- and post-spinal manipulation.

Hypothesis Two: Micro-organisms will be transferred from patients to chiropractors during spinal manipulation.

Objective Two: To identify the bacterial flora present on the chiropractic practitioners' hands using conventional identification tests, biochemical profiling and test kit staining characteristics and morphology.

Hypothesis three: The majority of micro-organisms isolated during the sampling procedure will consist of potentially pathogenic species.

Objective Three: To determine the efficacy of decontaminants used by the chiropractic practitioners against isolated bacteria using a modified Kirby Bauer technique.

Hypothesis Four: A decontaminant used by the chiropractors will be effective against the majority of bacteria isolated from chiropractors hands.

1.4 RATIONALE

The chiropractic profession uses manipulation of the spine and extremity joints during treatment, thus resulting in skin-to-skin contact between the practitioner and the patient. Research has shown that healthcare workers' hands serve as a source and vehicle of transport in the proliferation of micro-organisms within the healthcare sector, thus resulting in nosocomial infections (Allegranzi and Pittet 2009). Poor hand hygiene practices have been attributed by the WHO as a primary cause in the spread of micro-organisms within the healthcare environment (De Alwis *et al.* 2012). Proper hand hygiene protocols have been deemed as the most effective procedure that should be implemented by healthcare workers in order to limit the contamination and cross-transmission of pathogens occurring between patients and healthcare workers within these healthcare facilities (Kac *et al.* 2005; Rotter *et al.* 2009).

South Africa is a third-world, developing country that has a high incidence of immunocompromised patients infected with HIV/AIDS, therefore even normal bacterial flora poses a threat to these susceptible patients (Johansson 2007; Kapp 2007; Harling, Ehrlich and Myer 2008; Logtenberg 2009). Minimal studies on hand hygiene practices exist within the chiropractic profession at an international level, with no research piloted in a South African setting that has evaluated these practices. The importance of conducting this study within a chiropractic setting will verify potential bacterial flora present on chiropractors' hands during spinal manipulation of patients and the route of transfer thereof. It will identify the bacterial isolates collected in order to ascertain the possible pathogenicity of these organisms. The efficacy of hand decontaminants frequently used

by chiropractic practitioners will also be tested. Should these decontaminants prove ineffective, then alternative techniques may be implemented with the aim of reducing contamination during chiropractic manipulation.

1.5 ASSUMPTIONS

The researcher assumed that it takes approximately the same amount of time for each chiropractic practitioner to perform one spinal adjustment. It was also assumed that no contamination occurred during the collection of samples from the chiropractor's hands and the transportation of these samples to the microbiology laboratory.

1.6 LIMITATIONS

- Samples were not obtained at exactly the same time each day as chiropractic practitioners mostly begin practice at varying times each day.
- Observational bias may have occurred as the chiropractic practitioners may have altered their daily hand-washing routine simply because they were aware of being observed, thus altering the microflora on their hands.
- Only some of the bacterial flora present on the chiropractic practitioners' hands were identified to a genus level due to cost constraints.
- Only bacteria were assayed for in this study.
- During the enumeration of colonies found on the Tryptone Soy agar plates, those plates containing colony forming units (CFUs) ranging between 30 and 300 were used to ensure reliability and validity.

1.7 THESIS LAYOUT

Chapter One includes a synopsis of the literature relevant to this research study, outlining the aim, objectives and rationale forming the basis of the study. It also provides a critical overview of the intrinsic limitations and assumptions of the study. An in-depth review

pertaining to the literature related to this study is presented in Chapter Two, followed by the methodology of the study in Chapter Three. Chapter Four presents and analyses the results obtained, followed by a critical discussion of these results in keeping with the original aim and objectives of the study. Chapter Five proposes recommendations drawn from the outcomes of the study.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This literature review will provide an overview of the origins and control of bacterial contamination during spinal manipulation. It will comprehensively outline six topics that recurrently emerge throughout the literature. These topics include a brief background of the chiropractic profession, together with its principles and practice. It will highlight the presence of bacterial flora found on humans and how this may result in healthcare-associated infections. Assessing the mode of transmission of the micro-organisms and risk of contamination therewith will also be presented. It will further analyse the various intervention strategies employed against these micro-organisms in diverse healthcare settings. The sources utilized in this literature review were accessed via the DUT Summon search, Science Direct, Google and Google Scholar meta-search engines.

2.2 CHIROPRACTIC PRINCIPLES AND PRACTICE

The term chiropractic was derived from the Greek stem words, meaning 'done by hand' (Haldeman 2005). Daniel David Palmer, who is recognized as the father of chiropractic within the profession, defined it as being "a science of healing without drugs" (Ernst 2008). The inception of chiropractic dates back to 18 September 1895 when the first official spinal manipulation was performed (Meeker and Haldeman 2002; Haldeman 2005; Ernst 2008; Chiropractic Association of South Africa 2016). Chiropractic is thus defined by the WHO as,

"...a healthcare profession concerned with the diagnosis, treatment and prevention of disorders of the neuromusculoskeletal system and the effects of these disorders on general health. There is an emphasis on manual techniques, including joint adjustment and/or manipulation with a particular focus on subluxations."

This definition is in keeping with legislature supplied by the chiropractic profession's governing bodies the (World Federation of Chiropractic 2001; Chiropractic Association of South Africa 2016; Allied Health Professions Council of South Africa 2016 Act 63 of 1982).

Chiropractic adheres to a bio-psychosocial paradigm of health, which incorporates a holistic approach to the treatment of patients by utilizing the close interrelationship of the spine, the nervous system and the musculoskeletal system of the body (Meeker and Haldeman 2002; Chapman-Smith 2008; Ernst 2008). A subluxation is defined as dysfunctional movement within a joint capsule due to sprain or strain of the joint (Gatterman 1995). The subluxation adversely affects health as a result of neurological and vascular involvement (Gatterman 1995; Meeker and Haldeman 2002; Haldeman 2005; Chapman-Smith 2008; Ernst 2008). Subluxations can be rectified through spinal manipulations (Ernst 2008). Spinal manipulations, or adjustments, function by moving the joint found between two successive vertebrae by exerting a load or force on the joint. This allows it to move beyond its normal physiological range of motion into what is termed a 'paraphysiological' space, thus reinstating the normal physiological range of motion within the joint (Meeker and Haldeman 2002; Redwood and Cleveland 2003; Ernst 2008).

Chiropractic is currently the most popular and frequented complementary and alternative medicine (CAM) (Meeker and Haldeman 2002; Haldeman 2005; Logtenberg 2009). Although CAM is deemed as an alternative form of healthcare that is not integrated within mainstream medicine, it still has a functional role in the diagnosis of conditions as well as the treatment thereof (Blum *et al.* 2008). As declared by Meeker and Halderman (2002), however, the chiropractic profession has reached a crossroads between alternative and mainstream medicine. Some chiropractic practitioners have refused to be labelled as complementary and alternative therapists, as they see themselves as primary healthcare providers (Meeker and Haldeman 2002). Leach (2004) proffered that chiropractic has ascended to the third most frequented primary healthcare profession in the world following medicine and dentistry. Chiropractic practitioners are renowned for specialising in spinal health and other musculoskeletal conditions in multiple countries (Clare 2005; World Federation of Chiropractic 2016). Patients most commonly seek chiropractic treatment for musculoskeletal conditions with the majority being for lower back pain (approximately 60%); neck pain (20%); shoulder complains; extremity and arthritic pain; as well as headaches which include migraine-type headaches (10%). The remaining 10% of consultations are as a result of conditions provoked or triggered by neuromusculoskeletal disorders (Ernst 2008; Evans, Williams and Perko 2008). An estimated 11-19% of conditions treated by chiropractors are attributable to non-musculoskeletal causes (Ernst 2008; Logtenberg 2009).

According to the Allied Health Professions Council of South Africa (AHPCSA) (2016) the chiropractic scope of practice includes

...the treatment or prevention of any physical defect, illness or efficiency related to spinal, pelvic, spino-visceral and general neuromusculoskeletal conditions in any person by means of manipulation or adjustment, electrotherapy, exercise therapy, hydrotherapy, traction therapy, thermal therapy, vibration and immobilization therapy, neuro-muscular reflex therapy, massage therapy, acupuncture or acupressure and remedies, dietary advice or dietary supplementation.

Chiropractors also apply additional treatment including myofascial techniques, therapeutic exercises and rehabilitation techniques (Meeker and Haldeman 2002). Many of these treatment protocols require skin-to-skin contact between the practitioner and the patient. (Allegranzi 2009; Evans 2009). As Duse (2005) explained, South Africa has inadequate systems in place to identify the rate of healthcare-associated infections occurring during treatment, which is the first step toward establishing adequate infection control parameters. The epidemic of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is widespread in South Africa, with not only these patients serving as optimal hosts for healthcare-associated infections but also rendering them susceptible to normal bodily bacterial flora (Duse 2005; Johansson 2007; Logtenberg 2009).

2.3 PRESENCE OF BACTERIAL FLORA ON HUMANS

The human skin is constantly exposed to a myriad of bacterial flora on a daily basis (Lange-Asschenfeldt *et al.* 2011). Our skin is not only an organ, it is an organ with a multifaceted microbiological ecosystem where an interaction between micro-organisms and the host occurs (Fredericks 2001). The skin is colonized by bacterial flora that are categorized into resident, transient and infectious bacteria located on the epidermis (Rotter 2009; Lange-Asschenfeldt *et al.* 2011; Oluwole *et al.* 2013). The resident bacterial flora population, which consists of gram-positive *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Propionibacterium* spp. and gram negative *Acinetobacter* spp., vary based on their location in the body (Leyden *et al.* 1987; Roth and James 1988; Chiller, Selkin and Murakawa 2001; Lange-Asschenfeldt *et al.* 2011). Transient bacterial flora such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* tend to present only in atypical conditions (Charbonneau, Song and Liu 2010).

According to skin flora maps reported by Bibel and Lovell (1976), aerobic bacteria can colonise the skin from between 1 to 10^5 colony-forming units found per cm^2 of skin in various regions of the body. The axilla, groin and inter-toe web spaces have a higher moisture content with bacterial flora of up to 10^7 colony forming units per cm^2 ; regions with a lower moisture content have 10^4 colony forming units or less per cm^2 (Larson *et al.* 2000; Fredericks 2001). These bacterial communities tend to vary in genus as opposed to the bacteria residing in the throat, stomach or gut (Fredericks 2001). Bacterial flora found on skin has commensal, symbiotic or parasitic relationships relative to the host (Chiller, Selkin and Murakawa 2001). Certain bacterial flora are inherently harmful to the host, whilst others have an opposing effect and improve the body's immune system (Fredericks 2001; Ki and Rotstein 2008; Gallo and Nakatsuji 2011; Holbert *et al.* 2014).

Staphylococcus aureus, *Micrococcus luteus*, *Escherichia coli*, *Propionibacterium acnes* and *Acinetobacter* spp. are bacterial types that form part of the body's natural microflora (Chiller, Selkin and Murakawa 2001; Struelens, Denis and Rodriguez-Villalobos 2004). These commensal bacteria, as well as pathogens with low virulence rates, have the potential to cause pathogenic contagion within their host in cases of suppressed immune systems, thus subjugating the host's defence mechanism (Breathnach 2005; Moor and Ferguson 2006). Commensal bacteria act as the host's protective mechanism by protecting the body and immune system against assaults by pathogens, however when the host becomes immuno-compromised, as seen in HIV/AIDS patients, excess antibiotic treatment results in an exponential growth of these bacteria outside their natural habitat, which inevitably results in infection (World Health Organisation 2002).

The gram-positive genus *Staphylococcus* is identified microscopically by its gram-positive cocci that form in aggregated clusters (Chiller, Selkin and Murakawa 2001). *Staphylococcus epidermidis* and *Staphylococcus hominis* are widespread and frequently commensal bacteria found in the body; they are known to occasionally cause healthcare-associated infections in patients with synthetic heart valves and intravenous catheters (Charbonneau, Song and Liu 2010; Mandell, Bennette and Dolin 2010). Table 2.1 shows the most frequented location of these *Staphylococcus* organisms, which include the upper trunk and cranial region (Chiller, Selkin and Murakawa 2001). They are distinguished by their ability to produce excess slime (Chiller, Selkin and Murakawa, 2001). The Centers for Disease Control and Prevention (CDC) (2005) reiterated the role that organisms from the genus *Staphylococcus* play in the dissemination of skin infections in the United States of America (USA), resulting in an increased utilization of antibiotic treatment by patients.

As indicated in **Table 2.1** *Micrococcus luteus* is the most frequently occurring *Micrococcus* sp. found on the skin from the eight other species found, and rarely results in infections as they are commensal bacteria and are only known to cause opportunistic infections in immuno-compromised patients (Chiller, Selkin and Murakawa 2001). Other gram-positive skin commensal organisms include *Corynebacterium* spp., *Propionibacterium* sp., *Dermabacter* sp. and *Brevibacterium* sp. Gram-negative bacteria such as the *Acinetobacter* spp., commonly found in burn wounds, prefer dry areas in which to colonize (Chiller, Selkin and Murakawa 2001).

Staphylococcus aureus is a gram-positive bacterium commonly residing on the skin and nasopharyngeal passages of normal healthy individuals (Centers for Disease Control and Prevention 2005). It is frequently associated with the development of cutaneous infections, manifesting in serious atopic conditions that require antibiotic intervention (Banning 2005). It also possesses a pathogenic potential to cause a variety of life-threatening conditions, including respiratory, meningeal, osseous and cardiac-associated diseases (World Health Organisation 2005; Becker *et al.* 2007). This transient flora may colonize the epidermis of neonates; the external portion of the nostrils in approximately 20 - 40% of healthy individuals; as well as the skin of atopic patients (Strange *et al.* 1996; Banning 2005). Furthermore, it is also known to be the most common cutaneous bacterium associated with HIV infected patients, instigating cutaneous diseases with potential life-threatening complications (Berger 1993). Berger (1993) advised that the susceptibility of HIV patients to *Staphylococcus aureus* is due to the nasal carriage of this organism.

The human skin provides nutrients and conditions that are suitable for most pathogenic micro-organisms to thrive in, with certain bacteria having the capability to resist most of the cleaning protocols employed, thus contributing to their persistence survival and presence within an ecosystem (Centers for Disease Control and Prevention 2008; Oluwole *et al.* 2013). The 1940's saw an upsurge in the use of antibiotics such as penicillin in the treatment of infections, which adversely resulted in the development of antibiotic resistance by these infective organisms (Winter 2005). Owing to its ability to mutate, a certain strain of *Staphylococcus aureus* became unresponsive to the antibiotic compound methicillin, and is called methicillin resistant *Staphylococcus aureus* (MRSA) (Banning 2005). The MRSA has become a common pathogen causing nosocomial (hospital-acquired) infections and resulting in a burgeoning threat to healthcare on an international scale (Banning 2005). The issue of antibiotic resistance is more serious in

public hospitals than in the private sector, as these hospitals have a deficiency of resources and are over-populated, with 80% of the South African population making use of public healthcare facilities (About South Africa n.d.). Patients in healthcare facilities who acquire infections secondary to the presented condition treated for at that facility, have what is termed a healthcare-associated infection (World Health Organisation 2002; Meier, Stone and Gebbie 2008; Centers for Disease Control and Prevention 2005).

Table 2.1: Bacterial skin residents and their associated dermatoses (Chiller, Selkin and Murakawa 2001; Charbonneau, Song and Liu 2010; Farage, Miller and Maibach 2015).

| Bacteria | Location | Distinguishing features | Skin pathology |
|----------------------------|-----------------------|---------------------------------|-----------------------|
| Gram (+) | | | |
| Staphylococcus | | | |
| <i>S. epidermidis</i> | Upper trunk | Produce slime | Acne vulgaris |
| <i>S. hominis</i> | Glabrous skin | | |
| <i>S. haemolyticus</i> | | Produce slime | Meningitis |
| <i>S. capitis</i> | Head | | Endocarditis |
| <i>S. mitis</i> | | | Soft tissue infection |
| <i>S. warneri</i> | | | |
| <i>S. saprophyticus</i> | perineum | Cause UTI | Cystitis |
| <i>S. cohnii</i> | | | |
| <i>S. xylosus</i> | | | |
| <i>S. simulans</i> | | | |
| <i>S. saccharolyticus</i> | Forehead/antecubital | anaerobic | |
| Micrococcus | | | |
| <i>M. luteus</i> | | | |
| <i>M. varians</i> | | | |
| <i>M. lylae</i> | In children/cold temp | | |
| <i>M. kristinae</i> | In children | | |
| <i>M. nishinomiyaensis</i> | | | |
| <i>M. roseus</i> | | | |
| <i>M. sedentarius</i> | Pitted keratolysis | | |
| <i>M. agilis</i> | | | |
| Corynebacterium | | | |
| <i>C. minutissimum</i> | Intertriginous | Lipophilic/porphyrin | Erythrasma |
| <i>C. tenius</i> | Intertriginous | Lipophilic | Trichomycosis |
| <i>C. xerosis</i> | Conjunctiva | Lipophilic | Conjunctivitis |
| <i>C. jeikeium</i> | Intertriginous | Lipophilic/antibiotic resistant | |
| <i>Rhodococcus</i> | | Lipophilic | Granuloma in HIV |
| Propionibacterium | | | |
| <i>P. acnes</i> | Sebaceous gland | Lipophilic/anaerobic | Acne |
| <i>P. granulosum</i> | Sebaceous gland | Lipophilic/anaerobic | Severe acne |
| <i>P. avidum</i> | Axilla | Lipophilic/anaerobic | |
| Brevibacterium | Toe webs | Nonpophilic (large colony) | |
| Dermabacter | Pitted keratolysis | Nonpophilic (large colony) | |
| Gram (-) | | | |
| Acinetobacter | Dry areas | Gram-negative | Burn wounds |

2.4 HEALTHCARE-ASSOCIATED INFECTIONS

“A healthcare-associated infection has been defined as an infection that occurs in a patient, within a healthcare facility, in whom the infection is not present or incubating at the time of admission or that is acquired during the stay in the healthcare facility, but arises within 48 hours after discharge” (World Health Organisation 2002). Revelas (2012) described a healthcare-associated infection as being one that occurs within 48 hours of the patient being admitted to a healthcare facility; three days after being discharged; or 30 days following an operative procedure. These infections were previously referred to as ‘hospital-acquired infections’ as hospitals are domains for immunocompromised patients, allowing infections to spread readily (Meier, Stone and Gebbie 2008). This results in an increased length of hospital stays, the potential for disability, unforeseen higher medical costs and fatalities (Archibald and Jarvis 2007; Mehmood *et al.* 2014). Healthcare-associated infections remain a universal safety concern for healthcare workers and patients (World Health Organisation 2002).

Infection is a consequence that occurs between a susceptible host and an infectious pathogen, where factors such as pathogenicity, virulence, infectious dose as well as mode of transport of the infectious pathogen are critical in the outcome of infections (Boyce and Pittet 2002; Wilcox 2009). Wilcox (2009) further stated that in order for a healthcare-associated infection to be established, three fundamental elements are required to facilitate the development of an infection. These elements include a source, which serves as a reservoir for infectious pathogens; a susceptible host with a portal of entry; and a mode of transmission for the pathogens. The host-pathogen-environment triad determines the susceptibility of a host in acquiring a healthcare-associated infection. The host, together with microbial and environmental factors in a specific healthcare setting, will govern the distribution and development of healthcare-associated infections operating either systemically or locally (World Health Organisation 2002; Logtenberg 2009).

Healthcare-associated infections are rife in the 21st century due to hospitals accommodating a greater number of patients with weak immune systems. There has also been an increase in medical procedures that breach the body’s protective barriers with inadequate sanitization protocols being implemented by healthcare personnel (Revelas 2012). The status of the patient’s immune system at the time determines the individual’s outcome (Boyce and Pittet 2002). Host factors such as age and underlying disease (for example diabetes), HIV/AIDS, malignancy, transplants, and certain medication that alters

the normal bodily flora, may increase the patient's susceptibility to infection (Wilcox 2009, Cristina *et al.* 2013). Surgical devices including urinary catheters, endotracheal tubes and synthetic- based implants have the potential to facilitate the development of healthcare-associated infections due to the resultant breach in the barrier of the skin, thereby allowing access to potential pathogens (Hidron *et al.* 2008).

The occurrence of healthcare-associated infections in developed countries has manifested in 5 - 15% of patients admitted to regular hospital wards, with an incidence of 50% or more noted in patients in intensive care units (ICUs) (Vincent 2003). Revelas (2012) posited that they affect every one in ten patients admitted to hospital facilities. The magnitude of the problem remains an enigma in developing countries due to a lack of knowledge in the diagnosis of healthcare-associated infections and resources required to implement intervention strategies (Allegranzi and Pittet 2009). The Centers for Disease Control and Prevention (2016) confirmed an estimated two million healthcare-associated infections established each year in hospitals within the USA, resulting in a mortality rate of 90,000 deaths, with mortality and morbidity occurring in 5 - 10% of these cases (Diekema and Saubolle 2011).

Magill *et al.* (2014) conducted a survey within 183 hospitals in the USA, where 11,282 patients availed themselves for the study; of the total participants, 452 patients had one or more healthcare-associated infections present. Sources of these infections included respiratory disease (21.8%), infections due to surgical incisions (21.8%), and abdominal diseases (17.1%). Nejad *et al.* (2011) performed a systemic review of healthcare-associated infections in Africa, which yielded the following results: the prevalence of health care-associated infections in hospitals ranged from 2.5 – 14.8% in Algeria (Lamarque 2003); Burkina Faso (Dia *et al.* 2008); Senegal (Gosling *et al.* 2003); and the United Republic of Tanzania (Atif *et al.* 2006). Infection rates ranged between 5.7 – 45.8% in studies carried out in Nigeria and Ethiopia (Kesah *et al.*, 2004; Messele *et al.* 2009). Nigeria reported an incidence of 45.8%, equating to an incidence density of 26.8 infections per 1000 paediatric patients admitted to the surgical ward (Kesah *et al.* 2004).

Risk factors for the development and manifestation of these infections are subdivided into three categories, namely, iatrogenic factors, organizational factors or patient-related factors (Allegranzi and Pittet 2009). Iatrogenic risk factors comprise of both diagnostic and invasive procedures such as exploratory surgery, catheterization, and administration of prophylaxis, antibiotics and other medicinal administrations via oral or intravenous routes.

Organizational risk factors comprise of contamination of air-conditioning and water systems, along with the physical layout of the healthcare-facility (i.e. open hospital beds accommodating patients with contagious infections in close proximity to one another). Patient-related risk factors incorporate the time spent at the healthcare-facility, the severity and contagion rate of the illness, as well as a predisposed immunosuppressed individual (Kleinpell, Munro and Guiliano 2008; Allegranzi and Pittet 2009). Additional risk factors include geriatric patients due to their diminished immune systems (van den Biggelaar *et al.* 2004; Logtenberg 2009; Magill *et al.* 2014); neonatal patients, particularly premature babies owing to their undeveloped immune systems (Breathnach 2005; Srivastava and Shetty 2007; Logtenberg 2009); and patients who have been hospitalized for extended periods of time (Magill *et al.* 2014). Furthermore, the general health status of individuals predisposes them to acquiring infections; these include patients who are malnourished, overweight, have a history of chronic disease, or are dependent on either nicotine or alcohol (Heinzelmann, Scott and Lam 2002). Healthcare-associated infections not only contribute to morbidity and mortality rates, they have brought about an increase in multiple drug-resistance organisms, with more than 70% of these organisms resistant to no less than one commonly used drug. This exacerbates the challenge hospitals face in terms of clinical treatment of the patients and cross-contamination of these pathogens (Hidron *et al.* 2008).

Healthcare-associated infections were primarily caused by the *Streptococcus* spp. in the late 1800's, followed by gram-positive *Streptococcus* and *Staphylococcus aureus* through the next 50 to 60 years (Revelas 2012). In the 1970's, gram-negative *Bacillus* spp., including *Pseudomonas aeruginosa* and *Enterobacteria* species, were noted in the majority of healthcare-associated infections that were contracted. Gram-positive bacteria including coagulase-negative *Staphylococcus*, *Staphylococcus aureus* and *Enterococcus* spp. accounted for 34% of healthcare-associated infections occurring between 1990 and 1996 (Ponticelli 2007). *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Klebsiella pneumoniae* were gram-negative bacteria responsible for 32% of infections caused during the same era, resulting in abdominal upsets, while others resulted in nephritis or cystitis, respiratory infections and bronchial pneumonia (Revelas 2012; Centers for Disease Control and Prevention 2016). **Table 2.2** depicts a study conducted between January 2006 and October 2007 on the distribution of prominent pathogens associated in nosocomial infections as reported to the National Healthcare Safety Network (NHSN) (Hidron *et al.* 2008). The study aimed to describe the frequency of specific bacterial pathogens in the cause of device- and procedure-associated healthcare-associated infections. From the 28,502 cases of healthcare associated infections during

the period January 2006 to October 2007, a total of 33,848 pathogenic micro-organisms were noted; 29,448 (87%) were bacterial flora and the remainder 4,400 (13%) were fungi. Overall, 70% of the isolates enumerated were sub-divided into *Staphylococcus* (15%); *Staphylococcus aureus* (15%); *Enterococcus* species (12%); *Candida* spp. (11%); *E.coli* (10%); or *Pseudomonas aeruginosa* (8%) (Hidron *et al.* 2008).

Table 2.2: Pathogens initiating nosocomial infections, reported to the National Healthcare Safety Network (NHSN) between January 2006 and October 2007 (Hidron *et al.* 2008). Rank = 1: Most frequently occurring; Rank = 10: Least frequently occurring.

| Pathogen | Overall* No. (%) of pathogens | Rank | CLABSI No. (%) of pathogens | Rank | CAUTI No. (%) of pathogens | Rank | VAP No. (%) of pathogens | Rank | SSI No. (%) of pathogens | Rank |
|--------------------------------|-------------------------------------|------|-----------------------------------|------|----------------------------------|------|--------------------------------|------|--------------------------------|------|
| CoNS | 5,178 (15.3) | 1 | 3,900 (34.1) | 1 | 234 (2.5) | 7 | 79 (1.3) | 9 | 965 (13.7) | 2 |
| <i>Staphylococcus aureus</i> | 4,913 (14.5) | 2 | 1,127 (9.9) | 4 | 208 (2.2) | 8 | 1,456 (24.4) | 1 | 2,108 (30.0) | 1 |
| <i>Enterococcus species</i> | | 3 | | 2 | | 3 | | 10 | | 3 |
| <i>E. faecalis</i> | 1,177 (3.5) | | 627 (5.5) | | 335 (3.6) | | 21 (0.4) | | 194 (2.8) | |
| <i>E. faecium</i> | 1,888 (5.6) | | 942 (8.2) | | 562 (6.0) | | 38 (0.6) | | 345 (4.9) | |
| <i>Candida species</i> | | 4 | | 3 | | 2 | | 7 | | 8 |
| <i>C. albicans</i> | 2,295 (6.8) | | 673 (5.9) | | 1,361 (14.5) | | 140 (2.4) | | 115 (1.6) | |
| Other <i>Candida</i> spp. | | | | | | | | | | |
| <i>Escherichia coli</i> | 3,264 (9.6) | 5 | 310 (2.7) | 8 | 2,009 (21.4) | 1 | 271 (4.6) | 6 | 671 (9.6) | 4 |
| <i>Pseudomonas aeruginosa</i> | 2,664 (7.9) | 6 | 357 (3.1) | 7 | 938 (10.0) | 4 | 972 (16.3) | 2 | 390 (5.6) | 5 |
| <i>Klebsiella pneumonia</i> | 1,956 (5.8) | 7 | 563 (4.9) | 5 | 722 (7.7) | 5 | 446 (7.5) | 5 | 213 (3.0) | 7 |
| <i>Enterobacter species</i> | 1,624 (4.8) | 8 | 443 (3.9) | 6 | 384 (4.1) | 6 | 498 (8.4) | 3 | 293 (4.2) | 6 |
| <i>Acinetobacter baumannii</i> | 902 (2.7) | 9 | 252 (2.2) | 9 | 109 (1.2) | 9 | 498 (8.4) | 3 | 42 (0.6) | 9 |
| <i>Klebsiella</i> | 359 (1.1) | 10 | 99 (0.9) | 10 | 85 (0.9) | 10 | 128 (2.2) | 8 | 47 (0.7) | 9 |
| Other | 5,267 (15.6) | | 1,201 (10.5) | | 1,321 (14.1) | | 1,375 (23.1) | | 1,363 (19.4) | |
| Total | 33,848 (100) | | 11,428 (100) | | 9,377 (100) | | 5,960 (100) | | 7,025 (100) | |

CAUTI= catheter-associated urinary tract infection; CLABSI= central line– associated bloodstream infection; CoNS= coagulase-negative staphylococci; NOS= not otherwise specified; SSI= surgical site infection; VAP= ventilator-associated pneumonia.

2.5 MODES OF TRANSMISSION IN HEALTHCARE-ASSOCIATED INFECTIONS

As indicated by the CDC (2016) there are numerous categories of pathogenic organisms that can cause infections, namely, bacteria, fungi, viruses and parasites. The mode of transmission for these organisms varies based on the type of organism involved. For instance, *Mycobacterium tuberculosis* is transmitted via airborne routes. Organisms transmitted in this manner via the dissemination of airborne droplet particles containing infectious pathogens that have the ability to remain infective over time and distance, are then inhaled by a susceptible host who has not come into actual contact with the infected individual (Wilcox 2009). The influenza virus is transmitted via droplet spray, whereby respiratory droplets that have emanated from within an infected host's respiratory tract is transmitted to a susceptible host who is in close proximity to the infected individual via coughing, sneezing or talking (World Health Organisation 2002; Centers for Disease Control and Prevention 2005; Bolashikov and Melikov 2008). The transmission of healthcare-associated infections occurs via contact transmissions, which are sub-divided into indirect contact and direct contact (Centers for Disease Control and Prevention 2016).

2.5.1 Indirect contact transmission

Indirect contact transmission occurs when a pathogenic organism is transferred via an intermediate object or individual (Wilcox 2009). The CDC (2002) indicated that indirect contact transmission may occur via contaminated hands of healthcare workers after they come into contact with contaminated equipment or body-sites on infected patients. Healthcare devices may also transmit pathogens if they are contaminated and have not been adequately disinfected and sterilized (Bifero, Prakash and Bergin 2006; Logtenberg 2009). A systemic review carried out by Schabrun and Chipcase (2006) aimed to determine the contamination of healthcare equipment including stethoscopes, otoscopes, diagnostic ultrasound, auriscopes and interferential therapy. Contamination of equipment occurred in more than 70% of the 20 studies carried out. The most prevalent bacterial flora noted included commensal skin flora and environmental flora, including coagulase negative *Staphylococcus*, *Staphylococcus aureus*, *Pseudomonas* spp., *Acinetobacter* spp. and *Pasteurella* spp.

Micro-organisms possess the capability of being attached to inanimate objects including healthcare equipment utilised by healthcare workers, such as the ultrasound probe of therapeutic ultrasound machines, as well as the pads of interferential current therapy machines, which are both used extensively by chiropractors as modalities during treatment (Lambert *et al.* 2000; Moor and Ferguson 2006; Shiferaw *et al.* 2013). Once the contaminated equipment comes into contact with susceptible sites on a patient, these pathogens may be transferred to the patient's skin resulting in an infection (World Health Organisation 2002). The propagation of healthcare-associated infections from contaminated equipment is due to their ability to remain viable on inanimate objects, thus increasing the potential for contamination of other inanimate objects in the vicinity should they come into contact with them (Cozad and Jones 2003; Boone and Gerba 2007).

2.5.2 Direct contact transmission

This means of transmission occurs when an infected individual transfers pathogens to a susceptible, uninfected host via immediate contact without a transitional medium or individual who is contaminated (World Health Organisation 2002; Wilcox 2009; Centers for Disease Control and Prevention 2016). These types of transmissions can occur via direct epidermal contact, for example kissing, touching and sexual intercourse. According to Borkow and Gabbay (2007) healthcare-associated infections occur primarily due to direct contact transmission between the skin or mucosal lining of an infected individual and a susceptible host. The principal carriers are healthcare-workers with poor hand hygiene methods, whether it is through a lack of knowledge or inadequate sanitization techniques (Talaro and Talaro 1993; Kampf and Kramer 2004; Rotter 2009).

Pittet *et al.* (2006) investigated the link between healthcare workers' hands and healthcare-associated infections, identifying sequential steps that are required in the transmission of these infections via healthcare workers' hands. The five steps were categorised in this order:

1. Pathogenic organisms are present on the patient's skin or have been transferred to inanimate objects or healthcare equipment in the close vicinity.
2. The healthcare workers' hands become contaminated by these pathogens.
3. The pathogens remain viable by surviving for several minutes on the healthcare workers' hands.

4. Hand hygiene protocols employed by the healthcare worker is either insufficient, entirely omitted or the disinfectant utilized by the healthcare worker is inadequate.
5. Direct contact between the healthcare-workers' contaminated hands and the skin of the patient, inanimate objects or healthcare equipment in the room will initiate an infection.

Pathogenic micro-organisms associated with direct contact infections after hospitalization included commensal flora such as *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* spp. and *Acinetobacter* spp. (Vincent 2003). A systemic review carried out by Kramer, Schwebke and Kampf (2006) indicated that *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* (all 100%), *Candida albicans* (90%), *Rhino* virus (61%), hepatitis A virus (22- 33%) and *rota* virus (16%) were the most successful pathogenic organisms transferred during direct single hand contact. Patients themselves play a role in the spread of infection within the healthcare system by their ability to endogenously infect themselves via the transmission of pathogenic organisms to other parts of their own body, to the healthcare workers on duty, as well as to fellow patients in close proximity (Banfield and Kerr 2005; Centers for Disease Control and Prevention 2016).

2.6 CONTAMINATION OF HEALTHCARE WORKERS' HANDS

The human skin contains between 10^2 and 10^6 colony forming units of bacterial flora per cm^2 (Larson *et al.* 1998). As the day progresses commensal and pathogenic micro-organisms accrue on health workers' hands from their daily activities, such as direct contact with patients and their bodily fluids, diagnostic procedures and catheterization (Pittet *et al.* 1999; Pittet *et al.* 2006; Carling, Parry and Von Beheren 2008). Numerous studies have indicated that frequent routes of transmission can be attributed to the contaminated hands of healthcare-personnel, doctors and nurses, as well as other individuals involved with patient care (Khalifeh and Jafarpour 2003; Zobeiri and Karami-Martin 2004; Allegranzi and Pittet 2009). Longtin *et al.* (2014) expressed that current research indicates healthcare workers' hands are the principal route for cross-contamination.

In 1999 a study was conducted using samples obtained from 417 health workers' hands, of which 327 (78.4%) exhibited positive cultures for resident flora (75%), transient flora (14.5%) and infectious flora (10.5%) (Pittet *et al.* 1999). In another study conducted by

Khalifeh and Jafarpour (2003), samples collected from hospital members' hands included coagulase negative *Staphylococci* (72.9%); *Staphylococcus aureus* (30.5%); *Bacillus* (22%); *Klebsiella* (10.2%); *Streptococci* (3.4%); and *Escherichia coli* (1.7%). Khodavaisy *et al.*'s. (2011) study included 40 healthcare workers based in one hospital. The study resulted in contamination rates of 73.1% of hands with isolates including *Staphylococci* (23%); *Klebsiella spp.* (7.9%); *Enterobacter spp.* (4.7%); *Escherichia coli* (3.9%); *Acinetobacter spp.* (3.1%); and *Pseudomonas spp.* (2.3%).

It is noted that 50% of epidemic and non-epidemic strains of *Escherichia coli* survived after six minutes and *Klebsiella spp.* after two minutes (Fryklund, Tullus and Burman 1995). *Shigella dysenteriae* type one has the ability to survive on human hands for a period of one hour (Islam, Hossain and Khan 1997). Gloves have shown not to provide total protection against contamination, as the surface of the glove comes into contact with pathogens and inevitably results in the contamination of a patient's skin and any animate objects with which the healthcare worker comes into contact (Pittet *et al.* 2006).

Poor hand hygiene practices result in contamination, as healthcare workers' hands gradually increase in bacterial colonisation over the course of the day during patient care (Pittet *et al.* 1999; Pessoa-Silva, Dharan and Hugonnet 2004). Only a few studies have tested the efficacy of hand hygiene procedures, such as washing hands to decrease the load of pathogens found on hands. It can be assumed, however, that contaminated healthcare workers' hands remain as a means of transport for these pathogens within the healthcare environment due to poor hand hygiene practices (Pittet *et al.* 2006; Allegranzi and Pittet 2009). In the absence of proper hand hygiene protocols, and with an increased duration of patient contact, the potential for hand contamination increases, thus instigating healthcare-associated infections (Pittet *et al.* 2006). Based on these findings, optimal hand hygiene practices are deemed the cornerstone for the prevention of healthcare-associated infections (Allegranzi and Pittet 2009).

2.7 HAND HYGIENE PRACTICES OF HEALTHCARE WORKERS

Hand hygiene is a process whereby hands are rid of micro-organisms, both commensal and pathogenic, by using soap (antimicrobial or non-antimicrobial) together with water, in order to cleanse the surface of the hands (Busari *et al.* 2012; Oluwole *et al.* 2013). Pittet *et al.* (2004) conducted a study to identify the risk factors for non-adherence to hand hygiene practices and the beliefs and perceptions related to hand hygiene amongst 163

physicians. The overall adherence was 57% with a noticeable difference depending on what the physicians specialized in medically, ranging from 87% amid the interns and 23% amongst the anaesthesiologists. Adherence rates were higher when the physicians were aware of being observed (61%) to when they were not (44%). A questionnaire concerning the perceptions, beliefs and attitudes of the physicians towards hand hygiene indicated that 85% were aware of non-adherence posing a risk for cross-contamination to their patients, 77% said that they intended to abide by hand hygiene practices and 73% aimed to make progress in their adherence level. The physicians exhibited a positive attitude towards employing hand hygiene techniques when making direct contact with a patients skin, adjustment of intravenous devices, and making contact with various body sites on the same patient during a routine check-up (Pittet *et al.* 2004).

Previous studies conducted in both developed first-world countries and developing countries have indicated insufficient compliance with hygiene practices implemented by healthcare workers (Pittet *et al.* 2006). Poor hand hygiene compliance may be attributed to inadequate infrastructure and equipment required to enable proper hand washing practices, along with cultural and religious beliefs in some settings (Ahmed *et al.* 2006; Allegranzi and Pittet 2009). The WHO (2004) indicated that certain factors determine hand hygiene compliance of healthcare workers. These include: specific professionals such as a doctor, nursing assistant, physiotherapist or technician; specific environments in a hospital setting, especially the intensive care units; surgery and emergency medicine; insufficient staff members and overcrowding; and making use of protective gowns and gloves. Healthcare-associated infections have escalated over the last 30 years; however, these infections can be avoided (Centers for Disease Control and Prevention 2000; Pittet *et al.* 2006; Meier, Stone and Gebbie 2008). Preventative strategies such as improved hand hygiene practices have resulted in a considerable decline in infection transmission (Sacar *et al.* 2006; Howard *et al.* 2009; Mehmood *et al.* 2014).

Pittet *et al.* (2006) explored the correlation between improving awareness and initiating proper hand hygiene practices, and then assessing the outcomes of the infection rates. **Table 2.4** presents thirteen studies published between 1977 and 2005 which were conducted in a hospital setting that examined the implementation of new hand hygiene regulations and its consequence for healthcare-associated infections (Pittet *et al.* 2006). Significant reductions were noted in four of the studies conducted throughout the hospital. Of the five studies carried out within the adult intensive care units, four instances showed a decrease in infection rates. The four studies conducted in neonatal care units also

presented a reduction in infection rates due to improved hand hygiene practices (Pittet *et al.* 2006). Uneke *et al.* (2014) conducted a study to promote hand hygiene practices at a Nigerian teaching hospital and found compliance rates to be quite low, however after executing guidelines endorsed by the WHO on hand hygiene, higher rates of compliance were noted amongst the nurses (72.9%), midwives (65%) and doctors (59.7%). A recent study conducted in Cape Town, South Africa, implemented the WHO's 'five step' multimodal approach as an intervention strategy to healthcare personnel in eleven selected wards at the Groote Schuur Hospital over a three-month period (Patel *et al.* 2016). The compliance rates of healthcare personnel were assessed before and after applying the intervention strategy, and results obtained showed an improvement of 34% in 2014 to 76% in 2015 with regard to hand hygiene compliance before patient contact, and an improvement of 47% in 2014 to 82% in 2015 after patient contact (Patel *et al.* 2016).

Table 2.4: Hospital based studies investigating the relationship between hand hygiene protocols and the dissemination of nosocomial infections, 1975–2005 (Pittet *et al.* 2006).

| Reference | Type of hospital setting | Results obtained | Duration of follow-up |
|------------------------------|--------------------------|--|-----------------------|
| Casewell and Phillips (1977) | Adult ICU | Significant reduction ($p<0.001$) in the percentage of patients colonised or infected by <i>Klebsiella</i> spp | 2 years |
| Conly et al (1989) | Adult ICU | Significant reduction ($p=0.02$) in healthcare-associated infection rates immediately after hand hygiene promotion (from 33% to 12% and from 33% to 9%) | 6 years |
| Simmons et al (1990) | Adult ICU | No effect on healthcare-associated infection rates (no significant [$p<0.05$] improvement of hand hygiene adherence) | 11 months |
| Doebbeling et al (1992) | Adult ICU | Significant ($p<0.02$) difference between rates of healthcare-associated infection using two different hand hygiene agents | 8 months |
| Webster et al (1994) | NICU | Elimination of MRSA, when combined with multiple other infection control measures. Reduction of vancomycin use. Significant $p<0.02$ reduction of nosocomial bacteraemia (from 2.6% to 1.1%) using triclosan compared with chlorhexidine for handwashing | 9 months |

| | | | |
|------------------------|------------------------------|--|-----------|
| Zafar et al (1995) | Newborn nursery | Control of a MRSA outbreak using a triclosan preparation for handwashing, in addition to other infection control measures | 3.5 years |
| Larson et al (2000) | MICU/NICU | Significant (85%, $p=0.02$) relative reduction of VRE rate in the intervention hospital; insignificant (44%) relative reduction in control hospital; no significant change in MRSA | 8 months |
| Pittet et al (2000) | Hospital-wide | Significant ($p=0.04$ and $p<0.001$) reduction in the annual overall prevalence of healthcare associated infections (41.5%) and MRSA cross-transmission rates (87%). Active surveillance cultures and contact precautions were implemented during same time period | 5 years |
| Hilburn et al (2003) | Orthopaedic surgical unit | 36.1% decrease in infection rates (from 8.2% to 5.3%) | 10 months |
| MacDonald et al (2004) | Hospital-wide | Significant ($p=0.03$) reduction in hospital-acquired MRSA cases (from 1.9% to 0.9%) | 1 year |
| Swoboda et al (2004) | Adult intermediate care unit | Reduction in healthcare-associated infection rates (not significant, p value not reported) | 2.5 years |

| | | | |
|--------------------------------|---------------|---|-----------|
| Lam <i>et al.</i> (2004) | NICU | Reduction (not significant, $p=0.14$) in healthcare-associated infection rates (from 11.3 per 1000 patient-days to 6.2 per 1000 patient-days) | 6 months |
| Won <i>et al.</i> (2004) | NICU | Significant reduction ($p=0.003$) in healthcare-associated infection rates (from 15.1 per 1000 patient-days to 10.7 per 1000 patient-days), in particular of respiratory infections | 2 years |
| Zerr <i>et al.</i> (2005) | Hospital-wide | Significant ($p=0.01$) reduction in hospital-associated rotavirus infections | 4 years |
| Rosenthal <i>et al.</i> (2005) | Adult ICU | Significant ($p<0.001$) reduction in healthcare-associated infection rates (from 47.5 per 1000 patient-days to 27.9 per 1000 patient-days) | 21 months |
| Johnson <i>et al.</i> (2005) | Hospital-wide | Significant ($p=0.01$) reduction (57%) in MRSA bacteraemia | 36 months |

ICU=intensive care unit, NICU=neonatal ICU, MRSA=meticillin-resistant *Staphylococcus aureus*, MICU=medical ICU, VRE= vancomycin-resistant enterococci.

2.8 GUIDELINES FOR HAND HYGIENE PRACTICES

After assessing the compliance of hand hygiene protocols carried out by healthcare-workers globally, and thereafter evaluating their knowledge on the transmission of micro-organisms and hand washing ideologies, the WHO (2002) implemented international guidelines for hand hygiene practices. The strategy incorporated five main components. The first was to bring about a system of change whereby healthcare-workers had better access to alcohol-based hand rubs; the second strategy was to ensure better training and education regarding these hand hygiene methods. The third strategy was employed to monitor the implementation and performance of these hand hygiene practices. The fourth and fifth strategies were to display visual reminders in the allocated workplace and create a safe climate within the institution respectively (WHO 2002).

Insufficient research has been conducted in the past to analyse hand hygiene protocols of chiropractic practitioners. Interestingly, Evans *et al.* (2009), published guidelines with methods of obtaining optimum hygiene within the chiropractic profession. In order to disinfect hands, an alcohol-based gel containing at least 60% alcohol and an emollient for skin sensitivity needs to be used. It should also be available in all areas where skin-to-skin contact by hand occurs (Evans *et al.* 2009). When hands are visibly soiled, they need to be washed, as recommended by Evans *et al.* (2009), before using the alcohol-based hand hygiene product. The standards stipulated included removing any jewellery and watches and ensuring that clothing did not make contact with the sink. The water should be turned on and adjusted to warm, thereafter the hands should be washed up to and including the wrists. All areas of the hands and wrists should be washed for at least 20 seconds. In order to scrub between fingers, they should be interlaced. If hands were exposed to infectious material, a nail brush or stick should be used to scrub beneath fingernails. Hands and wrists should be well-rinsed and the procedure repeated if the hands were exposed to infectious material. Hands are then to be dried thoroughly with paper towel, which must be properly disposed of in an appropriate container. A dry paper towel should be used to turn off the water tap and to open the door. When using an alcohol-based hand hygiene product, Evans *et al.* (2009) advised dispensing a portion of gel the size of a [dime] R1 coin on the palm of the hand and to rub it over hands and wrists including each finger to the ends. After ensuring all surface areas of the hands have been covered, the hands should be allowed to air dry. Excess gel should not be wiped off. At least one of the two measures should be performed between each patient contact, whether in practice or when practicing adjustment techniques. In cases where either the doctor or patient has a visible skin lesion, gloves should be worn during the

examination and treatment, after which the gloves should appropriately be disposed of followed by immediate hand sanitising (Evans *et al.* 2009).

2.9 INTERVENTION STRATEGY FOR HAND HYGIENE

The term 'decontamination' is defined as the mechanical removal or destruction of micro-organisms and their debris (Gould 2000). Evidence for the efficacy of hand decontamination dates back to the mid-19th century, when the obstetrician named Ignaz Semmelweis carried out an epidemiological study in which he observed a noticeable decrease in the mortality rates of newborns due to hand washing procedures (Boyce and Pittet 2002; Shuhua *et al.* 2011). Various interventions exist in terms of products and practices used to decontaminate hands which may influence the removal of infectious micro-organisms that result in healthcare-associated infections and are deemed crucial to healthcare workers as they decrease the frequency of these infections occurring (Richards *et al.* 1999; Rotter *et al.* 2009).

Riaz, Ahmad and Hasnain (2009) outlined that soaps play an integral role in the elimination of micro-organisms, whether from the surface of skin or the surface of inanimate objects. They typically comprise of fats and emollients, however some soaps are supplemented with detergents to enhance their anti-bacterial activity (Friedman and Wolf 1996). **Table 2.5** depicts the results from a study carried out by Burton *et al.* (2011). Twenty subjects consented to purposefully contaminate their hands by deliberately touching door handles and railings in public areas, in order to investigate the effect that hand washing with water or soap only compared with no hand washing at all will have on the micro-organisms found. The outcomes revealed that hand washing with water alone substantially reduced the prevalence of bacterial flora. Hand washing using soap decreased the rates of faecal bacteria found on the hands, however, especially *Enterococcus* species. Table 2.5 also showed that hand washing with both soap and water significantly decreased the amount of multiple isolates found on contaminated hands as opposed to just using water alone (Burton *et al.* 2011).

Table 2.5: The efficacy of using soap, water only or no hand washing on faecal bacteria found on hands after self-contamination (Burton *et al.* 2011).

| Faecal Bacteria | No hand washing | Water only | Soap and water |
|-------------------------------|-----------------|------------|----------------|
| <i>Enterococcus</i> spp. | 46 (29%) | 24 (15%) | 4 (3%) |
| <i>Enterobacter amnigenus</i> | 14 (9%) | 4 (3%) | 4 (3%) |
| <i>Enterobacter cloacae</i> | 13 (8%) | 5 (3%) | 2 (1%) |
| <i>Shigella</i> spp. | 2 (1%) | 1 (1%) | 0 (0%) |
| <i>Klebsiella</i> spp. | 5 (3%) | 2 (1%) | 1 (1%) |
| <i>E. coli</i> | 0 (0%) | 0 (0%) | 1 (1%) |
| <i>Pantoea</i> spp. | 0 (0%) | 2 (1%) | 1 (1%) |
| Other bacteria | 70 (44%) | 36 (23%) | 13 (8%) |

Soaps that contain anti-microbial active ingredients have been shown to remove more bacteria in comparison with plain soap (Lucet, Rigaud and Mentre 2002). As shown through research conducted by Osborne and Grube (1982), anti-bacterial soaps have the potential to remove 65% to 85% bacteria from the surface of human skin. The Centers for Disease Control and Prevention (2006) stated that plain soaps have the ability to clean soiled hands and remove transient bacterial flora from hands, although they are not effective for the eradication of pathogenic micro-organisms. The CDC (2016) further suggested employing either anti-microbial soaps or waterless sanitizers after contact with patients, to prevent the spread of commensal micro-organisms as well as antibiotic resistant micro-organisms such as methicillin-resistant *Staphylococcus aureus*. A study conducted by Kac *et al.* (2005) evaluated the efficacy of hand rubbing with an alcohol based solution (containing 45% 2-propanol, 30% 1-propanol, 0.2% mecetronium ethylsulphate and emollients) and hand washing (using un-medicated soap), against transient bacterial flora found on 50 healthcare workers' hands in a hospital setting. A reduction was noted in bacterial contamination of both palms (99% hand rubbing versus 75% hand washing) and fingertips (98% hand rubbing versus 82% hand washing), with hand rubbing achieving a larger reduction in contamination levels (Kac *et al.* 2005).

2.10 CONCLUSION

Chiropractic treatment involves hand-to-skin contact between the practitioner and patient frequently throughout the day, therefore increasing the probability of healthcare-associated infections being established. Considering that chiropractors have become the most sought after complementary-care practitioners in South Africa, steps need to be taken in order to assure effective infection control parameters during the treatment of patients. In order to implement these practices, there is a need to determine the extent of infection occurring

during treatment. A few studies have identified organisms prevalent during treatment, the direction of transfer of these organisms, or evaluated the role of proper hand hygiene practices within the chiropractic profession internationally; no such studies have been conducted within the profession in a South African setting. This study therefore aims to determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post- spinal manipulation of patients. It further aims to classify the bacterial flora present on the chiropractic practitioners' hands, as well as to determine the efficacy of decontaminants used by the chiropractic practitioners' against isolated bacteria found on chiropractic practitioners' hands in private practice in South Africa.

CHAPTER 3: METHODOLOGY

3.1 STUDY DESIGN

The study was located in the quantitative experimental paradigm and conducted as a cross-sectional investigation, divided into three principal objectives. The first objective comprised of two main outcomes;

1. To determine and enumerate the presence of bacterial flora on chiropractors' hands before and after spinal manipulation from the samples collected and incubated on appropriate agar plates.
2. To determine the direction of transfer of bacterial flora (i.e. was the bacteria transferred from the chiropractor to the patient or from the patient to the chiropractor during spinal manipulation).

The second objective was to identify the bacterial flora obtained from the chiropractic practitioners' hands before and after manipulation and the incidence thereof.

The third objective was to determine the efficacy of decontaminants used by the chiropractic practitioners against the isolated bacteria identified using a modified Kirby-Bauer technique.

3.2 SAMPLING PROTOCOL

3.2.1 Sample Groups

The sampling method employed was convenience sampling. Potential participants were chosen because of their convenient accessibility and close proximity to the researcher and microbiology laboratory. The sample group comprised of 40 chiropractic practitioners from the greater Durban area. In order to determine the population size, a sample calculator from the survey system website was utilized. To achieve a confidence level of 95%, a sample size of 40 chiropractors was required for the study to be statistically significant. A list of potential candidates was collated and they were contacted telephonically by the researcher to solicit their participation in the research study. The participants who agreed to participate in the

research were then issued with a letter of information and consent to read and sign (Annexure A). The chiropractic practitioners were then stratified based on the regions of the spine that they manipulated. This categorization facilitated the comparison of colony-forming units (CFUs) found on the four regions of the spine outlined below:

- Region 1 - Cervical spine
- Region 2 -Thoracic spine
- Region 3 - Lumbar spine
- Region 4 - Sacroiliac joints

3.2.2 Sample Group Characteristics

Inclusion criteria:

- Chiropractic practitioners registered with the Allied Health Professions Council of South Africa (AHPCSA).
- Chiropractic practitioners who agreed to participate in the study and who signed informed consent forms.
- Chiropractic practitioners utilising spinal manipulation, where the chiropractor's hands are in direct contact with the patient's skin.
- Chiropractic practitioners with patients who did not require dry needling prior to the adjustment, as the use of alcohol to the area would have resulted in inaccurate findings.

Exclusion criteria:

- Chiropractic practitioners not registered with the Allied Health Professions Council of South Africa (AHPCSA).
- Chiropractic practitioners who did not consent to participate in the study.
- Chiropractic practitioners performing spinal manipulation over the clothing of the patient.
- Chiropractic practitioners performing extremity manipulation only.
- Chiropractic practitioners with patients who required dry needling prior to the adjustment, as the use of alcohol to the area would have resulted in inaccurate findings.

3.3 STUDY PROCEDURE

The researcher was allotted a convenient time by each consenting chiropractic practitioner to call at his or her practice. A single swab was taken of the chiropractic practitioner's hands pre- and post-spinal manipulation of their first, second or third patient of the day. This contingency guaranteed at least one of the patients fitting the inclusion criteria of the study. It also ensured that each of the chiropractor's hands was swabbed at approximately the same time each day. A letter was sent to the Head of Department: Biotechnology and Food Technology requesting permission to conduct the microbiological analysis of the study at the Durban University of Technology microbiology laboratory (Annexure B). The study procedure was then adapted to each of the three objectives.

3.3.1 Verification of Presence and Enumeration of Bacteria

Objective 1: To determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post- spinal manipulation of patients, by means of hand swabs, serial dilution and plate counts.

3.3.1.1 Hand-sampling protocol for bacterial contamination

The chiropractic practitioners' hands were swabbed according to published techniques (Logtenberg 2009; De Alwis *et al.* 2012). A sterile polyester swab (3.2 mm x 162.3 mm) (Whatman FTA collection products) dampened with a phosphate buffer solution was used to obtain samples from both hands of each participating chiropractic practitioner by rolling the moistened tip along the palm, fingers, and in-between the fingers (De Alwis *et al.* 2012). This procedure was performed before and after spinal manipulation of the patient. Each polyester swab containing the sample collected was then infused into a test tube containing 3 ml of Tryptic Soy broth (Biolab), which was shaken briskly to disperse the microbial flora collected on the swab into the broth (Logtenberg 2009). The growth of the bacteria collected was inhibited in the Tryptic Soy broth but remained in a state of stasis until the diluted bacterial flora were plated on media containing a carbon source. An alphanumeric system of labelling was implemented to label obtained specimens, to ensure and preserve the anonymity of the chiropractic practitioners. The specimens collected were transported to the microbiology laboratory to be serially diluted.

3.3.1.2 Serial dilution of samples

For each sample obtained pre-spinal manipulation, six test tubes were prepared in advance as the experiment was carried out in duplicate to avoid experimental error. Each of the test tubes contained 9 ml of saline solution (Sigma-Aldrich) and were labelled according to their dilutions, for example the first test tube was labelled 10^{-1} , the second 10^{-2} and the third 10^{-3} . The rationale behind these sequential dilutions was to reduce the number of micro-organisms to a lesser concentration, thus allowing the researcher to individually count the bacterial flora obtained from the original sample.

All the procedures were performed within a laminar flow cabinet to ensure an aseptic working environment, thereby reducing the possibility of contamination (LabtecBioflow II, South Africa). One ml was drawn aseptically from the original sample within the Tryptic Soy broth and was then dispensed into the test tube labeled 10^{-1} , after which the used tip from the pipette was discarded in the bio-hazard box and replaced with a new tip. The test tube was then placed on the vortex machine (Vortex Genie 2, Scientific Industries) for five seconds in order to thoroughly disperse the sample within the saline solution. Using the pipette, 1 ml was aseptically drawn from the test tube labeled 10^{-1} and dispensed into the test tube labeled 10^{-2} . This process was repeated one more time until a dilution of 10^{-3} was obtained. The identical process was performed using the post-manipulation sample, therefore resulting in a total of twelve test tubes for inoculation onto the Tryptic Soy agar plates. Tryptic Soy agar (TSA) (Merck) was the medium of choice as it allows for the cultivation of various types of fastidious and non-fastidious micro-organisms (Atlas 2010).

3.3.1.3 Spread plate technique for isolation and enumeration

A total of sixteen TSA plates were prepared, as four TSA plates were made in order to inoculate the original sample obtained pre- and post- spinal manipulation in duplicates, with the remaining twelve TSA plates used for the inoculation of each of the serial dilutions obtained from the pre- and post- spinal manipulation samples. These twelve TSA plates were labelled according to their respective dilutions from 10^{-1} to 10^{-3} in duplicates. Information such as the date, alphanumeric code, group, and whether the dilution utilized was from the pre- or- post- spinal manipulation sample was indicated on the plates. The original sample was inoculated first, where 0.1 ml from the original pre-manipulation sample (called the inoculum) was drawn up into a pipette and then aseptically inoculated onto two of the TSA plates. The identical process was repeated for the original post-manipulation

sample. A sterile hockey stick was used to spread the inoculum onto the entire surface of the TSA plate. This process was repeated for the inoculation of each of the serial dilutions obtained from the pre- and post- spinal manipulation samples. The inoculated TSA plates were then incubated in an inverted position at 37°C for 24 to 48 hours (Logtenberg 2009; Mehmood *et al.* 2014).

3.3.1.4 Verification of bacterial flora

The growth and presence of microbial colonies found on the TSA plates served as evidence of bacterial flora present on the chiropractic practitioners' hands.

3.3.1.5 Enumeration of bacterial flora

Colony forming units of viable microbial flora present on the surface of the TSA plates were counted after 24 to 48 hours of incubation using a colony counter. The colony forming units were then recorded on data collection sheet one (Annexure C) (Biology Online 2008). Agar plates containing only between 30 and 300 colony forming units were enumerated for optimum statistical reliability. Plates with colony forming units below 30 or above 300 were excluded. This procedure was implemented by Jones, Hoerle and Riekse (1995), Lecat *et al.* (2009), Rehman, Razzaq and Owais (2011) and Campos-Murguía *et al.* (2014).

In order to ascertain the viable count (ml^{-1}) of bacterial flora enumerated, the following calculation was utilised based on research by Penn (1991), Pollack *et al.* (2002), Reasoner (2003) and Harley (2008):

$$\text{Viable count (cfu/ml}^{-1}\text{)} = \left(\frac{\text{average or mean no. of cfu}}{\text{volume in ml} \times \text{optimum counting dilution}} \right)$$

3.3.1.6 The direction of transfer of bacterial flora

The direction of transfer was ascertained by comparing the viable count of bacteria found on the same chiropractor's hands pre- and post-spinal manipulation. If the viable count from the pre-manipulation reading was higher than the post-manipulation reading, it was indicative of a greater number of bacteria on the chiropractor's hands, which were then transferred to the patient's skin during manipulation. If, however, the viable count of the post-manipulation reading was higher than the pre-manipulation reading, bacteria were considered to be transferred from the patient's skin to the chiropractor's hands during the manipulation.

3.3.2 Classification of bacterial flora

Objective 2: To classify the bacterial flora present on the chiropractic practitioners' hands using conventional identification tests, biochemical profiling and test kit staining characteristics and morphology.

3.3.2.1 Isolation of bacteria

In order to identify the microbial flora enumerated from the pre- and post-manipulation samples, sub-cultures were made using the streak plate technique. A TSA plate was placed in the inverted position on the working surface within the laminar flow. A loop was passed through the flame of the bunsen burner until sterile, after which it was allowed to cool. Once cooled, the loop was dipped into the inoculum (single bacterial colony from samples collected). The TSA plate was lifted off the working surface and the inoculated loop was then drawn across approximately 30% of the surface of the plate in a zigzag formation in order to avoid overlapping of the previous streaks made. Rotating the TSA plate to 90°, the loop was re-sterilized in the flame and starting in the previously streaked section, the loop was drawn across the TSA plate again in a zigzag formation. The procedure was carried out once more ensuring the loop did not make contact with the previously streaked sections. All the plates were then incubated at 37°C for 24 hours (Penn 1991; Pollack *et al.* 2002).

3.3.2.2 Identification of bacteria

The sub-cultured and axenic bacterial cultures obtained were identified according to their macroscopic and microscopic characteristics. Macroscopic colony characteristics refer to the

characteristics of the bacterial colony when looking at it with the naked eye. The following features were evaluated (Swalaha 2013; Tille 2014):

- ❖ Size: measured in millimetres or described as pinpoint, small, medium or large.
- ❖ Shape (form): example - punctiform, circular, rhizoid, irregular or filamentous.
- ❖ Colour.
- ❖ Elevation: example - flat, raised, convex, umbonate and umbilicate.
- ❖ Margin: example - entire, undulate, lobate, curled or filamentous.
- ❖ Colour changes to the agar media caused by the microbial growth.
- ❖ Surface appearance of colony: example - smooth and glistening, rough, wrinkled or dry and powdery.
- ❖ Odour.

In order to identify the bacteria microscopically, pure cultures were gram stained order to distinguish between the gram-negative and gram-positive bacterial flora (DePietro 2013). The gram-positive bacteria were recognised by their blue/purple stain, whereas gram-negative bacteria stained pink (Tille 2014).

The gram stain procedure was performed as described by researchers Pollack *et al.* (2002), Forbes, Sahm and Weissfeld (2007) and Swalaha (2013). A slide containing a heat-fixed smear from the isolate bacteria was placed on the staining rack. The smear was first stained using crystal violet for 60 seconds. The crystal violet was then rinsed off using distilled water. The smear was stained with iodine for 60 seconds and rinsed off with distilled water. A few drops of ethanol were placed on the slide for fifteen seconds to decolourise the smear, before being rinsed off with distilled water in order to halt the decolourization process. Finally the smear was counter-stained with safranin for 30 seconds before being rinsed off with distilled water and blotted dry with a paper towel. The slide was viewed under a Nikon light microscope at 1000× magnification, where the microscopic characteristics were observed and noted on data collection sheet two (Annexure D).

Once pure cultures of bacteria were obtained they were inoculated onto selective media in order to determine their genus and species. Potential pathogenic bacteria, such as gram-positive *Staphylococcus aureus*, were inoculated onto Mannitol Salt Phenol Red agar (Sigma-Aldrich) as well as Baird Parker agar (Biolab) (Atlas 2010). Gram-negative bacilli, *Pseudomonas* spp. and *E. coli* were inoculated onto MacConkey agar (Sigma-Aldrich), as it

aids in the isolation and differentiation of lactose-fermenting and non-lactose-fermenting enteric bacilli (Tille 2014). These were further differentiated from each other using the oxidase test. Changes observed in the selective media were also noted on data collection sheet two (Annexure D).

3.3.3 Efficacy of decontaminants

Objective 3:- To determine the efficacy of decontaminants used by the chiropractic practitioners against isolated bacteria using a modified Kirby-Bauer technique.

3.3.3.1 Modified Kirby Bauer technique

In order to ascertain the efficacy of decontaminants frequently used by the chiropractic practitioners to cleanse their hands, a modified Kirby-Bauer technique comprising of the disk diffusion method was utilized (Pollack *et al.* 2002).

The five most frequently used decontaminants were:

- ❖ Dettol original hand wash.
- ❖ D-Germ hand disinfectant.
- ❖ TCP liquid disinfectant.
- ❖ Dis-chem instant hand sanitizer.
- ❖ Radox herbal hand wash.

3.3.3.2 Preparation of Mueller-Hinton agar plates and inoculation

Pure cultures obtained by sub-culturing the bacteria found in the original sample were stored using 50% of glycerol in micro bank vials at -70°C (Davies Diagnostics, South Africa). The sub-cultures were then inoculated in Nutrient broth (Biolab) and were incubated at 37°C for 24 hours (Cos *et al.* 2006). A spectrophotometer (Biochrom LibraS21) was used in order to obtain a MacFarland standard of 0.5 absorbance, which ensured a standardized bacterial cell concentration of each bacterium being tested. A suspension of 100 microliters of each inoculum was plated onto Mueller-Hinton agar plates (Fluka, Biochemika) using a pipette. A sterile swab was utilised to spread the inoculum across the entire surface of the Mueller-Hinton agar plate.

3.3.3.3 Determination of efficacy of decontaminants

Whatman Number 1 filter paper was cut into circular disks with a diameter of 5 mm and placed in an open sterile petri-dish within the laminar flow cabinet (LabtecBioflow II, South Africa). The disks were then impregnated with 0.1 ml of each of the decontaminants being tested and left to dry. Once dried, three disks were placed onto the pre-inoculated Mueller Hinton agar plates using sterilized forceps and incubated at 37°C for 24 hours. The process was repeated until each organism found was tested against all five decontaminants. Ciprofloxacin (Fluka, Biochemika) (3 mg/mL) was used as the positive control in this experiment. Following the 24-hour incubation period, the TSA plates were examined for the presence of a zone of inhibition around each filter paper disk. Once noted, the diameter of each zone of inhibition was measured in millimetres, thus determining the efficacy of each decontaminant against each isolate bacteria found, and was then recorded on data collection sheet three (Annexure E).

3.4 Statistical analyses

The data collected from the chiropractor's hands was analysed with SPSS version 24.0 and Stata version 13.0 (SPSS Inc., Chicago, Illinois, USA). The results presented the descriptive statistics in the form of graphs, cross tabulations and other figures for the quantitative data that was collected. Inferential techniques included the use of the paired t-Test and the Wilcoxon Signed Ranks test, which was then interpreted using *p*-values. The *p*-value was generated from a test statistic. A significant result was indicated with ' $p < 0.05$ '. A variable calculating the difference in the number of bacteria found on the chiropractors' hands pre- and post-spinal manipulation was created by subtracting the quantity of bacteria found post-manipulation from the quantity found pre-manipulation. If this figure was negative, it indicated that the bacteria were transferred from the patient to the chiropractor's hands; if this figure was positive, the bacterial transfer occurred from the chiropractor to the patient.

CHAPTER 4: RESULTS AND DISCUSSION

The aim of this study was to determine the presence and control of bacterial contamination during spinal manipulation among chiropractors in private practice in a South African setting. The results obtained are presented and discussed within this chapter in accordance with the three study objectives:

Objective One: To determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post-spinal manipulation of patients, by means of hand swabs, serial dilution and plate counts.

Objective Two: To classify the bacterial flora present on the chiropractic practitioners' hands using conventional identification tests, biochemical profiling and test kit staining characteristics and morphology.

Objective Three: To determine the efficacy of decontaminants used by the chiropractic practitioners against isolated bacteria using a modified Kirby Bauer technique.

4.1 DATA

4.1.1 Primary data

The primary data was obtained from the microbiological analyses of the samples obtained from the chiropractors' hands and was recorded on three data collection sheets. The number of CFUs present on the chiropractors' hands pre- and post-spinal manipulation were recorded on Data collection sheet one (Annexure C). The sub-cultured and axenic bacterial cultures obtained were identified according to their macroscopic and microscopic characteristics, and recorded on data collection sheet two (Annexure D). Data collection sheet three (Annexure E) was used to record the results obtained from the modified Kirby Bauer test. The diameter of each zone of inhibition was measured in millimetres, thus determining the efficacy of each decontaminant against each bacterial isolate.

4.1.2 Secondary data

Secondary data was obtained from the analysis of the primary data, as well as various sources of related literature, journal articles, books and online sources.

4.2 ABBREVIATIONS RELEVANT TO THIS CHAPTER

A: Bacterial growth was absent

HAI: Healthcare associated infection.

n: Refers to the sample size.

p: Refers to the *p*-value, which is the probability of getting the output observed (Bowers 2002).

P: Bacterial growth was present

Region 1: Cervical Spine.

Region 2: Thoracic Spine.

Region 3: Lumbar Spine.

Region 4: Sacroiliac joint.

Std. deviation: Standard deviation

Z: Indicates the distance and the direction of a point from the mean in terms of standard units. The deviations are standardised or placed on a common scale (Blair and Taylor 2008).

4.3 STATISTICAL CONCEPTS USED IN THIS CHAPTER

2-tailed p -value: The p -value is the probability of obtaining a result as observed when the null hypothesis is true. These results can occur by chance, equally often in either direction. To allow for this, a two-sided or two-tailed p -value is calculated (Campbell *et al.* 2007).

Mean: It is calculated by dividing the sum of the values by the number of values in the set. In contrast to the median, every sample value is used to calculate the mean and it can be influenced by extreme values called outliers. This may produce a mean which is not very representative of the general mass of data (Bowers 2002).

p -value: This p -value differs from the 2-tailed p -value as it is compared to the pre-determined significance level (0.05) and serves as a measure of the weight or strength of evidence for rejection of the null hypothesis, with large values implying no evidence and small values indicating strong evidence. If the p is less than or equal to 0.05 the data is considered highly significant and the null hypothesis is rejected. If the p -value is greater than 0.05, there is insufficient evidence to reject the null hypothesis (Gardiner 1997; Campbell *et al.* 2007).

Standard deviation: The standard deviation is the quantity expressing the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points are usually close to the mean of the set of values. A high standard deviation indicates that the data points are spread out over a wider range of values. (Bowers 2002; Campbell *et al.* 2007).

4.4 RESULTS

In order to ascertain the viable count (ml^{-1}) of bacterial flora enumerated, the following calculation was utilised based on research by Penn (1991), Pollack *et al.* (2002), Reasoner (2003), Harley (2008) and Logtenberg (2009):

$$\text{Viable count (cfu/ml}^{-1}\text{)} = \left(\frac{\text{average or mean no. of cfu}}{\text{volume in ml} \times \text{optimum counting dilution}} \right)$$

All the figures used in the tables within this chapter are the viable count (cfu/ml) of bacterial flora enumerated from the chiropractors' hands.

4.4.1 Objective One: To determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post-spinal manipulation of patients, by means of hand swabs, serial dilution and plate counts.

4.4.1.1 Region of the spine manipulated

In order to ascertain the regions of the patients' spine manipulated by the 40 chiropractic practitioners, the areas of the spine that were manipulated were grouped into four regions. Due to the fact that the samples collected were from actual patients of these practitioners, the researcher was unable to pre-determine which areas of the spine would be manipulated. Patients who visit chiropractors usually seek treatment for low back pain (approximately 60%), neck, extremity and arthritic pain (20%), headaches (10%) and a wide variety of conditions caused, aggravated or mimicked by neuromusculoskeletal disorders (10%) (Meeker and Haldeman 2002; Ernst 2008; Evans *et al.* 2008; Logtenberg 2009).

The figure below indicates the regions of the spine that were manipulated (**Figure 4.1**). A little less than a quarter of the chiropractors (22.5%) had worked only on the thoracic spine. This was followed by cervical and thoracic spine manipulations (17.5 %) and cervical spine manipulations only (15%). The groups infrequently worked on were the cervical and lumbar spine (2.5%) and the thoracic spine, lumbar spine and sacroiliac joints (2.5%).

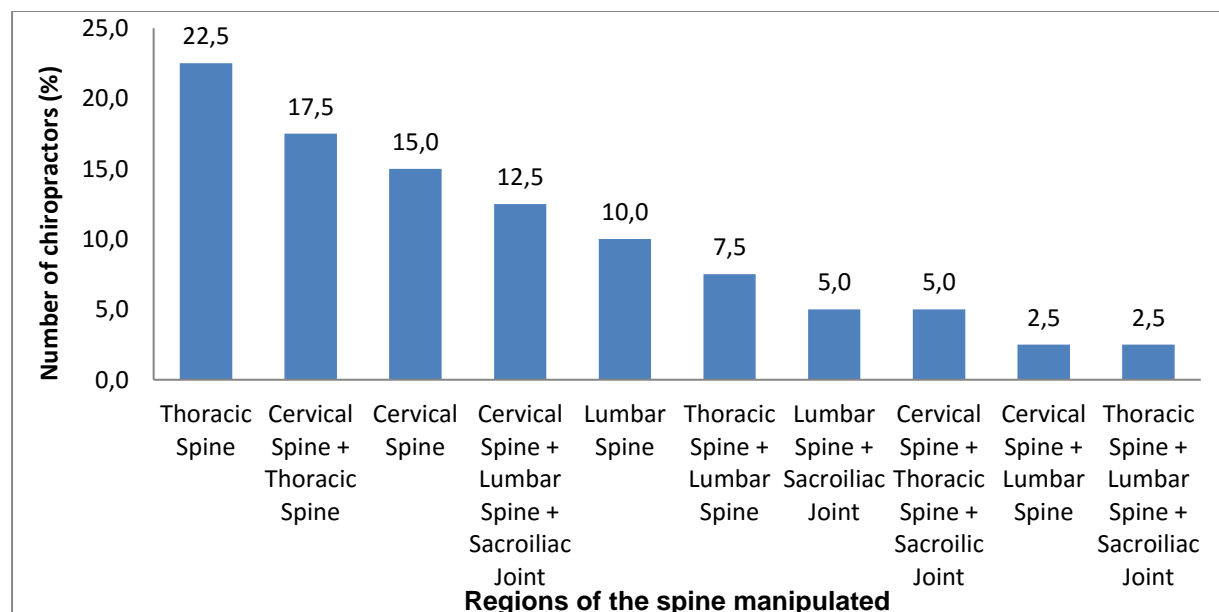


Figure 4.1: Distribution of regions of the spine manipulated by 40 chiropractors of 40 patients during a single treatment session.

4.4.1.2 The presence of bacterial flora on the chiropractors' hands

4.4.1.2.1 Comparison of viable count pre- and post-spinal manipulation

A total of 658,200 cfu/ml⁻¹, with a mean of 16,456 (27,718) cfu/ml⁻¹ was enumerated from the 40 chiropractors' hands. According to Larson (1998) the human skin contains between 10² and 10⁶ colony-forming units of bacterial flora per cm². The heterotrophic plate counts post-spinal manipulation were higher (496,380 cfu/ml⁻¹) than the pre-manipulation readings (161,820 cfu/ml⁻¹). Some post readings show significantly higher levels, whilst other post-manipulation levels are lower (but still greater than the pre-manipulation levels). Using a paired t-Test, a significant difference was noted in the viable count of bacteria found on the chiropractors' hands before and after manipulation ($p<0.001$).

It was evident that more bacteria was being transferred from the patient to the chiropractor during spinal manipulation. The transfer of bacteria from the patient to the chiropractor occurred in 70% of the cases, whereas the remaining 30% of the cases saw chiropractors transferring bacteria to their patients. **Table 4.1** illustrates the transfer of bacteria that occurred during the spinal manipulation of patients. In order to ascertain the direction of transfer, when the viable count from the pre-manipulation reading was higher than the post-manipulation reading it was indicative of a greater number of bacteria on the chiropractor's hands, which was transferred to the patient's skin during manipulation. This occurred in twelve (30%) of the cases (**Table 4.1**). This may be attributed to the chiropractors' hands being contaminated by a previous patient. It could also indicate poor hand hygiene protocols by the chiropractors between patients. As the day progresses, commensal and pathogenic micro-organisms accrue on health workers' hands from their daily activities, such as direct contact with patients and their bodily fluids (Pittet *et al.* 1999; Pittet *et al.* 2006; Carling, Parry and Von Beheren 2008).

When the viable count of the post-manipulation reading was higher than the pre-manipulation reading, a negative value was obtained. This occurred in 28 (70%) of the cases, where bacteria was considered to be transferred from the patient's skin to the chiropractor's hands during the manipulation. This could be due to poor personal hygiene habits of the patient or the number of spinal manipulations that the specific patient received, thus increasing the surface area with which the chiropractor made contact.

Table 4.1: The direction of transfer of bacteria occurring during spinal manipulation of 40 patients by 40 chiropractors during a single treatment session. Negative values indicated a transfer of bacteria from the patient's skin to the chiropractor's hands. Positive values (*) indicated a transfer of bacteria from the chiropractor's hands to the patient's skin.

| Chiropractor (Alphanumeric code) | Pre-Post Test (Direction of transfer) | Heterotrophic plate count | |
|-------------------------------------|--|------------------------------|-------------------------------|
| | | Pre Test (ml ⁻¹) | Post Test (ml ⁻¹) |
| *C110 | 1110 | 3090 | 1980 |
| C132 | -690 | 1620 | 2310 |
| C102 | -28500 | 1500 | 30000 |
| C125 | -690 | 1530 | 2220 |
| C120 | -4110 | 1200 | 5310 |
| C133 | -37410 | 1290 | 38700 |
| C119 | -960 | 960 | 1920 |
| C130 | -4710 | 1590 | 6300 |
| C139 | -61260 | 1440 | 62700 |
| C112 | -150 | 2160 | 2310 |
| C127 | -750 | 1320 | 2070 |
| C104 | -63150 | 1050 | 64200 |
| C124 | -1110 | 1470 | 2580 |
| C108 | -540 | 1020 | 1560 |
| C128 | -28800 | 1500 | 30300 |
| C117 | -570 | 2070 | 2640 |
| *C103 | 660 | 1860 | 1200 |
| *C137 | 1380 | 2010 | 630 |

| | | | |
|-------|--------|-------|-------|
| C129 | -33000 | 36300 | 69300 |
| C121 | -36660 | 2940 | 39600 |
| C107 | -450 | 1200 | 1650 |
| C115 | -30720 | 1980 | 32700 |
| C126 | -960 | 1620 | 2580 |
| C135 | -1080 | 1050 | 2130 |
| *C101 | 870 | 2430 | 1560 |
| C109 | -150 | 960 | 1110 |
| *C123 | 30 | 1170 | 1140 |
| C118 | -810 | 2130 | 2940 |
| *C134 | 28320 | 30600 | 2280 |
| C106 | -300 | 1470 | 1770 |
| *C116 | 3120 | 4260 | 1140 |
| *C129 | 480 | 1050 | 570 |
| *C111 | 1710 | 3180 | 1470 |
| *C131 | 27420 | 29700 | 2280 |
| C105 | -3000 | 2130 | 5130 |
| *C140 | 390 | 1140 | 1050 |
| C122 | -31200 | 2100 | 33300 |
| C138 | -27030 | 3270 | 30300 |
| C114 | -1200 | 1200 | 2400 |
| *C136 | 210 | 1260 | 1050 |

4.4.1.2.2 Total percentage of viable count

Staphylococci were most prevalent in the pre-spinal manipulation readings accounting for 53% of the colonies, followed by Micrococci with 39%, bacilli with 4%, *Staphylococcus aureus* with 3% and Streptococci with 1%. *Pseudomonas* spp. was present but they were uncommon. *E. coli* was not present pre-manipulation. The post-manipulation readings included a high prevalence of Micrococci accounting for 57% of the colonies, followed by Staphylococci with 32%; *Pseudomonas* spp. with 5%; *E.Coli* with 3%; *Staphylococcus aureus* with 2%; and Bacilli with 1%. Streptococci were present but uncommon.

In a study conducted by Khalifeh and Jafarpour (2003) samples collected from hospital members' hands included coagulase negative *Staphylococci* (72.9%); *Staphylococcus aureus* (30.5%); *Bacillus* (22%); *Klebsiella* (10.2%); *Streptococci* (3.4%); and *Escherichia coli* (1.7%). Khodavaissy *et al's.* (2011) study included 40 healthcare workers based in one hospital. The study resulted in contamination rates of 73.1% of hands with isolates including *Staphylococci* (23%); *Klebsiella* spp. (7.9%); *Enterobacter* spp. (4.7%); *Escherichia coli* (3.9%); *Acinetobacter* spp. (3.1%); and *Pseudomonas* spp. (2.3%).

4.4.1.3 Individual bacteria present pre- and post-manipulation

Table 4.2 illustrates the total viable count, mean and standard deviation for the individual bacteria found on the chiropractors' hands pre- and post-manipulation. It further indicates the number of chiropractors on which each bacterial isolate was located. *Staphylococcus*, *Micrococcus* and *Bacillus* were most commonly found on the chiropractors' hands pre-manipulation, before they made any contact with their patient, therefore indicating that these bacterial isolates originated from the chiropractor. The occurrence of *Pseudomonas* was minimal pre-manipulation, as it was found only on one chiropractor's hands. *E.coli* was not present on any of the chiropractors' hands before making contact with their patients.

Table 4.2 and **Table 4.3** depict the presence (P) and absence (A) of individual bacterial growth post-manipulation. The post-manipulation readings are derived from samples collected from the chiropractors' hands after they had made contact with their patients' spine. *Staphylococcus* and *Micrococcus* were also the highest number of bacterial isolates found on the patients' skin. *Staphylococcus epidermidis* and *Staphylococcus hominis* are the most widespread frequently occurring commensal bacteria found in the body and are known to occasionally cause healthcare-associated infections in patients with synthetic heart valves and intravenous catheters (Mandell, Bennette and Dolin 2010). *Micrococcus luteus* is the

most frequently occurring *Micrococcus* sp. found on the skin from the eight other species found and rarely result in infections, as they are commensal bacteria and have only known to cause opportunistic infections in immuno-compromised patients (Chiller, Selkin and Murakawa 2001).

Table 4.2: Bacteriological counts (means) pre- and post-manipulation for each bacterium.

| | n | Viabale count (cfu/ml ⁻¹) | Mean | Std. Deviation |
|--|----|--|-------|----------------|
| Pre Test (ml ⁻¹) | 40 | 161820 | 4046 | 8193 |
| Post Test (ml ⁻¹) | 40 | 496380 | 12410 | 19525 |
| <i>Staphylococcus</i> sp. Pre-test | 28 | 85860 | 3066 | 6459 |
| <i>Staphylococcus</i> sp. Post-test | 20 | 158910 | 7946 | 13230 |
| <i>Micrococcus</i> sp. Pre-test | 26 | 62040 | 2386 | 4943 |
| <i>Micrococcus</i> sp. Post-test | 31 | 279330 | 9011 | 15609 |
| <i>Bacillus</i> sp. Pre-test | 13 | 7200 | 554 | 531 |
| <i>Bacillus</i> sp. Post-test | 9 | 6930 | 770 | 656 |
| <i>Streptococcus</i> sp. Pre-test | 2 | 1200 | 600 | 594 |
| <i>Streptococcus</i> sp. Post-test | 3 | 1140 | 380 | 121 |
| <i>Pseudomonas</i> sp. Pre-test | 1 | 420 | 420 | 0 |
| <i>Pseudomonas</i> sp. Post-test | 6 | 22560 | 3760 | 7015 |
| <i>E. coli</i> Pre-test | 0 | 0 | 0 | 0 |
| <i>E. coli</i> Post-test | 8 | 16050 | 2006 | 3031 |
| <i>Staphylococcus aureus</i> Pre-test | 4 | 5400 | 1350 | 1945 |
| <i>Staphylococcus aureus</i> Post-test | 5 | 11430 | 2286 | 2970 |

n=number of chiropractors.

Table 4.3: Bacteria found during spinal manipulation occurring in specific regions of the spine.

| Bacteria | Group (Spinal region) | | | | | | | | | | | | |
|------------------------------|-----------------------|----------------|--------------|------------------|---------------------------------|-------------------------------|-----------------------------------|-------------------------------|---------------------------------|--|--|--|-------------------------|
| | Cervical Spine | Thoracic Spine | Lumbar Spine | Sacroiliac Joint | Cervical Spine + Thoracic Spine | Cervical Spine + Lumbar Spine | Cervical Spine + Sacroiliac Joint | Thoracic Spine + Lumbar Spine | Lumbar Spine + Sacroiliac Joint | Cervical Spine + Thoracic Spine + Sacroiliac Joint | Cervical Spine + Lumbar Spine + Sacroiliac Joint | Thoracic Spine + Lumbar Spine + Sacroiliac Joint | Number of times present |
| <i>Staphylococcus</i> | P | P | P | A | P | A | A | P | P | P | P | P | 9 |
| <i>Micrococcus</i> | P | P | P | A | P | P | A | P | P | P | P | A | 9 |
| <i>Bacillus</i> | P | P | P | A | P | A | A | A | A | A | A | P | 5 |
| <i>Streptococcus</i> | A | P | A | A | P | A | A | A | A | A | A | A | 2 |
| <i>Pseudomonas</i> | P | P | P | A | P | A | A | A | A | A | P | A | 5 |
| <i>E. coli</i> | A | A | P | A | A | P | A | P | P | P | P | A | 6 |
| <i>Staphylococcus aureus</i> | P | P | A | A | P | A | A | A | A | P | A | A | 4 |

A= Growth was absent; P= Growth was present

4.4.1.4 Comparison of mean values pre- and post-manipulation

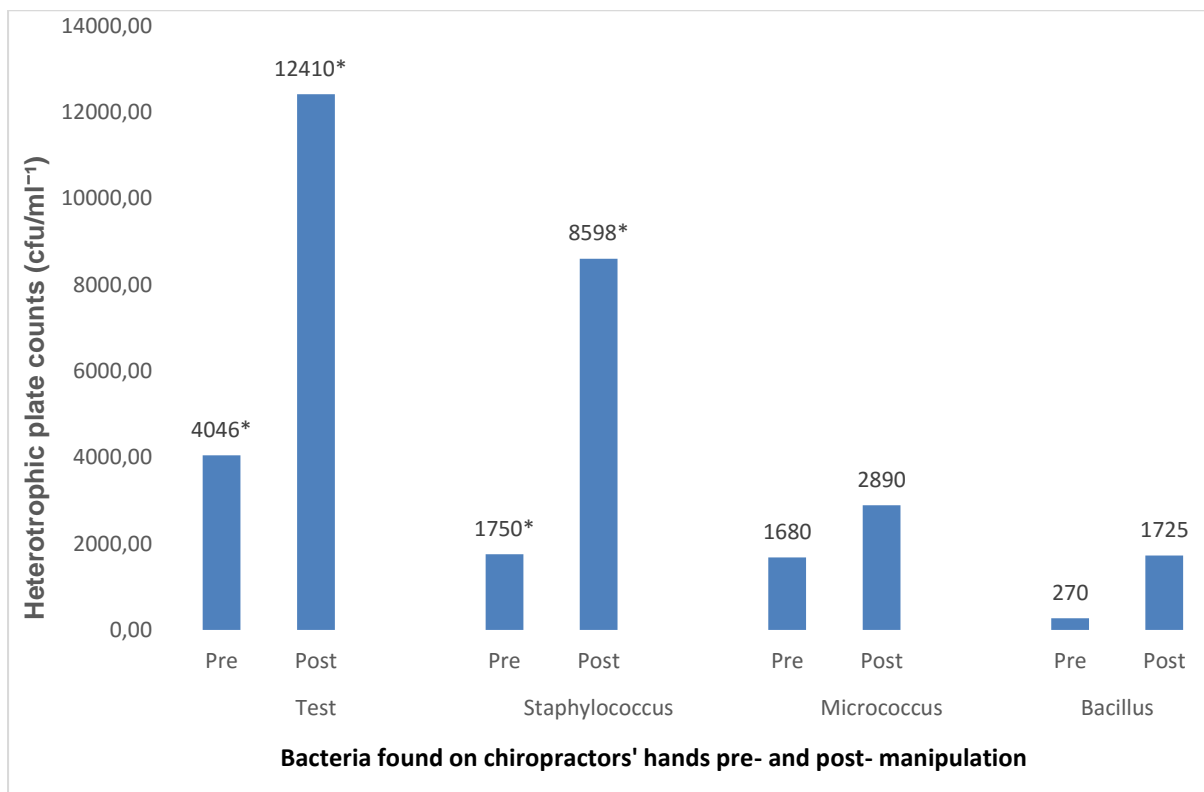
In order to determine whether the levels of bacteria pre- and post-manipulation were significant, a Wilcoxon paired test was conducted (**Table 4.4**). The analysis included only valid pairs of results. In some instances, the Wilcoxon Signed Ranks Test could not be performed, as paired readings were not observed and no statistical analysis could be carried out for those bacteria. A significant difference was only noted in the heterotrophic plate count ($p=0.004$) and the viable count of *Staphylococcus* ($p=0.015$). There was no statistical significant difference noted with viable counts of *Micrococcus* and *Bacillus*.

Figure 4.2 represents the mean values of paired readings for the heterotrophic plate counts, *Staphylococcus*, *Micrococcus* and *Bacillus*. The variance of the mean values before and after manipulation is noted, with the post-manipulation readings higher than the pre-manipulation readings in each case.

Table 4.4: Wilcoxon signed ranks Test to establish difference in mean values for valid paired results pre- and post-spinal manipulation by 40 chiropractors of 40 patients during a single treatment session.

| | | Mean No. of bacteria (cfu/ml ⁻¹) | Wilcoxon Signed Ranks Test Sig. value |
|----------------------------|-----------|---|---|
| Heterotrophic plate counts | Pre-test | 4046 | |
| | Post-test | 12410 | .004* |
| <i>Staphylococcus</i> sp. | Pre-test | 1750 | |
| | Post-test | 8598 | .015* |
| <i>Micrococcus</i> sp. | Pre-test | 160 | |
| | Post-test | 2890 | .409 |
| <i>Bacillus</i> sp. | Pre-test | 270 | |
| | Post-test | 1725 | .180 |

*means are significantly different at p<0.05



*means are significantly different at p<0.05

Figure 4.2: Mean values of paired readings for valid paired results pre- and post-manipulation by 40 chiropractors of 40 patients over a one month period.

The region of the spine most frequently manipulated was the thoracic spine (**Table 4.5**), however the highest readings of micro-organisms noted were found in the lumbar and sacroiliac regions of the spine. This may be attributable to the close location to the intergluteal cleft, and thus the perineum.

Table 4.5: Mean values of heterotrophic plate count pre- and post-manipulation in the various regions of the spine of the 40 patients manipulated by the 40 chiropractors during a single treatment session.

| Spinal Region | | Pre-Test (cfu/ml ⁻¹) | Post-Test (cfu/ml ⁻¹) |
|--|-----------------------|----------------------------------|-----------------------------------|
| Cervical Spine | n | 6 | 6 |
| | Mean | 5985 | 1865 |
| | Std. Deviation | 11620 | 703 |
| Thoracic Spine | n | 9 | 9 |
| | Mean | 4897 | 1820 |
| | Std. Deviation | 9648 | 683 |
| Lumbar Spine | n | 4 | 4 |
| | Mean | 1635 | 10005 |
| | Std. Deviation | 490 | 15249 |
| Cervical Spine + Thoracic Spine | n | 7 | 7 |
| | Mean | 1920 | 19684 |
| | Std. Deviation | 1099 | 24594 |
| Cervical Spine + Lumbar Spine | n | 1 | 1 |
| | Mean | 3090 | 1980 |
| | Std. Deviation | | |
| Thoracic Spine + Lumbar Spine | n | 3 | 3 |
| | Mean | 1160 | 2210 |
| | Std. Deviation | 272 | 337 |
| Lumbar Spine + Sacroiliac Joint | n | 2 | 2 |
| | Mean | 2340 | 15885 |
| | Std. Deviation | 1188 | 20386 |
| Cervical Spine + Thoracic Spine + Sacroiliac Joint | n | 2 | 2 |
| | Mean | 1395 | 5805 |
| | Std. Deviation | 276 | 700 |
| Cervical Spine + Lumbar Spine + Sacroiliac Joint | n | 5 | 5 |
| | Mean | 9132 | 47340 |
| | Std. Deviation | 15212 | 18124 |
| Thoracic Spine + Lumbar Spine + Sacroiliac Joint | n | 1 | 1 |
| | Mean | 2160 | 2310 |
| | Std. Deviation | | |
| Total | n | 40 | 40 |
| | Mean | 4046 | 12410 |
| | Std. Deviation | 8193 | 19525 |

4.4.2 Objective Two: To classify the bacterial flora present on the chiropractic practitioners' hands pre and post- spinal manipulation using conventional identification tests, biochemical profiling and test kit staining characteristics and morphology.

4.4.2.1 Identification of the bacterial flora

The identity of seven genera of bacterial flora was established through the samples obtained from the 40 chiropractic practitioners' hands pre- and post-spinal manipulation. **Figure 4.3** depicts the macroscopic colony morphology of small (between 1 and 3 mm) punctiform white colonies that were circular in shape. Microscopic characteristics illustrated Gram-positive cocci arranged in grape-like clusters, indicative of the genus *Staphylococci*. *Staphylococcus epidermidis* and *Staphylococcus hominis* are the most widespread commensal bacteria found in the body, constituting more than 50% of normal skin flora; they are known to occasionally cause health care-associated infections in patients with synthetic heart valves and intravenous catheters (Archer 1995; Oumeish, Oumeish and Bataineh 2000). The most frequented location of staphylococci includes the upper trunk and cranial region (Chiller, Selkin and Murakawa 2001). The CDC (2005) reiterated the role that organisms from the genus *Staphylococcus* play in the dissemination of skin infections in the United States, resulting in an increased utilization of antibiotic treatment by patients.

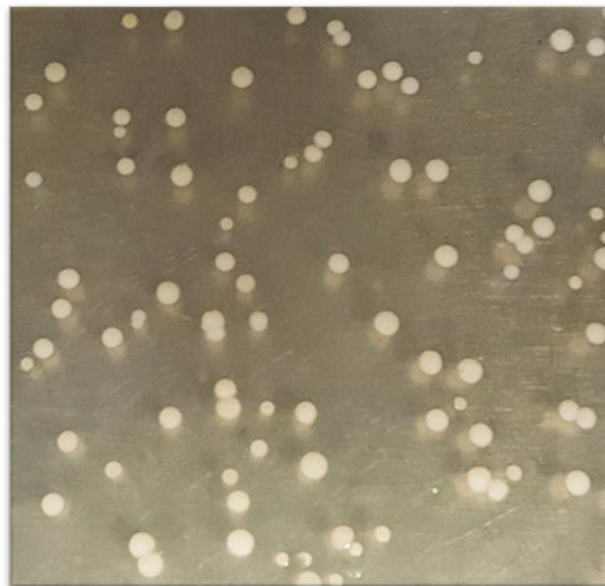


Figure 4.3: Colony morphology of *Staphylococcus* sp. showing small (between 1 and 3 mm) punctiform white colonies that was circular in shape grown on TSA for 24 hours at 37°C.

The second colony depicted the macroscopic morphology commonly seen in the genus micrococci (**Figure 4.4**). This included medium to large (between 3 mm and 10 mm) circular colonies that appeared yellow in colour. Their microscopic characteristics also appeared as gram-positive cocci, however they were distinguished from Staphylococci by performing the oxidation-fermentation test (Madigan, Martinko and Parker 2000). *Micrococcus luteus* is the most frequently occurring *Micrococcus* species found on the skin from the eight other species found, and also forms part of the body's natural constituents (Chiller, Selkin and Murakawa 2001).

Under normal circumstances, these commensal bacterial flora protect the host from the colonization of pathogenic bacteria. If, however, the host's immune system becomes compromised, as seen in patients with HIV/AIDS due to the excessive use of antibiotics, this results in exponential growth of these bacteria outside their natural habitat, inevitably resulting in infection (World Health Organisation 2002; Breathnach 2005; Moor and Ferguson 2006).

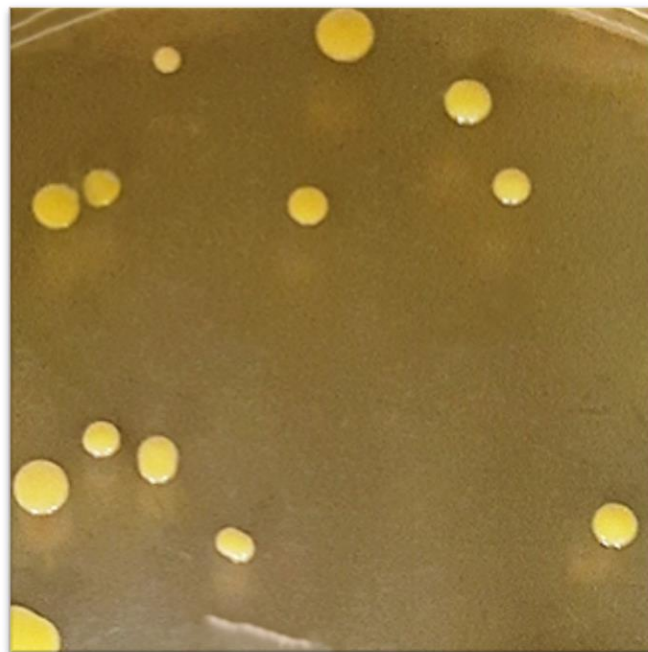


Figure 4.4: Colony morphology of *Micrococcus* sp. showing medium to large (between 3 mm and 10 mm) circular colonies that appeared yellow in colour grown on TSA for 30 hours at 37°C.

The macroscopic morphology of the third bacterial colony appeared as large (between 8 mm and 13 mm), irregular, and grey-white in colour with lobate margins (**Figure 4.5**). Microscopic findings showed gram-positive rod chains of the genus bacilli. A study conducted by Khalifeh and Jafarpour (2003) ascertained that 22% of organisms isolated from hospital members' hands were *Bacillus*. *Bacillus cereus* is a bacterium found in soil and food that is harmful to humans and may result in food-borne illnesses resulting in severe nausea, vomiting and diarrhoea (Koneman *et al.* 1997; Baveja 2005; Greenwood *et al.* 2007; Centers for Disease Control and Prevention 2016).

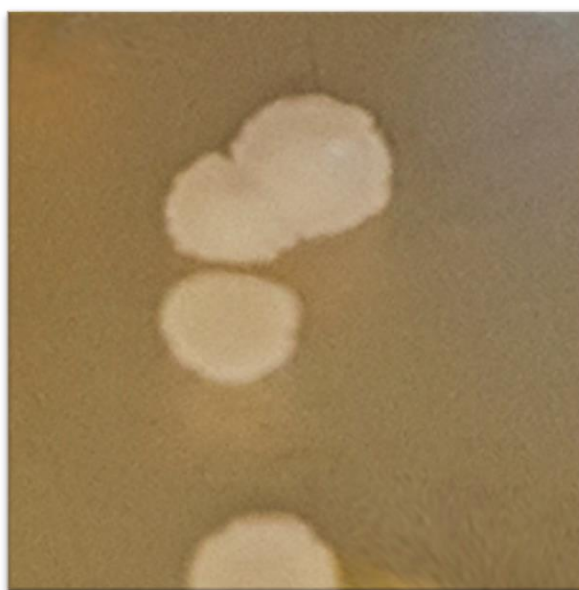


Figure 4.5: Colony morphology of *Bacillus* sp. showing large (between 8 mm and 13 mm), irregular, and grey-white in colour with lobate margins grown on TSA for 24 hours at 37°C.

The fourth bacterial colony, depicted in **Figure 4.6**, was small (1 mm), circular and pale yellow in colour. Microscopic characteristics matched those of the gram-positive cocci arranged in grape-like clusters indicative of the genus *Staphylococcus*. The isolates were then inoculated onto selective media such as Mannitol Salt Phenol Red agar (Sigma-Aldrich) as well as Baird Parker agar (Biolab) (Atlas 2010). **Figure 4.7** illustrates the Mannitol Salt Phenol Red agar turning yellow, thus serving as a positive test for the presence of *Staphylococcus aureus*. In order to authenticate these findings, the isolates were then inoculated onto the Baird Parker agar which turned black as a result of the reduction of tellurite in the medium, verifying the presence of *Staphylococcus aureus* as seen in **Figure 4.8**. *Staphylococcus aureus* is a gram-positive bacterium commonly residing on the skin and

nasopharyngeal passages of normal healthy individuals (Centers for Disease Control and Prevention 2005). It is frequently associated with the development of cutaneous infections, manifesting in serious atopic conditions that require antibiotic intervention (Banning 2005). It also possesses the pathogenic potential to cause a variety of life-threatening conditions, including respiratory, meningeal, osseous and cardiac-associated diseases (World Health Organisation 2005; Becker *et al.* 2007). This transient flora may colonize the epidermis of neonates, the external portion of the nostrils in approximately 20 - 40% of healthy individuals, as well as the skin of atopic patients (Strange *et al.* 1996; Banning 2005). It is also known to be the most common cutaneous bacterium associated with HIV-infected patients, instigating cutaneous diseases with potential life-threatening complications (Berger 1993).

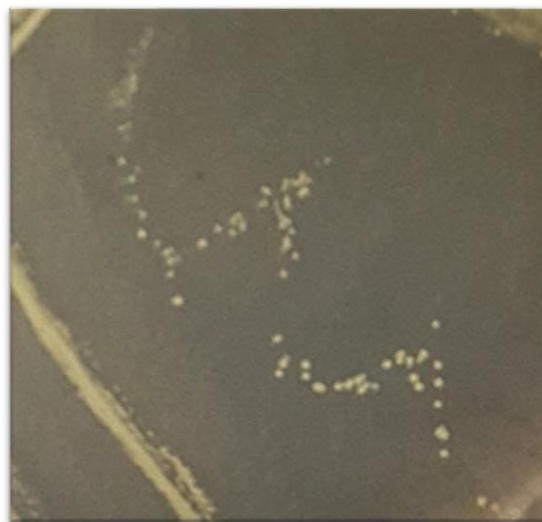


Figure 4.6: Colony morphology of *Staphylococcus aureus* showing small (1 mm), circular colonies pale yellow in colour grown on TSA for 36 hours at 37°C.

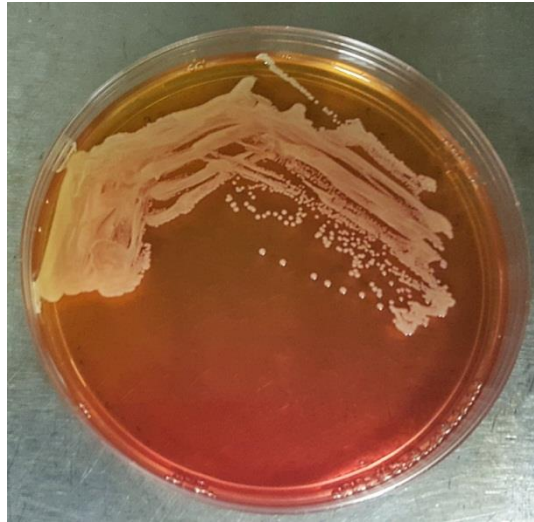


Figure 4.7: Mannitol Salt Phenol Red agar turns yellow indicative of the presence of *Staphylococcus aureus*.

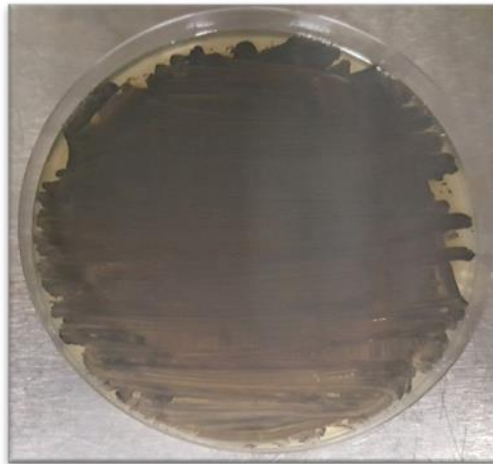


Figure 4.8: Baird Parker agar depicting a black growth indicative of *Staphylococcus aureus* as a result of the reduction of tellurite in the medium.

Figure 4.9 displays small (between 2 mm and 4 mm), punciform, circular, white colonies macroscopically. The microscopic findings demonstrated short, gram-negative rods suggestive of the bacterium *Escherichia coli*. *E. coli*, as it is commonly known, is a bacterial flora that also forms part of the body's natural constituents (Chiller, Selkin and Murakawa

2001; Struelens, Denis and Rodriguez-Villalobos 2004). In order to validate these findings, the isolates were inoculated onto MacConkey agar, where they grew pink in colour due to their ability to ferment lactose and produce acid, as seen in **Figure 4.10**. *E. coli* was one of the common pathogens responsible for healthcare-associated infections occurring between 1990 and 1996 resulting in diarrhoea, urinary tract infections, respiratory illness and pneumonia (Revelas 2012; Centers for Disease Control and Prevention 2016).

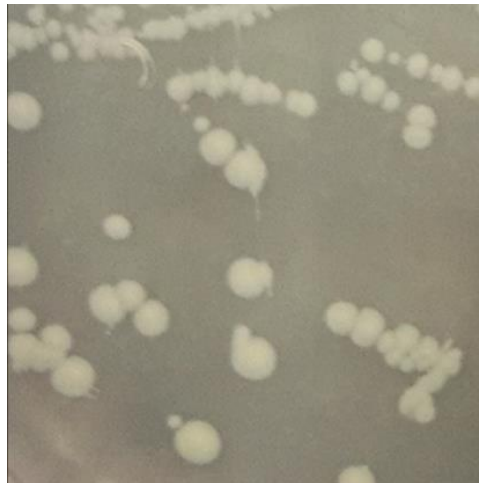


Figure 4.9: Colony morphology of *E. coli* showing small (between 2 mm and 4 mm), punctiform, circular, white colonies grown on TSA for 36 hours at 37°C.



Figure 4.10: *E. coli* producing acid, thus lowering the pH of the MacConkey agar to below 6.8 and resulting in the appearance of pink colonies due to lactose fermentation.

The macroscopic morphology of the sixth colony appeared as medium sized (between 4 mm and 7 mm), circular colonies that were white-beige in colour. Microscopic characteristics included gram-negative rods indicative of the *Pseudomonas* spp. The oxidase and catalase tests performed on these isolates were both positive. Between January 2006 and October 2007, a total of 33,848 pathogenic micro-organisms were instigated in the dissemination of healthcare- associated infections, with *Pseudomonas aeruginosa* responsible for 8% of those infections (Hidron *et al.* 2008). *Pseudomonas aeruginosa* is known to be an antimicrobial-resistant pathogen and is commonly related to ventilator-associated pneumonia and various sepsis syndromes (Hidron *et al.* 2008).

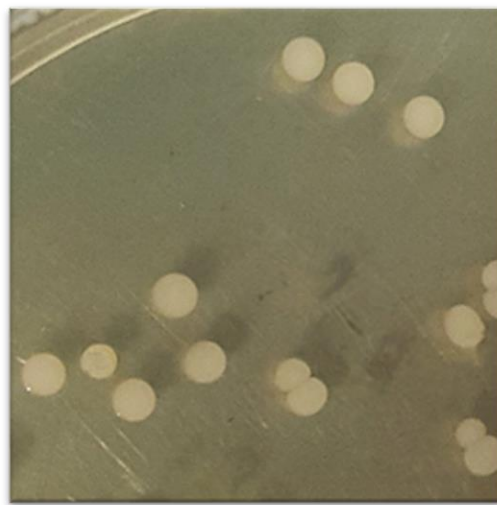


Figure 4.11: Colony morphology of *Pseudomonas* sp. Showing medium sized (between 4 mm and 7 mm), circular colonies that were white-beige in colour grown on TSA for 24 hours at 37°C.

Figure 4.12 macroscopically illustrates small (1 mm) circular colonies that appear grey-white in colour. Microscopic characteristics include gram-positive cocci in chains associated with the *Streptococcus* spp. A negative catalyse test was produced. Healthcare-associated infections were primarily caused by the *Streptococcus* species in the late 1800's (Revelas 2012).



Figure 4.12: Colony morphology of *Streptococcus* sp. Showing small (1 mm), circular colonies that appeared grey-white in colour grown on TSA for 36 hours at 37°C.

4.4.2.2 Pathogenicity of bacteria isolated

As highlighted by the CDC (2005) organisms from the genus *Staphylococcus* are one of the most common causes of minor skin infections in the USA, with a minority of these infections becoming severe and requiring antibiotic treatment. *Staphylococcus aureus* and *Streptococcus* are found on the skin and in the nasopharyngeal regions of healthy individuals (Chiller, Selkin and Murakawa 2001; Struelens, Denis and Rodriguez-Villalobos 2004); both have the potential to cause skin infections. *Streptococcus* spp. and *Pseudomonas* spp. are causative bacteria associated in the pathogenesis of pneumonia (Revelas 2012; Centers for Disease Control and Prevention 2016). *E. coli* are the bacteria prevalent in urinary tract infections, resulting in cystitis and nephritis (Revelas 2012; World Health Organisation 2005). The prevalence of micrococci in such high quantities is attributed to the fact that it forms part of the body's natural constituents (Chiller, Selkin and Murakawa 2001; Struelens, Denis and Rodriguez-Villalobos 2004). These commensal bacteria, with their low virulence rates have the potential to cause pathogenic contagion within their host in cases of suppressed immune systems, thus subjugating the host's defence mechanism (Breathnach 2005; Moor and Ferguson 2006). Under normal circumstances, these commensal bacteria protect the host from colonisation by pathogenic flora. In cases when the host becomes immuno-compromised (as seen in HIV/AIDS patients) excess antibiotic treatment results in an exponential growth of these bacteria outside their natural habitat, which inevitably results in infection (World Health Organisation 2002).

4.4.3 Objective Three: To determine the efficacy of decontaminants used by chiropractic practitioners, against isolated bacteria using a modified Kirby Bauer technique.

4.4.3.1 Decontaminants commonly used by chiropractic practitioners

The researcher selected the five most frequented decontaminants used by chiropractic practitioners in private practice. These included Dettol original hand wash (**Figure 4.13**); D-Germ hand disinfectant (**Figure 4.14**); TCP liquid disinfectant (**Figure 4.15**); Dis-Chem instant hand sanitizer (**Figure 4.16**); and Radox herbal hand wash (**Figure 4.17**).



Figure 4.13: Dettol original hand wash.



Figure 4.14: D-Germ hand disinfectant.

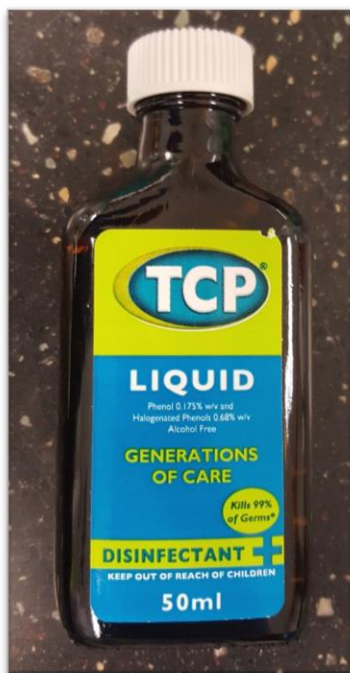


Figure 4.15: TCP liquid disinfectant.



Figure 4.16: Dis-Chem hand sanitizer.



Figure 4.17: Radox herbal hand wash.

4.4.3.2 Testing the efficacy of decontaminants used by chiropractic practitioners, against isolated bacteria using a modified Kirby Bauer technique.

The Kirby Bauer technique was used to ascertain the sensitivity and resistance of micro-organisms to various antibiotics. The test may be modified and used to determine the resistance of micro-organisms to decontaminants. This simple and inexpensive test is utilised to visually represent the sensitivity of bacteria to the decontaminants via a zone of inhibition (Pollack *et al.* 2002; Logtenberg 2009). Pre-standardized zones of inhibition have been established for individual antibiotics. The results can be categorized as sensitive, intermediate or resistant based on the resultant size of the zones of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the micro-organism being tested is deemed to be sensitive to the antibiotic which is characterized by a large zone of inhibition. Conversely, if the observed zone of inhibition is smaller than the standard size of the zone of inhibition, the micro-organism is considered to be resistant. An intermediate zone of inhibition indicates micro-organism resistance occurring between the sensitive and resistant categories.

Table 4.6 represents the mean zone of inhibition of the decontaminants against the bacteria isolated from the pre- and post- spinal manipulation readings. The overall mean zone of inhibition for each decontaminant was then compared to the positive control used (ciprofloxacin). A significant difference was noted between the performance of the positive control and the decontaminants on the bacteria isolated. None of the decontaminants tested, besides D-Germ hand disinfectant, were as effective against the bacteria isolated as the positive control. The decontaminants were most effective against gram-positive bacteria such as *Bacillus*, *Micrococcus*, *Staphylococcus*, *Staphylococcus aureus* and *Streptococcus* sp. (Figure 4.18). *E. coli* (Figure 4.19) and *Pseudomonas*, both gram-negative bacteria, were resistant to the decontaminants. Logtenberg (2009) conducted a study to assess chiropractic beds as reservoirs for micro-organisms at a chiropractic teaching clinic. It was found that the disinfectant employed to clean the beds was most effective against Gram-positive staphylococci, micrococci and bacilli than the gram-negative *Serratia*. This is possibly due to the inability of the disinfectant to penetrate into the cells found in gram-negative bacteria owing to their extra lipopolysaccharide layer (Denyer and Stewart 1998).

Table 4.6: Mean of three zones of inhibition of decontaminants against seven bacterial genera identified on 40 chiropractors' hands during spinal manipulation (Zone of inhibition for a positive control of ciprofloxacin at (3 mg/mL)).

| Bacteria isolated | Dettol original hand wash | D-GERM hand disinfectant | TCP liquid disinfectant | Dis-Chem instant hand sanitizer | Radox herbal hand wash | Positive Control– Ciprofloxacin (antibiotic) |
|------------------------------|---------------------------|--------------------------|-------------------------|---------------------------------|------------------------|--|
| <i>Bacillus</i> sp. | 10.90 | 15.30 | 6.00 | 0.00 | 8.60 | 34.50 |
| <i>Escherichia coli</i> | 0.00 | 13.30 | 0.00 | 0.00 | 0.00 | 36.00 |
| <i>Micrococcus</i> sp. | 9.30 | 14.00 | 7.30 | 0.00 | 7.30 | 32.00 |
| <i>Pseudomonas</i> sp. | 6.00 | 9.20 | 4.60 | 0.00 | 3.30 | 30.50 |
| <i>Staphylococcus</i> sp. | 21.00 | 29.40 | 14.00 | 0.00 | 17.50 | 36.50 |
| <i>Staphylococcus aureus</i> | 8.60 | 15.00 | 0.00 | 0.00 | 7.00 | 20.00 |
| <i>Streptococcus</i> sp. | 2.30 | 5.20 | 0.00 | 0.00 | 0.00 | 26.00 |
| Overall Mean | 8.30 | 14.49 | 4.56 | 0.00 | 6.24 | 30.79 |
| Standard Deviation | 6.82 | 7.52 | 5.18 | 0 | 6.06 | 5.98 |

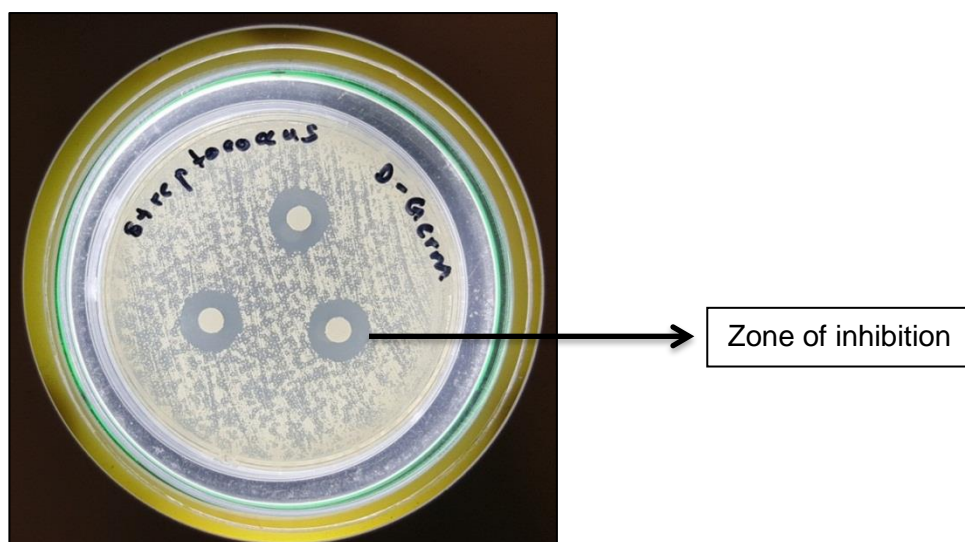


Figure 4.18: Modified Kirby Bauer test indicating the zones of inhibition using *Streptococcus* isolated from the chiropractors' hands against D-Germ hand disinfectant.

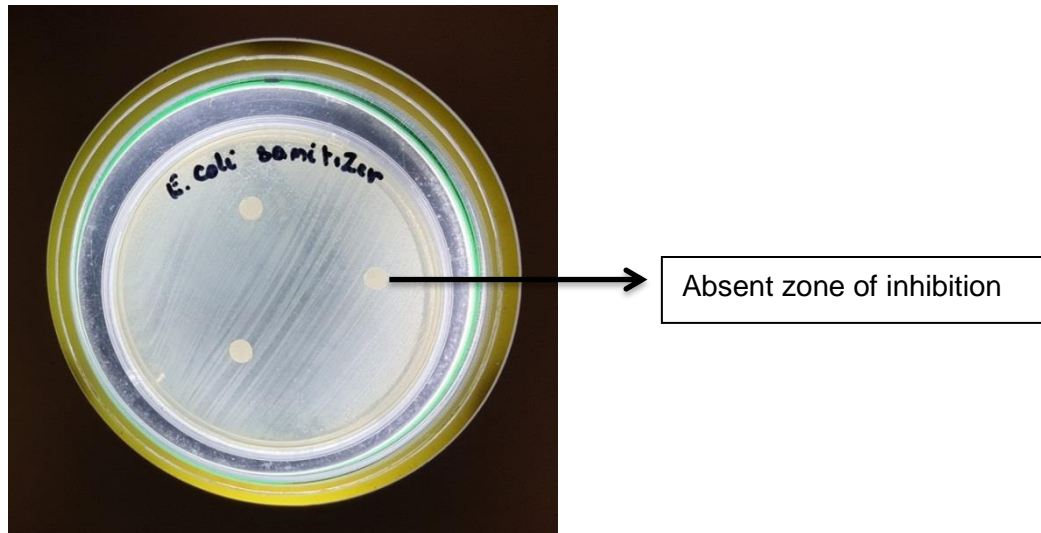


Figure 4.19: Modified Kirby Bauer test indicating the zones of inhibition using *E. coli* isolated from the chiropractors' hands against Dis-Chem instant hand sanitizer.

Ciprofloxacin (Fluka, Biochemika) (3 mg/mL) was used as a positive control in this experiment in order to ascertain the antimicrobial susceptibility of each bacterial isolate found pre- and post-spinal manipulation (**Table 4.7**). The test was conducted in duplicates for each bacterium (**Figure 4.20**). An average zone of inhibition was determined, and then compared with a standardized range of inhibition. This enabled the researcher to establish the antimicrobial susceptibility of each bacterium and compare these values to those generated from the modified Kirby Bauer test conducted using the decontaminants.

Table 4.7: The antimicrobial susceptibility of isolated bacteria tested against 3 mg/mL ciprofloxacin using the Kirby Bauer method.

| Organism | Positive Control – Ciprofloxacin (antibiotic) | | | | Antimicrobial susceptibility | | |
|------------------------------|---|---------------------------|---------------------------------|---------------------------------|------------------------------|--------------|-----------|
| | Zone of inhibition 1 (mm) | Zone of inhibition 2 (mm) | Average zone of inhibition (mm) | Normal range of inhibition (mm) | Susceptible | Intermediate | Resistant |
| <i>Escherichia coli</i> | 37 | 35 | 36 | 30 - 40 | √ | | |
| <i>Bacillus</i> | 35 | 34 | 34.5 | 25 - 35 | √ | | |
| <i>Staphylococcus aureus</i> | 23 | 23 | 23 | 21 - 27 | | √ | |
| <i>Micrococcus</i> | 33 | 31 | 32 | 30 - 38 | √ | | |
| <i>Streptococcus</i> | 26 | 26 | 26 | 22 - 28 | √ | | |
| <i>Staphylococcus</i> | 37 | 36 | 36.5 | 30 - 40 | √ | | |
| <i>Pseudomonas</i> | 30 | 31 | 30.5 | 25 - 33 | √ | | |



Figure 4.20: Kirby Bauer test indicating the zones of inhibition using *Micrococcus* isolated from the chiropractors' hands against the positive control (ciprofloxacin).

Table 4.8 ranks the overall mean of the zone of inhibition for each decontaminant tested. It was noticed in **Figure 4.21** that the control had the highest overall mean and was most effective, while the Dis-Chem instant hand sanitizer ($p=9.71$) was the least effective decontaminant tested. The bacteria isolated were most susceptible to the D-Germ hand disinfectant, followed by Dettol original hand wash and Radox herbal hand wash. The bacteria were also resistant to the TCP liquid disinfectant and the Dis-Chem instant hand sanitizer, each demonstrating zone of inhibitions 4.56mm and 0.00mm respectively. *E. coli*, *Pseudomonas*, *Staphylococcus aureus* and *Streptococcus* were the most resistant bacteria to the decontaminants used by chiropractors.

Table 4.8: Overall mean of zone of inhibition for the positive control (ciprofloxacin) and each decontaminant used by the chiropractors.

| Decontaminants | Overall Mean (mm) |
|--|-------------------|
| Positive Control – Ciprofloxacin (antibiotic) | 30.79 (5.98) |
| D-GERM hand disinfectant | 14.49 (7.52) |
| Dettol original hand wash | 8.30 (6.82) |
| Radox herbal hand wash | 6.24 (6.06) |
| TCP liquid disinfectant | 4.56 (5.18) |
| Dis-Chem instant hand sanitizer | 0.00 (0) |

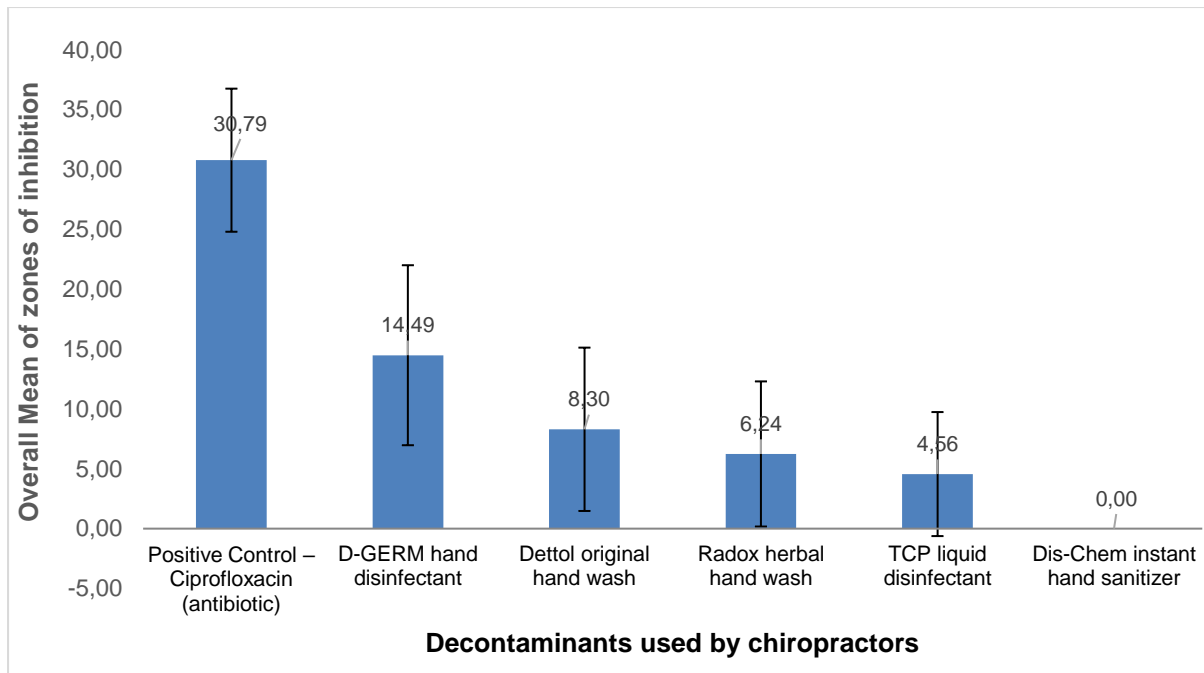


Figure 4.21: Mean and standard deviation of the zones of inhibition for each decontaminant used by chiropractors (+/- 1 standard deviation).

Table 4.9 illustrates paired comparisons of each decontaminant against each other. The Wilcoxon test is used (instead of the paired t-test) due to the non-parametric nature of the data. There was no significant difference noted between the D-Germ hand disinfectant and Dettol original hand wash, Radox herbal hand wash and Dettol original hand wash, and Dis-Chem instant hand sanitizer and TCP liquid.

Table 4.9: Paired comparisons of each decontaminant against one another.

| Wilcoxon Signed Ranks Test | p-value |
|---|---------|
| D-GERM hand disinfectant - Dettol original hand wash | 0.612 |
| TCP liquid disinfectant - Dettol original hand wash | 0.046* |
| Dis-Chem instant hand sanitizer - Dettol original hand wash | 0.043* |
| Radox herbal hand wash - Dettol original hand wash | 0.249 |
| TCP liquid disinfectant - D-GERM hand disinfectant | 0.018* |
| Dis-Chem instant hand sanitizer - D-GERM hand disinfectant | 0.018* |
| Radox herbal hand wash - D-GERM hand disinfectant | 0.042* |
| Dis-Chem instant hand sanitizer - TCP liquid disinfectant | 0.068 |
| Radox herbal hand wash - TCP liquid disinfectant | 0.043* |
| Radox herbal hand wash - Dis-Chem instant hand sanitizer | 0.028* |

*means are significantly different at $p < 0.05$

4.5 HYPOTHESES

The following deductions were made pertaining to the hypotheses made in chapter one:

Hypothesis One: Micro-organisms will be present on the chiropractic practitioners' hands pre- and post-spinal manipulation.

Hypothesis One was accepted as microbial growth was evident on 100% of the chiropractors' hands both pre- and post-spinal manipulation. A total of 658,200 cfu/ml⁻¹ was enumerated from the samples collected from the chiropractors' hands during manipulation.

Hypothesis Two: Micro-organisms will primarily be transferred from patients to chiropractors during spinal manipulation.

Hypothesis Two was accepted as the heterotrophic plate counts post-spinal manipulation were higher (496,380 cfu/ml⁻¹) than the pre-manipulation readings (161,820 cfu/ml⁻¹). Some post-manipulation readings show significantly higher levels whilst other post-manipulation levels are lower (although still greater than the pre-manipulation levels). This is an indication of a larger quantity of bacteria being transferred from the patient to the chiropractor during spinal manipulation. The transfer of bacteria from the patient to the chiropractor occurred in 70% of the cases, whereas the remaining 30% of the cases saw chiropractors transferring bacteria to their patients.

Hypothesis Three: The majority of micro-organisms isolated during the sampling procedure will consist of pathogenic species.

Hypothesis Three was accepted as the majority of the identified organisms were either primary or opportunist pathogens. These included *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *E. coli*, *Streptococcus* spp. and *Staphylococcus aureus*.

Hypothesis Four: The decontaminant used by the chiropractors will be effective against the majority of isolated bacteria.

Hypothesis Four is rejected as none of the tested decontaminants were as effective against the bacteria isolated as the positive control. It can therefore be deduced that each bacterial isolate is resistant to the decontaminants currently used by chiropractic practitioners in practice.

CHAPTER 5: EVALUATION OF THE RESEARCH, CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

In the previous chapter the results were presented and critically appraised in accordance with the aim and objectives of the research. This is the final chapter which comprises of the conclusions drawn from the research study, coupled with the limitations encountered and recommendations for future research and for the chiropractic profession.

5.1 Evaluation of the research

The aim of this study was to determine the presence of bacterial flora on the hands of chiropractic practitioners, as well as to determine the direction of transfer of the bacterial flora during spinal manipulation. Additionally, it also aimed to evaluate the efficacy of decontaminants used by the practitioners against the isolated bacteria cultivated from their hands. This study was conducted on chiropractors in private practice in South Africa as a cross-sectional investigation based in the quantitative experimental paradigm. The research question, aims and objectives were identified and discussed in previous chapters.

The first objective aimed to determine the presence and direction of transfer of bacterial flora on chiropractic practitioners' hands pre- and post-spinal manipulation of patients, by means of hand swabs, serial dilutions and plate counts. Bacteria were found on 100% of the 40 chiropractic practitioners' hands pre- and post-spinal manipulation. A significant difference was observed in the viable count of bacteria post-manipulation (70%), as opposed to pre-manipulation (30%). The direction of transfer was greater from the patient to the chiropractor in 70% of the instances sampled. The means between the counts on chiropractors' and patients was noted as significant, therefore, overall, the direction of transfer was from the patient to the chiropractor during spinal manipulation.

The second objective sought to classify the bacterial flora present on the chiropractic practitioners' hands using conventional identification tests, biochemical profiling and test kit staining characteristics and morphology. The bacteria identified included *Staphylococcus*, *Micrococcus*, *Bacillus*, *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas* and *E. coli*. Staphylococci were most prevalent in the pre-spinal manipulation readings accounting for 53% of the colonies, followed by micrococci with 39%, bacilli with 4%, *Staphylococcus aureus* with 3% and streptococci with 1%. *Pseudomonas* spp. were present but uncommon.

E. coli were not present on the chiropractors' hands in any of the samples obtained pre-manipulation. The post-manipulation readings constituted a high prevalence of micrococci, accounting for 57% of the colonies, followed by staphylococci with 32%, *Pseudomonas* spp. with 5%, *E. coli* with 3%, *Staphylococcus aureus* with 2% and bacilli with 1%. Streptococci were present but uncommon. Micrococci increased from 39 to 57% prevalence indicating a transfer from patients to chiropractors. Also *E. coli*, although absent on chiropractors hands was found on hands after manipulation and seemed to have been transferred to the chiropractors. *Staphylococcus aureus*, dropped by one percent post-manipulation indicating a small transfer to patients from chiropractors.

The third objective was to determine the efficacy of decontaminants used by the chiropractic practitioners, against the total number of bacteria isolated both pre- and post-spinal manipulation using a modified Kirby Bauer technique. The decontaminants tested were most effective against Gram-positive bacteria (96% of total bacteria isolated) such as *Bacillus*, *Micrococcus*, *Staphylococcus*, *Staphylococcus aureus* and *Streptococcus*. *E. coli* and *Pseudomonas* spp., both Gram-negative bacteria (4% of total bacteria isolated), were resistant to the decontaminants. The Gram-negative bacteria isolated were most susceptible to the D-Germ hand disinfectant with a resultant zone of inhibition that had an overall mean of 14.49 (7.52) mm, while the Dis-Chem instant hand sanitizer was the least effective decontaminant tested as it did not inhibit the growth of any of the bacteria it was tested against. Ciprofloxacin was the antibiotic used as a positive control. None of the decontaminants tested were as effective against the bacteria isolated as the positive control. A significant difference was noted between the performance of the positive control and the decontaminants on the bacteria isolated. The poor performance of the decontaminants may be owing to the fact that most were alcohol-based and the individual decontaminants may have evaporated before bacterial growth occurred. Alternatively, each bacterial isolate found on the chiropractors' hands could be resistant to most of the decontaminants currently used by these practitioners in practice. Conclusions were drawn in accordance with the three study objectives.

5.2 Conclusions drawn from the research study

A total of 658,200 cfu/ml⁻¹, with a mean of 16,456 (27,718) cfu/ml⁻¹ was enumerated from the 40 chiropractors' hands. The heterotrophic plate counts post-spinal manipulation were higher (496,380 cfu/ml⁻¹) than the pre-manipulation readings (161,820 cfu/ml⁻¹). It was evident in 28 (70%) of the cases that more bacteria were being transferred from the patients'

skin to the chiropractors' hands during spinal manipulation. The majority of the identified organisms being transferred to the chiropractors' hands were either primary or opportunistic pathogens, with 92% being Gram-positive bacteria and the remaining 8% Gram-negative bacteria. The Gram-positive bacteria isolated were most susceptible to the D-Germ hand disinfectant, followed by Dettol original hand wash and Radox herbal hand wash. The Gram-negative bacteria isolated (*Pseudomonas* and *E. coli*) were most resistant to the TCP liquid disinfectant and the Dis-Chem instant hand sanitizer, the latter being the least effective at inhibiting bacterial growth. Ciprofloxacin was used as the positive control in the experiment. None of the tested decontaminants were as effective against the bacteria isolated as the positive control. It had the highest overall mean zones of inhibitions and was most effective against all bacteria tested. *E. coli*, *Pseudomonas*, *Staphylococcus aureus* and *Streptococcus* were the most resistant bacteria to all five decontaminants tested as their growth was not inhibited during the modified Kirby-Bauer test carried out. This is a cause for concern considering the poor performance of these decontaminants presently used by the chiropractic practitioners in practice as each of these bacteria have been instigated in the dissemination of healthcare-associated infections. These bacteria are associated with life-threatening conditions, including respiratory illness and pneumonia, cystitis and nephritis, cardiac-associated diseases and various sepsis syndromes (World Health Organisation 2005; Becker et al. 2007; Hidron et al. 2008; Revelas 2012; Centers for Disease Control and Prevention 2016).

5.3 Research Limitations

- Samples were not obtained at exactly the same time each morning, as most chiropractic practitioners begin practice at varying times each day.
- Observational bias may have occurred, as the chiropractic practitioners may have altered their daily hand-washing routine because they were aware of being observed, thus altering the microflora on their hands.
- Only two (*Staphylococcus aureus* and *E. coli*) of the bacterial flora present on the chiropractic practitioners' hands were identified to a genus level due to cost constraints.
- Only bacteria were assayed for in this study.

- During the enumeration of colonies found on the Tryptone Soy agar plates, those plates containing colony-forming units between 30 and 300 were utilised to ensure reliability and validity.
- The alcohol-based decontaminants rapidly evaporated off the disks during the Kirby Bauer technique and may not have been present for the duration of the incubation period, resulting in low results for the efficacy of the decontaminant.
- This research was conducted in order for the researcher to acquire an academic qualification. This resulted in limitations to the study in terms of both budget and time.

5.4 Recommendations

5.4.1 Recommendations for future research

- A larger sample group should be used in future studies in order to avoid Type II errors. This is a false negative, and occurs when the null hypothesis is accepted when in fact it is actually false. The probability of a correct decision, in other words to reject the null hypothesis, is termed power. Power increases as the level of significance increases and as the sample size increases (Gardiner 1997; Campbell, Machin and Walters 2007; Blair and Taylor 2008).
- The research took place within the Durban Metropolitan Area. This limited the study as it only focused on a certain group of chiropractic practitioners. If the study was conducted with chiropractors from different regions and socio-economic backgrounds, it may have altered the results obtained.
- This research study only evaluated contamination that occurred during spinal manipulation. Research could be piloted on the manipulation of the extremity joints.
- Research needs to be conducted in order to assess the presence of micro-organisms on diagnostic equipment (for example stethoscopes, sphygmomanometer and thermometers) that chiropractic practitioners come into contact with, as well as the therapeutic machines used on their patients in order to treat them (Transcutaneous Electrical Nerve Stimulation machines, ultrasound machines and Interferential Current machines). Diagnostic equipment contaminated with micro-organisms has

the potential to act as a source of microbial transfer (Walker, Gupta and Cheesebrough 2006; Jain *et al.* 2013; Shiferaw *et al.* 2013). Without adequate infection control, these micro-organisms have the ability to survive for prolonged periods of time on inanimate objects (Kramer, Schwebke and Kampf 2006).

- Research could be conducted to assess the uniforms worn by chiropractors. Munoz-Price *et al.* (2012) expressed that healthcare workers' uniforms have the potential to serve as reservoirs for pathogenic organisms. Even though the healthcare workers have washed their hands, they are potentially re-infecting them by touching their contaminated uniforms.

5.4.2 Recommendations for the chiropractic profession

- Chiropractors need to be educated on the important role of hand hygiene practices during treatment of their patients in order to prevent healthcare-associated infections.
- Due to the significant bacterial transfer occurring during spinal manipulation, chiropractors should decontaminate their hands both pre- and post-manipulation.
- The infective nature of some of the pathogens found on patients' skin during the research study predisposed the chiropractor to infection. The researcher advocates decontaminating the patient's skin before treatment, in order to decrease the risk of contagion.

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Annexure A:



LETTER OF INFORMATION AND CONSENT

Welcome to my research study.

Principal Investigator/s/researcher: Fariya Amod (B: Tech)

Co-Investigator/s/supervisor/s: Dr. F.M. Swalaha (D: Tech)

Prof. P. Reddy (Ph.D)

Brief Introduction and Purpose of the Study: This study seeks to determine the presence of microorganisms during chiropractic spinal manipulation.

Outline of the Procedures: The researcher will visit the chiropractor's practice at an appointment scheduled by the receptionist. Swabs will then be taken of the chiropractor's hand pre- and post-spinal manipulation of the patient being treated which will require a maximum of five minutes of the chiropractor's time.

Risks or Discomforts to the Participant: None.

Benefits: Your contribution to this study, by volunteering to partake, will help us as chiropractors to build on our knowledge with regards to practitioner-patient hygiene, thus resulting in increased health care success rates in the future.

Reason/s why the Participant May Be Withdrawn from the Study:

You have the right to withdraw from the study at any given time without any adverse consequences.

Remuneration: None.

Costs of the Study: None.

Confidentiality: The results obtained from this study will be used for research purposes only. The individuals that are directly involved in this study (Dr. F.M. Swalaha, Prof. P. Reddy and Fariya Amod) will have access to these records. Confidentiality will be maintained throughout the study and no personal information or identifiers will be used in the write up of the dissertation. After a period of five years the data collected will be destroyed through shredding.

Research-related Injury: None.

Persons to Contact in the Event of Any Problems or Queries:

Please contact the researcher (Fariya Amod - 083 478 6505), supervisors (Dr. F.M. Swalaha - (031) 3732689 or Prof. P. Reddy (031) 373 280) or the Institutional Research Ethics administrator on 031 373 2900. Complaints can be reported to the DVC: TIP, Prof F. Otieno on 031 373 2382 or dvctip@dut.ac.za.

Thank you for participating in my research study.

Kind regards,

Fariya Amod (Master's student)



CONSENT

Statement of Agreement to Participate in the Research Study:

I hereby confirm that I have been informed by the researcher, Fariya Amod, about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: REC 115/15,

I have also received, read and understood the above written information (Participant Letter of Information) regarding the study.

I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.

In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the researcher.

I may, at any stage, without prejudice, withdraw my consent and participation in the study.

I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

| | | | |
|---------------------------------|-------------|-------------|-------------------------------------|
| | | | |
| Full Name of Participant | Date | Time | Signature / Right Thumbprint |

I, Fariya Amod herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

| | | |
|--------------------------------|-------------|------------------|
| | | |
| Full Name of Researcher | Date | Signature |

| | | |
|---|-------------|------------------|
| | | |
| Full Name of Witness (If applicable) | Date | Signature |

| | | |
|------------------------------------|-------------|------------------|
| | | |
| Full Name of Legal Guardian | Date | Signature |

(If applicable)

Annexure B: Letter to the head of Biotechnology and food technology



Professor Kugen Permaul

Head of the Biotechnology Department

Department of Applied Sciences

25 January 2016

Dear Sir,

My name is Fariya Amod and I am currently a 7th year Chiropractic student at the Durban University of Technology. I am in the process of completing my masters in Chiropractic which therefore requires me to complete a dissertation.

Healthcare workers' hands are known vehicles for transmission of healthcare-associated pathogens from patient to patient within the healthcare environment resulting in nosocomial infections. Therefore I have chosen to evaluate the presence of bacterial flora on Chiropractic Practitioners' hands pre- and post- spinal manipulation. The study will be based in the quantitative paradigm conducted as a cross-sectional investigation which will be subdivided into three main objectives, namely, verifying the presence of bacterial flora on the hands of Chiropractic practitioners', classifying the bacterial flora enumerated and determining the efficacy of decontaminants used by Chiropractic Practitioners.

The samples obtained from the Chiropractors' hands will be processed and analysed in the Microbiology laboratory, located on the Steve Biko campus, with the help of a laboratory technician. The results obtained will be recorded on allocated data collection sheets with random alphanumeric coding in order to preserve anonymity and ensure unbiased results.

I therefore require the use of the microbiology laboratory for a period of four months (February 2016 to May 2016) in order to process my data collected. I require no funding from the Biotechnology Department for the materials used in my research project as I am willing to bear these costs.

In accordance with ethical approval, permission from the Head of the Biotechnology Department is sought for the use of the Microbiology laboratory in order to carry out this research project as the faculty of Health Sciences research and ethics committee requires approval from the Head of the Biotechnology Department for use of this facility.

Your favourable response to the above matter will be greatly appreciated.

Yours sincerely,

Requested by: Ms. F. Amod

Date

Student No: 21010122

Recommended by: Dr F.M. Swalaha

Supervisor

Date

Recommended by: Prof P. Reddy

Co-Supervisor

Date

Approved By: Prof: Kugen Permaul

Head of the Biotechnology Department

Date

Annexure C:

Data collection sheet one:

DATE:

CODE:

GROUP:

PATIENT:

Pre- Manipulation reading ☐

Post- Manipulation reading ☐

| Serial dilution concentration | Undiluted Bacterial Culture | -1 | -2 | -3 |
|---|--------------------------------|----|----|----|
| | | | | |
| Number of colonies in 1 st set | | | | |
| Number of colonies in 2 nd set | | | | |
| Average number of colonies | | | | |

*TNTC = too numerous to count, TFTC = too few to count

Viable count (ml⁻¹)

= (Average number of cfu ÷ volume plated in ml) × optimum counting dilution

= (÷ 0,1ml) × optimum counting dilution

= () × ()

=

Adapted from (Logtenberg 2009)

Annexure D:

Data collection sheet two:

DATE:

CODE:

GROUP:

PATIENT:

Pre- Manipulation reading

☐

Average no. of colonies enumerated:

Post- Manipulation reading

☐

Serial dilution concentration:

Macroscopic colony morphology:

| |
|---------------------|
| Size: |
| Shape (form): |
| Colour: |
| Elevation: |
| Margin: |
| Surface Appearance: |
| Odour: |

Microscopic colony morphology:

| Gram stain | |
|-----------------|-----------------|
| <u>Positive</u> | <u>Negative</u> |
| | |

| |
|-----------------------------|
| Genus: |
| Specie: |
| Morphological shape: |
| Selective Media: |

Adapted from (Logtenberg 2009)

Annexure E:

Data collection sheet 3:

Modified Kirby Bauer

| Organism | Dettol original hand wash | | | |
|------------------------------|---------------------------|----------------------|----------------------|----------------------------|
| | Zone of inhibition 1 | Zone of inhibition 2 | Zone of inhibition 3 | Average zone of inhibition |
| <i>Escherichia coli</i> | | | | |
| Bacillus | | | | |
| <i>Staphylococcus aureus</i> | | | | |
| Micrococcus | | | | |
| Streptococcus | | | | |
| Staphylococcus | | | | |
| Pseudomonas | | | | |

| Organism | D-GERM hand disinfectant | | | |
|------------------------------|--------------------------|----------------------|----------------------|----------------------------|
| | Zone of inhibition 1 | Zone of inhibition 2 | Zone of inhibition 3 | Average zone of inhibition |
| <i>Escherichia coli</i> | | | | |
| Bacillus | | | | |
| <i>Staphylococcus aureus</i> | | | | |
| Micrococcus | | | | |
| Streptococcus | | | | |
| Staphylococcus | | | | |
| Pseudomonas | | | | |

| Organism | TCP liquid disinfectant | | | |
|------------------------------|-------------------------|----------------------|----------------------|----------------------------|
| | Zone of inhibition 1 | Zone of inhibition 2 | Zone of inhibition 3 | Average zone of inhibition |
| <i>Escherichia coli</i> | | | | |
| Bacillus | | | | |
| <i>Staphylococcus aureus</i> | | | | |
| Micrococcus | | | | |
| Streptococcus | | | | |
| Staphylococcus | | | | |
| Pseudomonas | | | | |


| Organism | Dis-Chem instant hand sanitizer | | | |
|------------------------------|---------------------------------|----------------------|----------------------|----------------------------|
| | Zone of inhibition 1 | Zone of inhibition 2 | Zone of inhibition 3 | Average zone of inhibition |
| <i>Escherichia coli</i> | | | | |
| Bacillus | | | | |
| <i>Staphylococcus aureus</i> | | | | |
| Micrococcus | | | | |
| Streptococcus | | | | |
| Staphylococcus | | | | |
| Pseudomonas | | | | |

| Organism | Radox herbal hand wash | | | |
|------------------------------|------------------------|----------------------|----------------------|----------------------------|
| | Zone of inhibition 1 | Zone of inhibition 2 | Zone of inhibition 3 | Average zone of inhibition |
| <i>Escherichia coli</i> | | | | |
| Bacillus | | | | |
| <i>Staphylococcus aureus</i> | | | | |
| Micrococcus | | | | |
| Streptococcus | | | | |
| Staphylococcus | | | | |
| Pseudomonas | | | | |

Control:

| Organism | Positive Control – Ciprofloxacin (antibiotic) | | | | Antimicrobial susceptibility | | |
|------------------------------|---|---------------------------|---------------------------------|---------------------------------|------------------------------|--------------|-----------|
| | Zone of inhibition 1 (mm) | Zone of inhibition 2 (mm) | Average zone of inhibition (mm) | Normal range of inhibition (mm) | Susceptible | Intermediate | Resistant |
| <i>Escherichia coli</i> | | | | | | | |
| Bacillus | | | | | | | |
| <i>Staphylococcus aureus</i> | | | | | | | |
| Micrococcus | | | | | | | |
| Streptococcus | | | | | | | |
| Staphylococcus | | | | | | | |
| Pseudomonas | | | | | | | |

Annexure F: IREC Approval

| | | |
|---|---|---|
|  |  | <p>Institutional Research Ethics Committee Faculty of Health Sciences Room MS 49, Mansfield School Site Gate 8, Ritson Campus Durban University of Technology</p> <p>P O Box 1334, Durban, South Africa, 4001</p> <p>Tel: 031 373 2900 Fax: 031 373 2407 Email: lavishad@dut.ac.za http://www.dut.ac.za/research/institutional_research_ethics</p> <p>www.dut.ac.za</p> |
|---|---|---|

23 October 2015

IREC Reference Number: **REC 115/15**

Ms F Amod
P O Box 1197
Umhlanga Rocks
4320

Dear Ms Amod

Origins and control of bacterial contamination during spinal manipulation

I am pleased to inform you that Provisional Approval has been granted to your proposal REC 115/15 subject to:

- Obtaining and submitting the necessary gatekeeper permission/s to the IREC.

Full approval is subject to meeting the above condition.


The Proposal has been allocated the following Ethical Clearance number **IREC 125/15**. Please use this number in all communication with this office.


Approval has been granted for a period of two years, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the IREC. This form must be submitted to the IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Yours Sincerely


Professor J K Adam
Chairperson: IREC



2015-10-23

INSTITUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA

Annexure G: IREC Permission to proceed

| | | |
|---|---|---|
|  |  | Institutional Research Ethics Committee Faculty of Health Sciences Room MS 49, Mansfield School Site Gate 8, Ritson Campus Durban University of Technology P O Box 1334, Durban, South Africa, 4001 Tel: 031 373 2900 Fax: 031 373 2407 Email: lavishad@dut.ac.za http://www.dut.ac.za/research/institutional_research_ethics www.dut.ac.za |
|---|---|---|

19 February 2016

IREC Reference Number: **REC 115/15**

Ms F Amod
P O Box 1197
Umhlanga Rocks
4320

Dear Ms Amod

Origins and control of bacterial contamination during spinal manipulation

The Institutional Research Ethics Committee acknowledges receipt of your gatekeeper permission letter.

Please note that **FULL APPROVAL** is granted to your research proposal. You may proceed with data collection.

Yours Sincerely,



Professor J K Adam
Chairperson: IREC


2016-02-19
INSTITUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA