Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants

by

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I, Johmari Logtenberg, declare that this dissertation is a representation of my own work in both conception and execution, except where acknowledgements indicate the contrary.

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DEDICATION

I would like to dedicate this dissertation to:

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ABSTRACT

Background: Healthcare-associated infections (HCAIs) are a global concern as they affect millions of people worldwide. Poor hygiene practices and the use of microbial contaminated medical equipment by healthcare workers (HCWs) are common contributing factors to the development of HCAIs, which result in additional hospital costs, prolonged hospital stays, development of antibacterial resistance and increases in mortality and morbidity. Because Chiropractic students (CSs) at the Durban University of Technology Chiropractic Day Clinic (DUT CDC) make use of diagnostic equipment during their consultations with patients, this study aimed to determine if the stethoscopes and sphygmomanometers that were used in the DUT CDC served as reservoirs for bacterial growth, including antibiotic-resistant bacteria, and to correlate the findings with the hygiene practices of CSs and the efficacy of disinfectants.

Method: This quantitative study comprised of two phases: a phase one pre-test post-test design and a phase two cross-sectional descriptive questionnaire design. Phase one required the collection of bacterial samples from the stethoscopes and sphygmomanometers of 29 CSs before and after performing the physical assessments on new patients (58 samples). These bacterial samples were incubated and analysed. The bacterial isolates were enumerated, identified and, where appropriate, tested for antibiotic-resistance. The modified AOAC use dilution method was used to test the efficacy of the selected disinfectants. Phase two required 29 CSs to complete the research questionnaire. The data were initially captured onto Excel spreadsheets and subsequently analysed using IBM SPSS version 24.0 (*p*-value <0.05 was considered statistically significant), with the application of Spearman's rank correlation, an one-way ANOVA evaluation, Tukey post hoc and paired *t*-tests.

Results: Although the majority of the CSs was knowledgeable and regarded disinfection as important, only 13.8% applied adequate disinfection practices. The most common reasons that were stated were inadequate education or training, forgetfulness, lack of time, and disinfectant unavailability. Bacterial growth was present on 96.6% and 100.0% of the pre-test and post-test stethoscope samples respectively, and on 94.8% and 100.0% of the pre- and post-test sphygmomanometer samples respectively. The total colony-forming units (CFUs) for both the post-test readings were higher compared to their respective pre-test samples. Paired *t*-tests indicated significantly ($p < 0.01$) higher mean values for the post-stethoscope group only, with a greater distribution of the total CFUs for stethoscope samples at the diaphragms' edge. The bacteria that were isolated from both sets of pre- and post-test samples consisted predominantly of coagulase negative staphylococci (CoNS), *Micrococcus* spp. and *Staphylococcus aureus*, while the minority consisted of *Bacillus* spp., C*orynebacterium* spp., coliforms and *Escherichia coli*. Overall, the majority of the bacteria was considered potentially pathogenic, except for the post-test sphygmomanometer sample.

The disinfectant efficacy testing revealed results that were in contrast with the literature, which caused the researcher to question the validity and reliability of the modified AOAC use dilution method in this study. Chloramphenicol was the most effective antibiotic with a bacterial susceptibility rate of 95.7%, (Ciprofloxacin (93.2%), Vancomycin (80.8%), Amoxicillin (AMO) (69.5%) and Erythromycin (57.7%)). The resistance of *Micrococcus* spp. isolates to the various antibiotics was of concern. The high resistance levels of CoNS and *S. aureus* to AMO suggests the presence of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant CoNS isolates. The most susceptible specie in general was *Micrococcus* spp. at 60.9%, followed by *S. aureus* at 59.6%, *E. coli* at 53.8%, and CoNS at 48.7%, while the least susceptible was coliforms at 36.9%. No correlations ($p > 0.05$) were identified between the mean CFU isolates from the CSs' equipment and their reported average disinfection rate.

Conclusions and recommendations: Both stethoscopes and sphygmomanometers were contaminated with non-pathogenic and potential pathogenic bacteria (some were resistant to multiple antibiotic classes). Although knowledgeable about equipment disinfection procedures, only 13.8% of the CSs reported disinfecting their pieces of equipment after examining every patient. The provision of adequate equipment disinfection education, the placement of visual reminders and accessible disinfectants will assist in improving the practice of adequate equipment disinfection. Moreover, equipment disinfection before and after every patient consultation will minimise cross-contamination and thus the risk of the development of HCAIs.

Key concepts: Chiropractic, bacteria, contamination, stethoscopes, sphygmomanometer, disinfection practices, antibiotic resistance, bacterial reservoirs, healthcare-associated infections

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GLOSSARY

Agar: A gelatinous substance consisting of complex sulphated polysaccharides obtained from red algae that is used as a solidifying agent in the preparations of biological culture media (Atlas 2010).

Antibiotic: A substance produced either synthetically or by a microorganism that, at a low concentration, can kill or prevent the growth of other microorganisms (Dreyer *et al.* 2013).

Antibiotic class: Consists of different drugs with similar chemical and pharmacological properties (Anderson 2016).

Antibiotic resistant bacteria: Bacteria that have a capacity to render an antibiotic ineffective in spite of previous sensitivity to the drug (World Health Organization 2016).

Antibacterial resistance: The ability of bacteria to undergo alterations to reduce or eliminate the effectiveness of drugs, chemicals or other agents used to cure or prevent infections (United States Department of Health: Centers for Disease Control and Prevention 2013).

Attitude: A settled way of thinking or feeling about something (Oxford living dictionaires 2017).

Autoclave: Apparatus for sterilization of objects with the use of steam under pressure (Park Talaro and Chess 2014).

Bacteria: A simple microscopic single-celled organism that lacks a true nucleus (Comptroller and Auditor General 2009).

Bacterial interference: Mechanism involving competition for nutrients or receptors on host cells and mutual inhibition by toxic products, antibiotic materials or bacteriocins (Brooks *et al*. 2013).

Broth: General term used for liquid growth medium containing proteins and other nutrients needed to culture bacteria (Atlas 2010).

Cleaning: Removal of foreign, organic or inorganic materials from a surface or an object through manual or mechanical means (United States Department of Health: Centers for Disease Control and Prevention 2016).

Colony: Growth of a microorganism visible with the naked eye on a solid culture medium (Tille 2014).

Colony-forming units: Indication of the number of viable bacteria in a sample as represented by the formation of colonies (Biology online 2008).

Contact transmission: Transmission of the pathogen by contact of the source or reservoir of the pathogen with the host (Willey *et al.* 2014).

Contamination: Presence of microorganisms in area where they do not belong (Swalaha 2013).

Cross-contamination: The unintentional transfer of bacteria from one patient to another with a harmful effect as a result of the usage of improper or unsterile equipment, procedures or products (Russotto *et al.* 2015).

Culture: A strain of a microorganism in a growth medium (Pollack *et al.* 2002).

Developed country: A country which is developed in terms of economy, technology and industrialization. Also referred to as a first world country. Characteristics include a high standard of living, high gross domestic product, excellent medical, transportation, communication and educational facilities, superior housing and living conditions (Surbhi 2015).

Developing country: A country that has undertaken initial levels of industrial development. Also referred to as a third world country. Characteristics include low human development index, low gross domestic product, high illiteracy rate, poor educational, transportation, communication and medical facilities, high mortality and birth rates, poor living conditions, high levels of unemployment and poverty (Surbhi 2015).

Disinfectant: A chemical or agent that destroys or inhibits microorganisms that cause disease, but not bacterial spores (Comptroller and Auditor General National Audit Office 2000).

Disinfection: Process of destruction and elimination of all vegetative pathogens, except bacterial spores, on inanimate objects (Swalaha 2016).

Droplet transmission: Microorganisms are spread in droplet nuclei that travel short distances during coughing, sneezing or laughing (Swalaha 2016).

Endemic: The constant presence of a disease or infectious agent within a given geographic area or population (World Health Organization 2015).

Endospore: An extremely, heat- and chemical-resistant, dormant spore that develops within some Gram-positive bacteria such as *Bacillus* and *Clostridium* (Willey *et al.* 2014).

Fomite: Any inanimate object that is not in itself harmful, but may be contaminated with pathogens and thus can transmit disease (Tille 2014).

Genus: A taxonomic group that is below a family and above a species level. It includes groups of species that are structurally similar or phylogenetically related (Biology-online 2016a).

Gram-negative bacteria: Bacteria characterized by their cell envelopes, which consists of a peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane (Biology-online 2014a).

Gram-positive bacteria: Bacteria whose cell walls are mainly composed of peptidoglycan and lack an outer membrane (Biology-online 2014b).

Hand hygiene: Any method used in order to remove and/or destroy microorganisms located on the hands (World Health Organization 2009).

Hawthorne effect: Changes in the behaviour of research participants in response to their awareness of being studied either through answering questionnaires or being directly observed. However, little is known about its mechanisms of effects and its magnitude (McCambridge, Witton and Elbourne 2014).

Healthcare-associated infection: An infection that a patient contracts while being treated for another condition in a hospital or any other healthcare facility (World Health Organization 2011).

Host: An organism that harbours and support the growth and multiplication of another organism (Willey *et al.* 2014).

Immunodeficiency: Failure of the immune system to adequately protect the body as a result of a disease, chemotherapy, irradiation or administration of certain medications (Tille 2014).

Incidence: The number of new events/episodes or new patients acquiring an infection per 100 patients in a population for a defined period of time in a given time period (World Health Organization 2015).

Incubation: Allowing microorganisms to grow at a constant optimal temperature (Park Talaro and Chess 2014).

Infection: Invasion by and multiplication of pathogenic microorganisms in body tissue resulting in disease (Comptroller and Auditor General 2009).

Inoculated: Having introduced microorganisms into a culture medium (Swalaha 2013).

Inoculum: Substance containing bacteria from a pure culture which is used to start a new culture (Pollack *et al.* 2002).

Knowledge: Facts, information, and skills acquired through experience or education; the theoretical or practical understanding of a subject (Oxford living dictionaries 2017a).

Macroscopic morphology: The appearance of bacterial growth as seen by the visible eye on agar plates (Swalaha 2013).

Microflora: Microorganisms that live in or on the skin of healthy people in set anatomical locations (Tille 2014).

Microorganism: A microscopic organism such as bacteria, fungi, protozoa and viruses (Park Talaro and Chess 2014).

Microscopic morphology: The shape and arrangement of cells as seen under a light microscope (Swalaha 2013).

Morbidity: The state of being diseased, or in a reduced state of health (Comptroller and Auditor General National Audit Office 2000).

Mortality: Death (Comptroller and Auditor General 2009).

Nosocomial infection: See healthcare-associated infection.

Pathogen: Organisms that are capable of causing an infection and/or disease (Swalaha 2016).

Perception: The ability to see, hear, or become aware of something through the senses (Oxford living dictionaries 2017b).

Practice: The actual application or use of an idea, belief, or method, as opposed to theories relating to it (Oxford living dictionaries 2017c).

Prevalence: The total number of infection episodes or infected patients per 100 patients of a specific disease in a given population at a given point in time (Comptroller and Auditor General 2009).

Pure culture: A culture consisting of only one type of microorganism (same genus and specie) (Swalaha 2013).

Reservoir: A site or carrier that harbours pathogens and serves as a source from which other individuals can be infected (Tille 2014).

Resident microorganisms: Microorganisms that are distributed in set areas of the body on a permanent basis and that have the ability to re-establish themselves if disturbed. They assist in preventing the growth of pathogens through bacterial interference (Brooks *et al.* 2013).

Risk factor: Any attribute, characteristic or exposure of an individual that increases the likelihood of them developing a disease or injury (World Health Organization 2015).

Species: A group of organisms comprised of similar individuals capable of exchanging genes or interbreeding. The taxonomic group species is the principal natural taxonomic unit and is ranked below genus (Biology-online 2016b).

Sterile: A sterile item that is totally free from any viable microorganisms, spores and other pathogenic agents, thus preventing any disease transmission associated with the usage of that item (Willey *et al.* 2014).

Sterilization: Procedures that remove or eradicate all living cells, spores and acellular entities from an object, fluid or habitat (Willey *et al.* 2014).

Transient microorganisms: Microorganisms which are only temporarily present on the skin as they are derived from the environment (Brooks *et al.* 2013).

Viable count: Number of living cells as represented by the formation of colonies on agar media (Boundless microbiology 2015).

Virulence: Degree of pathogenicity or disease-producing ability of a microorganism (Tille 2014).

Zone of inhibition: The clear region around the paper disc saturated with an antimicrobial agent on the agar surface (Biology-online 2008).

ABBREVIATIONS AND SYMBOLS USED IN THIS DISSERTATION

ABR:	Antibacterial resistance
AHPB:	Associated Health Professions Board
AMO:	Amoxicillin
ARB:	Antibiotic resistant bacteria
ARM:	Antibiotic resistant microorganisms
B:	Sphygmomanometer
BSI:	Blood stream infections
C:	Chloramphenicol
CDC:	Chiropractic Day Clinic
CFU:	Colony-forming unit
CFUs:	Colony-forming units
CIP:	Ciprofloxacin
CoNS:	Coagulase-negative staphylococci
CS:	Chiropractic student
CSs:	Chiropractic students
DUT:	Durban University of Technology
DUT CDC:	Durban University of Technology Chiropractic Day Clinic
E:	Erythromycin
ECCE:	European Council on Chiropractic Education
EMB:	Eosin Methylene-blue
EPA:	United States Environmental Protection Agency
F:	*F*-distributions
HCAI:	Healthcare-associated infection
HCAIs:	Healthcare-associated infections

HCWs: Healthcare workers

ICUs: Intensive care units

IREC: Institutional Research and Ethics Committee

KAP: Knowledge, attitudes and practices

MAC: MacConkey agar

MHA: Mueller-Hinton agar

mmHg: Millimetre of mercury

MRSA: Methicillin-resistant *Staphylococcus aureus*

MSA: Mannitol Salt agar

N/A: Not applicable

n: Refers to the sample size

NB: Nutrient broth

p: Refers to the *p*-value, which is the probability of getting the output observed

R^2: Coefficient of determination

r_s: Spearman's rho

S: Stethoscope

spp.: Species

SSI: Surgical site infections

t: *t*-value

TSA: Tryptic Soy agar

TSB: Tryptic Soy broth

USA: United States of America

USDH: CDCP: United States Department of Health: Centers for Disease Control and Prevention

UTI: Urinary tract infections

VA: Vancomycin

WFC: World Federation of Chiropractic

WHO:	World Health Organization
*:	Statistically significant

CHAPTER 1: INTRODUCTION

1.1 Introduction to the study

Healthcare-associated infections (HCAIs) are infections that patients contract during their stay in a healthcare facility while being treated for an unrelated condition (World Health Organization 2011). HCAIs are a global concern as indicated by a report that was released by the World Health Organization (WHO) (2011). This report stated that HCAIs affect an estimated 4,1 million Europeans each year. In the year 2002 alone, as many as 1,7 million Americans were affected. The ramifications of HCAIs are far reaching as this phenomenon results in an additional financial burden on the healthcare system, prolonged hospital stay, development of antimicrobial resistance and an increase in mortality and morbidity (World Health Organization 2015).

Bacteria commonly associated with HCAIs include *Staphylococcus aureus*, *Klebsiella* species (spp.), *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter* spp. and *Enterococcus* spp. (World Health Organization 2015). Kramer, Schwebke and Kampf (2006) found that the majority of these bacteria are capable of surviving up to several months on inanimate objects located within a healthcare facility. Russotto *et al.* (2015) established that contaminated fomites, such as stethoscopes and sphygmomanometers, could serve as reservoirs for bacterial growth and as sources for bacterial transfer to patients.

Even though most of the bacteria isolated from these two types of diagnostic equipment were found to be constituents of the human skin's microflora, a number of pathogenic bacteria with some exhibiting antibacterial resistance (ABR) were identified by various researchers (de Gialluly *et al.* 2006; Uneke *et al.* 2010; Gupta *et al.* 2014; Pal *et al.* 2015). Antibiotic resistance in different types of microorganisms results in the treatment of common infectious diseases becoming more challenging, while in some cases impossible (World Health Organization 2016). In the United States of America (USA), Methicillin-resistant *Staphylococcus aureus* (MRSA) were implicated in causing 80 461 HCAIs and contributing to 11 285 deaths in 2013 alone (United States Department of Health: Centers for Disease Control and Prevention 2017b).

The findings listed above highlight the importance of adequate infection control protocols. In this context, the World Health Organization (2011) reported that inadequate hygiene practices contributed towards the transmission of microorganisms. This pertains to the practice of both good hand hygiene and the adequate disinfection of diagnostic equipment. Davis (2009) established that various types of alcohol are effective against *E. coli, S. aureus, Ps. aeruginosa, Streptococcus pyogenes* and *Serratia marcescens.* It has therefore been recommended that alcohol be used for the disinfection of non-critical items such as stethoscopes and sphygmomanometer.

Gupta *et al.* (2014) determined that the disinfection of stethoscopes with 70% ethanol resulted in a significant decrease (86.7%) in bacterial counts. In a similar study, Núñez *et al.* (2000) reported a reduction of 99.8% in colony forming units (CFUs) after the disinfection with isopropyl alcohol compared to a reduction of 98.2% when ethanol was used.

It is strongly recommended to disinfect stethoscopes before and after each patient is examined (Saloojee and Steenhoff 2001; Jain *et al.* 2013; Shiferaw *et al.* 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014). However, in a study conducted by Tang *et al.* (2011), only 8.0% of healthcare workers (HCWs) reported disinfecting their stethoscopes before and after examining every patient. Pal *et al.* (2015) identified that a staggering 84% of participating HCWs had never disinfected their stethoscopes. Healthcare workers cited lack of time, forgetfulness, insufficient education, absence of visual reminders and limited access to disinfectants as reasons that hindered the practice of adequate stethoscope disinfection (Tang *et al.* 2011; Muniz *et al.* 2012; Saunders, Hryhorskyj and Skinner 2013).

Zaghi *et al.* (2013) determined that the placement of disinfectants and visual reminders at strategic locations could significantly increase the rates of stethoscope disinfection by HCWs. Both Grecia, Malanyaon and Aquirre (2008) and Uneke *et al.* (2014) established that an education campaign relating to stethoscope disinfection could have a positive influence on disinfection compliance and on reducing the contamination levels of stethoscopes.

Chiropractic students (CSs) at the Durban University of Technology Chiropractic Day Clinic (DUT CDC) make use of diagnostic equipment during their consultation with new patients. Therefore, the purpose of this study was to determine if the stethoscopes and sphygmomanometers used by the CSs in the DUT CDC served as reservoirs for bacterial growth, including antibiotic-resistant bacteria that may act as a source of microbial transfer, and to determine the hygiene practices of the students as well as the efficacy of disinfectants.

1.2 Research questions

- Do the stethoscopes and sphygmomanometers used by the CSs in the DUT CDC serve as reservoirs for bacterial growth and subsequently act as sources of bacterial transfer?
- Are any of the bacteria isolated from the above equipment resistant to currently used antibiotics?
- What are the hygiene practices of CSs and the efficacy of disinfectants?

1.3 Aim of the research

The aim of the study was to determine if the stethoscopes and sphygmomanometers used by the CSs in the DUT CDC served as reservoirs for bacterial growth and subsequently acted as sources of bacterial transfer, including pathogenic and antibiotic-resistant bacteria, and to determine the hygiene practices of students and the efficacy of disinfectants.

1.4 Objectives

1. To determine the presence, quantities and identification of bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC before and after 58 new patient consultations using conventional bacterial plate count techniques and identification tests.
2. To assess the efficacy of disinfectants used by CSs in the DUT CDC for cleaning stethoscopes and sphygmomanometers using a modified AOAC use dilution method.
3. To determine the presence and quantities of antibiotic-resistant bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC using therapeutic antibiotics with the disk diffusion assay to determine bacterial susceptibility to antibiotics.
4. To evaluate the hygiene practices of 29 CSs regarding the disinfection of their stethoscopes and sphygmomanometers using a structured questionnaire.
5. To determine if CSs' hygiene practices correlated with the data obtained regarding the bacterial contamination of equipment such as stethoscopes and sphygmomanometers.

1.5 Context and rationale

Sources of bacterial transfer resulting in HCAIs include medical personnel and the equipment they use. Such infections may either be due to poor hand hygiene or to the use of contaminated equipment such as stethoscopes and sphygmomanometers (Walker, Gupta and Cheesbrough 2006; Jain *et al.* 2013; Shiferaw *et al.* 2013). Both potential pathogenic and non-pathogenic bacteria have been found in hospital and chiropractic settings (Evans *et al.* 2008; Logtenberg 2009; Jain *et al.* 2013; Shiferaw *et al.* 2013; Campos-Murguia *et al.* 2014; Longtin *et al.* 2014). At the DUT CDC, these bacteria may arise from cross-contamination when CSs examine patients from either the private or public sector. This study aimed to determine the presence, quantities and identification of such bacteria, including antibiotic-resistant bacteria that occurred on stethoscopes and sphygmomanometers used by CSs in the DUT CDC. The investigation was in line with similar studies that had been conducted in hospital settings. Concerted efforts by the researcher could trace no published work relating to bacterial sampling of diagnostic equipment in a chiropractic clinic setting.

Burnham *et al.* (2009) concluded that changes in the disinfection policies implemented at the Western States Chiropractic College, after the initial sampling of adjustment tables, resulted in a decrease in the total number of bacterial colonies when re-sampling was done four months later. In the context of the latter finding, it was envisaged that the results of the current study would indicate the effectiveness of the current hygiene practices implemented in the DUT CDC. These findings will be made available to lecturers, clinicians and CSs at the DUT CDC as it is thought that, when applied, their hygiene practices will be improved. The results may also inform similar practices in other healthcare settings.

This study determined participating CSs' hygiene practices as well as their exposure to information or education regarding adequate hygiene practices. The Chiropractic Department was made aware of the possible need for the improvement of hygiene education and practices, and therefore the researcher was given permission to conduct the study in the DUT CDC setting. As Hyder (2012) found a strong correlation between previous exposure to education regarding stethoscope hygiene and the implementation of stethoscope cleaning, it was thought that the findings of this study could inform best practices and increase awareness of the importance of good hygiene practices to prevent potential bacterial cross-contamination from patient to patient and from patient to chiropractor. Chiropractors can then take the necessary precautions to minimise the risk of potential infection of their patients and themselves.

1.6 Delimitations of the study

- CSs who were aware which exact diagnostic equipment would be sampled for the investigation were excluded from the study as this could bring the validity and integrity of the study's results into question.
- CSs who had completed their Durban University of Technology (DUT) patient number requirements prior to the commencement of the data collection period were excluded from the study, as their names had been taken off the clinic roster.
- Representative CFUs were used for the identification of the bacteria instead of each colony-forming unit (CFU) being analysed.
- Bacteriological samples were collected only from the diaphragm of the stethoscopes and the designated area of the cuff of the sphygmomanometers. These areas for sample collection were specifically chosen on the basis that they would have multiple exposure to patients' skin as well as the longest contact time with patients' skin during the performance of physical examinations.

- Due to financial constraints the diaphragm of the stethoscopes and the designated area of the cuff of the sphygmomanometers were assessed for the presence of bacteria and not for fungi, protozoa or viruses.

1.7 Brief outline of the Dissertation

Chapter 1 is preceded by the title page, acknowledgements, glossary, abstract and the table of contents. Chapter 1 provides an introduction to the research study and introduces the aims, objectives, research problem, context and rationale of the project. The chapter is concluded with an outline of the dissertation.

Chapter 2 presents a comprehensive review of the literature. Each component of the study is introduced and discussed in detail.

Chapter 3 provides and in-depth elucidation of the research methodology that was employed in the execution of this research study.

Chapter 4 commences with a report of the results that were obtained. The results are subsequently discussed with regards to their relevance and the findings are correlated with the aims and objectives of the study. The results are compared with those of existing literature in the process of validating the data.

Chapter 5 presents the conclusions that were reached and illuminates the limitations that impacted the study. Recommendations are offered based on the findings.

The reference list and appendices are attached to comprehensively inform the reader of the scope of the study.

CHAPTER 2: LITERATURE REVIEW

2.1 Chiropractic Healthcare

2.1.1 Definition

Chiropractic healthcare is defined by the World Federation of Chiropractic (2009) as "a health profession concerned with the diagnosis, treatment and prevention of mechanical disorders of the musculoskeletal system, and the effects of these disorders on the function of the nervous system and general health. There is an emphasis on manual treatments including spinal adjustment and other joint and soft-tissue manipulation".

2.1.2 History of chiropractic healthcare in South Africa

In the early 1900s, only four or five chiropractors from American origin were practising in South Africa. As the profession grew, legislation to regulate this form of healthcare was sought. Consequently, Act 76 of 1971 granted recognition to the 176 qualified chiropractors who were practising in South Africa at that time. However, no new chiropractor immigrating to South Africa after the set deadline would receive recognition (Chiropractic Association of South Africa 2015).

It was only in 1982 that the chiropractic profession obtained statutory recognition in South Africa with the implementation of Act 63 of 1982, which stated that the Associated Health Professions Board (AHPB) would govern the chiropractic profession. Only after the approval of the Association for Health Service Professions Amendment Act in 1985, was the Chiropractic Register re-opened. This allowed for the registration of new chiropractors whose qualifications met the standards set by the AHPB (Chiropractic Association of South Africa 2015).

The then Technikon Natal enrolled its first chiropractic students in 1989 with the Witwatersrand Technikon following suit a few years later (Korporaal 2013). Today these institutions are known as the Durban University of Technology and the University of Johannesburg respectively and they remain the only establishments in South Africa to offer tertiary programmes in chiropractic training and education (Chiropractic Association of South Africa 2015).

2.1.3 Scope of chiropractic practice in South Africa

The regulations as discussed in terms of the Associated Health Service Professions Act 63 of 1982 (South Africa 1982) define the scope of practice of chiropractors as follows:

- The physical assessment of any person, which may include the evaluation and interpretation of X-ray plates, in order to diagnose such a person with any illness, physical defect or deficiency.
- The prevention or treatment of any illness, physical defect or deficiency in any person with regards to pelvic, spinovisceral, spinal and general neuromusculoskeletal conditions by implementing:
    - Manipulation or adjustment
    - Electrotherapy
    - Exercise therapy
    - Hydrotherapy
    - Traction therapy
    - Thermal therapy
    - Vibration therapy
    - Immobilization therapy
    - Neuro-muscular reflex therapy
    - Massage therapy
    - Acupuncture or acupressure therapy
    - Dietary advice or dietary supplementation and
    - Remedies.

2.1.4 Durban University of Technology chiropractic curriculum

In a report compiled by the World Federation of Chiropractic (2012) (WFC), the chiropractic programme offered by DUT (Appendix X), is one of 41 across 16 countries which the WFC recognises. On the 27th of May 2017, the European Council on Chiropractic Education (ECCE) accredited the programme for an extended five year cycle (European Council on Chiropractic Education 2017). This was the third cycle of ECCE accreditation.

The diploma component establishes a solid foundation in the basic sciences with the practical component of Diagnostics introduced in the third year of study, where CSs are taught how to perform a physical examination that incorporates the use of standard diagnostic instrumentation (Faculty of Health 2017). During the fourth year of study, weekly hospital visits are conducted during which CSs are given the opportunity to interact with patients and perform case histories and physical examinations under the supervision of an appropriately qualified chiropractor and lecturer in Diagnostics IV. This prepares CSs for when they start consulting patients in the DUT CDC as part of their fifth year curriculum.

2.2 Durban University of Technology Chiropractic Day Clinic

2.2.1 Overview

The DUT CDC started operating in February 1994 (Drews 1995). The purpose of the DUT CDC is to provide eligible CSs and student interns with an infrastructure that allows them to obtain clinical experience by providing chiropractic services to the surrounding Durban community (Department of Chiropractic and Somatology 2017).

In order to complete the Clinical Chiropractic V curriculum, a fifth year chiropractic student (CS) must consult with a minimum of 35 new patients and conduct a minimum of 350 follow-up consultations (Department of Chiropractic and Somatology 2017). These consultations must take place under the supervision of qualified and registered chiropractors as stated in the Allied Health Service Professions Act 63 of 1982 (South Africa 1982).

At the time of the study, Clinic Director, Dr Korporaal, indicated in an e-mail communication on 3 March 2016 that in 2015 the DUT CDC averaged approximately 12 500 consultations per year.

2.2.2 Demographics of patients

McDonald (2014) performed a retrospective cross-sectional survey at the DUT CDC in order to determine the demographic characteristics of the patients attending the clinic at pre-selected timeframes. In order to obtain an overview of the patients and the conditions that were presented at the DUT CDC at the time, salient characteristics are outlined in Table 1 on the following page.

Table 1: Characteristics distribution of patients attending DUT CDC for the years 2000, 2006 and 2011 as determined by McDonald (2014)

Characteristics	Options	2000 *n*= 629	2006 n= 391	2011 n= 291
Age	Mean age	37.0 years	39.8 years	37.8 years
		%	**%**	**%**
Gender	Female	50.5	51.2	50.9
	Male	49.5	48.8	49.1
Ethnicity	White	64.2	46.3	49.5
	Indian	27.2	40.9	34.0
	Black	6.4	10.2	15.8
	Coloured	2.2	2.6	0.7
Occupation	Clerical	17.0	22.3	23.0
	Student	19.7	14.8	26.8
	Sales	12.2	15.1	11.0
	Housewife	10.3	10.5	8.2
	Manual	7.5	11.5	6.9
	Retired	7.6	7.2	6.9
	Teacher	5.2	5.4	5.2
	Unemployed	5.2	4.1	4.1
	Executive	5.9	3.6	3.4
	Medical	4.1	3.6	2.4
	Other	3.7	2.0	1.7
	Public service	1.4	0.0	0.3
Medical aid	No	56.1	63.2	58.4
	Yes	43.9	36.8	41.6
Main complaint	Low back pain	30.7	40.7	35.7
	Neck/ headaches	39.3	33.8	27.8
	Mid back	8.3	9.7	7.2
	Ankle	9.2	3.6	7.9
	Knee	7.9	3.1	8.9
	Shoulder	6.8	4.6	6.2
	Hip	3.7	1.3	3.4
	Wrist	2.2	1.3	0.7
	Other	1.1	0.5	1.4
	Elbow	0.8	1.5	0.7
Duration of main complaint	Chronic	52.9	45.8	51.2
	Acute	33.2	32.2	25.1
	Sub-acute	13.8	22.0	23.7

n= number of sampled patients per year, % = percentage

2.2.3 New patient procedure

2.2.3.1 Overview

The 'new patient' procedure is followed when it is the patient's first consultation at the DUT CDC, or if the person is an existing patient, but has not received treatment at the clinic over the last six months. The CS is required to complete a case history, physical and regional examinations (Department of Chiropractic and Somatology 2017).

2.2.3.2 Physical examination

After completing the case history and discussing it with the on-duty chiropractic clinician, the CS will perform a physical examination. With regard to the relevance to this research study, only the aspects of the physical examination that required the use of a stethoscope and sphygmomanometer will be discussed.

At the time of the study, Bates's *Guide to physical examination and history taking* and Macleod's *Clinical examination* were recommended for Diagnostic III and Diagnostic IV students (Durban University of Technology 2015). The protocol for the selected aspects of the physical examination as outlined in these two books (Bickley and Szilagyi 2013; Douglas, Nicol and Robertson 2013) are described below:

- Measuring blood pressure
    - The patient is positioned either seated or supine.
    - The brachial artery is palpated to confirm that it does have a viable pulse.
    - With the patient's arm supported and the elbow slightly flexed, the cuff of the sphygmomanometer is applied around the patient's arm with the centre of the cuff bladder positioned over the brachial artery.
    - While palpating the radial artery, the cuff is inflated. The pressure reading (X) on the manometer is noted at the point the pulse becomes impalpable. Reading X presents an estimation of the systolic pressure. The cuff is then deflated completely.
    - The diaphragm of the stethoscope is then placed over the brachial artery. The cuff is inflated to reading X + 30 mmHg.
    - The cuff is deflated slowly until a regular tapping sound can be heard. The pressure reading at which this occurs represents the systolic pressure. Deflation of the cuff is continued until the tapping sound disappears which indicates the diastolic pressure.
    - The procedure is repeated on the other arm.

- Auscultation during the respiratory examination
    - Auscultation and percussion allow the examiner to assess the lung tissue and the pleural space.
    - The patient is instructed to breathe deeply through an open mouth while the examiner auscultates each lung alternatively with the use of the stethoscope's diaphragm.
    - The following regions of each lung are auscultated: front of the chest from above the clavicle down to the sixth rib, the area from the axilla down to the eighth rib, and 5 cm on either side of the spine from above the scapula down to the eleventh rib.
    - The examiner assesses the patient's normal breathing sounds and listens for any adventitious sounds. If an abnormality is suspected, the examiner will use the diaphragm over the area in order to assess for transmitted voice sounds.

- Auscultation during the cardiovascular examination
    - Auscultation allows the examiner to assess the heart sounds, referred to as the 'lub-dub sounds', for any abnormalities such as murmurs.
    - The four heart valves are evaluated by placing the stethoscope's diaphragm at the heart apex (mitral area), the lower left sternal border (tricuspid area), the left (pulmonic area) and upper right sternal (aortic area) borders.
    - If any abnormalities are suspected, the bell of the diaphragm can be used and the patient will be re-positioned accordingly.

- Auscultation during the abdominal examination
    - The patient is placed in a supine position.
    - The abdomen is auscultated in order to assess the bowel sounds and determine the presence of abdominal bruits or friction rubs.
    - The bowel sounds are assessed by placing the diaphragm of the stethoscope in each quadrant of the abdomen. The examiner will listen in each spot for at least 30 s. Here after, the bell is placed over the aorta, renal, iliac and femoral arteries to assess for bruits and over the liver and spleen to listen for friction rubs.

2.3 The human skin

The skin is the largest organ of the human body as it covers an area of two m² and weighs between four and five kilograms. The skin, together with hair, nails, sensory receptors, oil and sweat glands, forms the integumentary system (Tortora and Derrickson 2011).

2.3.1 Functions

According to Colledge *et al.* (2010), Moore, Dalley and Agur (2010), Douglas, Nicol and Robertson (2013) and Willey *et al.* (2014), the role of the skin, together with the other components of the integumentary system, is as follows:

- Protection: It serves as a barrier that protects the body from environmental effects, for example mechanical injuries, chemicals, UV radiation and invading microbes. The mechanisms involved to prevent microbes from penetrating the skin include: thick layer of keratinocytes and constant shedding of outer epithelial cells. The acidic pH and high concentration of sodium chloride on the epidermal surface make it an unfavourable environment for microbial colonization. Secretions from the sebaceous and sweat glands have antimicrobial properties.
- Containment: It holds the body's structures in place.
- Temperature regulation: This is done through the secretion and evaporation of sweat, dilation or constriction of blood vessels and pilo-erection or pilo-relaxation.
- Synthesis and storage of vitamin D.
- Maintenance of fluid balance: It prevents loss of water, macromolecules and electrolytes.
- Sensation: The skin contains multiple sensory endings and superficial nerves which play a role in conducting sensory inputs such as touch, pressure, temperature and pain.
- Immune response: Langerhans cells and macrophages have roles in the immune response against foreign antigens.
- Grip: Epidermal ridges, as seen on the palms, fingers, soles and toes, increase friction in that area, thus generating more grip for the feet or hands.

2.3.2 Microflora on the skin

The human skin is constantly exposed to the external environment and thus to numerous microorganisms. Normal flora or microflora represent the microorganisms that live in or on the skin of healthy people in set anatomical locations (Tille 2014).

Human skin is said to support an estimated 10^{12} bacteria at a time. The majority of these bacteria have a mutualistic relationship with the human body, with both parties benefiting from the association. The microorganisms located in or on the skin can be categorised either as resident or transient microorganisms. Resident microorganisms are permanently distributed in set areas of the body and have the ability to re-establish themselves if disturbed. These microorganisms assist in preventing the growth of pathogens through microbial interference. In contrast, transient microorganisms are only temporarily present on the skin as they are derived from the environment (Brooks *et al.* 2013).

Destruction or suppression of the normal microflora will result in a void that could be filled by opportunistic environmental microorganisms which may become pathogenic. At the same time, microorganisms which are regarded as microflora could, under certain circumstances, become pathogenic and cause disease (Grice and Segre 2011).

The following microorganisms are classified as normal microflora of the skin: Coagulase-negative staphylococci (CoNS) such as *Staphylococcus epidermis*, *α*-hemolytic and non-hemolytic streptococci, *S. aureus*, *Bacillus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Propionibacterium* spp., *Peptostreptococcus* spp., *Candida* spp. and *Acinetobacter* spp. (Brooks *et al.* 2013; Willey *et al.* 2014).

2.4 Healthcare-associated infections

2.4.1 Definition

A healthcare-associated infection (HCAI) is defined by the WHO (2011) as "an infection that a patient contracts while being treated for another condition, in a hospital or any other healthcare facility".

2.4.2 Prevalence and impact

2.4.2.1 Overview

The total impact of HCAIs is multifactorial and includes prolonged hospital stays, additional financial burden for health systems and patients, increased antimicrobial resistance and increased mortality and morbidity (World Health Organization 2015).

A report compiled by the WHO (2011) determined that HCAIs affect 4,1 million Europeans every year, resulting in 16 million additional days in hospital, 37 000 deaths, and contribute to an extra 110 000 deaths and cost an estimated €7 billion. Comptroller and Auditor General (2009) stated that in 2004 in England alone, 300 000 people were affected by HCAIs. The latter source also reported a financial burden on the healthcare system of more than £1 billion per year. In comparison, in 2002 in the United States of America, 1,7 million patients were affected by HCAIs which resulted in approximately 99 000 deaths and cost an estimated US$6,5 billion (World Health Organization 2011).

Even though global estimates regarding HCAIs were not available, the data suggested that millions of patients were affected each year. The published data also affirm that HCAIs pose a greater burden and safety risk for patients and HCWs in developing countries compared to developed countries (Allegranzi *et al.* 2011; World Health Organization 2011).

2.4.2.2 Developing countries versus developed countries

Allegranzi *et al.* (2011) found that, in contrast to developed countries, it was difficult to determine the exact endemic burden of HCAIs on developing countries, as 66.0% of these countries had no published data on this topic. However, the available data suggest that HCAIs pose a greater burden and safety risk for patients and HCWs in developing countries than in developed countries, which is concurred by the World Health Organization (2011). At the time of the latter study (2011), the average prevalence of HCAIs in Europe was 7.1%, whereas in resource-limited settings it averaged 15.5%. The difference with regards to incidences of intensive care unit-acquired infection was a pooled density of 47.9 per 1 000 patient-days in developing countries, whereas in the USA it was an estimated 13.6 per 1 000 patient-days. In developing countries, the following are regarded as potential risk factors for a high burden of HCAIs: inadequate environmental hygienic conditions, insufficient equipment, poor infrastructure, understaffing, overcrowding, paucity of knowledge and application of basic infection-control measures, and scarcity of local and national guidelines and policies. It was found that in developing countries, only 20.0% of HCWs practised good hygiene (Allegranzi *et al.* 2011).

2.4.2.3 South Africa

South Africa, similar to other African countries, lacks data on the impact of HCAIs (Duse 2005). Dramowski, Whitelaw and Cotton (2016) conducted a study at the Tygerberg Children's Hospital to investigate paediatric HCAIs. They recorded that 417 HCAI events transpired in 296 patients during 325 hospitalizations. The prevalence of HCAIs was 24.1%, while the incidence density was 31.1 per 1 000 patient-days. It was also found that 7.4% of the HCAI events had resulted in death. Dramowski, Whitelaw and Cotton (2016) calculated the total direct cost of HCAIs to be US$ 371 887 (R5 470 458) per annum as a result of 2 275 additional hospitalization days, a further 2 365 days of antibiotic therapy, and 3 575 extra laboratory investigations being performed. This meant an average cost of US$892 per HCAI event, which translates to R13 121.

2.4.3 Risk factors associated with HCAIs

According to a report compiled by WHO (2011), the most common factors independently associated with HCAI occurrence in developed countries are: age > 65 years, admission to intensive care units (ICUs) in frail state, longer than seven days' hospital stay, placement of a central venous or indwelling urinary catheter, insertion of an endotracheal tube, undergoing surgery, neutropenia, trauma-induced immunosuppression, McCabe score 'fatal', and impaired functional or coma status. The above risk factors were also identified in developing countries with the addition of the following: malnutrition, age < 1 year, low birth weight, parenteral nutrition, and two or more underlying diseases. Although not confirmed as independent risk factors, the following also contribute towards the occurrence of HCAIs in developing countries: insufficient financial support, overcrowding, inadequate numbers of trained personnel focusing on infection control, understaffed hospitals, and limited equipment and supplies (World Health Organization 2011).

In a study done by Dramowski, Whitelaw and Cotton (2016) in a South African setting, HCAIs were significantly associated with admission to paediatric ICUs, malnutrition, human immunodeficiency virus (HIV) infection, McCabe score 'fatal', comorbidities, indwelling devices, and blood transfusions.

2.4.4 Most common bacteria implicated in HCAIs

S. aureus is associated with normal skin microflora. For this reason it is commonly found in the nasopharynx, skin, perineal area and anterior nares. It can be transmitted by traumatic introduction (for example catheter or surgical wounds), fomites, direct human contact, or by aerosol transmission. It is capable of causing a wide spectrum of diseases and infections such as scalded skin syndrome, toxic shock syndrome, carbuncles, impetigo, bacteraemia, endocarditis and osteomyelitis (Tille 2014). According to a WHO (2011) report, *S. aureus* is the most frequently bacteria associated with intensive care unit-acquired infections. *S. aureus* possesses various virulence factors which enable it to cause an infection in its host. One of these factors is the ability to produce enzymes which render antibiotics ineffective. The two strains of *S. aureus* which are widely known for their antibiotic resistance are MRSA and Vancomycin-resistant *Staphylococcus aureus* (World Health Organization 2011). With regard to MRSA, patients most at risk are infants, the elderly, burn victims, recipients of an organ transplant, chronically ill patients, patients receiving chemotherapy, intravenous drug and steroids users, and patients diagnosed with HIV or diabetes mellitus (Green *et al.* 2012). *S. aureus* are resilient bacteria and have been found to be able to survive for between seven days to seven months on dry, inanimate surfaces (Kramer, Schwebke and Kampf 2006).

Coagulase-negative staphylococci (CoNS) are the most common bacteria associated with HCAIs. These bacteria are significantly less virulent than *S. aureus* and are seen more as opportunistic pathogens. As such, CoNS are more commonly associated with infections related to medical procedures and practices (Tille 2014).

The natural habitats of *Klebsiella* spp. and *E. coli* include the gastrointestinal tract of humans as part of the normal bowel flora. Their modes of transmission depend largely on the type of infection. In non-gastrointestinal infections that are common in the case of a hospital setting, transmission occurs by direct contact or through endogenous spread (Tille 2014). Kramer, Schwebke and Kampf (2006) stated that *Klebsiella* spp. can survive on an inanimate surface for any period between two hours to 30 months, while *E. coli* can survive for any period between 90 minutes and 16 months. It has been found that *E. coli* is the most common pathogen associated with urinary tract infection (UTI) that is acquired during a stay in a healthcare facility (World Health Organization 2011). *Klebsiella* spp. can result in pneumonia, blood stream infection and UTI. Both *Klebsiella* spp. and *E. coli* are capable of building resistance to antibiotics, especially against carbapenems (World Health Organization 2011; United States Department of Health: Centers for Disease Control and Prevention 2013).

Ps. aeruginosa is commonly found in the hospital environment in moist areas such as sinks, showers and respiratory equipment. Transmission occurs via exposure to contaminated medical equipment, direct contact and penetrating wounds (Tille 2014). It was also determined that *Ps. aeuginosa* can survive for up to 16 months on an inanimate surface before dissecation occurs (Kramer, Schwebke and Kampf 2006). It is associated with the following HCAIs: pneumonia, UTI, osteomyelitis, endocarditis, bacteraemia and surgical site infections (SSI) (Tille 2014).

Acinetobacter spp. is widely distributed in nature, but is commonly found in the hospital environment. *Acinetobacter baumannii* is typically the species associated with HCAIs and can cause wound, respiratory and genitourinary infections (Tille 2014). Factors that put patients at risk of contracting an *Acinetobacter* spp. infection include prior antibiotic exposure, intensive care unit admission, use of a central venous catheter and mechanical ventilation, or haemodialysis use (Cuhna 2016). According to Hota (2004), *Acinetobacter* spp. is capable of surviving up to 33 days on plastic laminated surfaces, while Kramer, Schwebke and Kampf (2006) found that it could survive up to five months on an inanimate surface.

Enterococcus spp. form part of the normal flora located in the human gastrointestinal and the female genitourinary tract. Transmission occurs via direct human contact, contaminated medical equipment and by endogenous spread if these bacteria gain access to a sterile site (Tille 2014). It has been estimated that *Enterococcus* spp. could survive for up to four months on non-living surfaces (Kramer, Schwebke and Kampf 2006). *Enterococcus faecalis* and *Enterococcus faecium* account for more than 90% of infections caused by *Enterococcus* spp. and are associated with UTI, endocarditis, bacteraemia and wound infections (Fraser 2017).

The review of the above literature showed that many pathogenic bacteria are capable of surviving for extended periods on contaminated fomites and can be transmitted to patients via direct contact.

2.4.5 Non-invasive sources contributing towards the potential spread of infections

Bifero, Prakash and Bergin (2006) argued that, for an inanimate object to become a reservoir for microorganisms, two factors must be present: first, inadequate cleaning and disinfection protocols which allow for an increase in microbial load and, secondly, the physical contact of the object's surface with the exposed skin of the patient. A vast body of literature supports the involvement of contaminated inanimate objects and surfaces for the transmission of pathogens to patients (Uneke *et al.* 2010; Jain *et al.* 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014; Russotto *et al.* 2015).

2.4.5.1 Stethoscopes

Longtin *et al.* (2014) stated that a stethoscope may be substantially contaminated after a single physical examination. Burrie (2011) affirmed that stethoscopes have a potential moderate to high risk of transmitting infections, especially in elderly and susceptible patients.

Table 2 below summarizes the findings of some studies that investigated the microbial contamination of stethoscopes.

Table 2: Summary of results of various studies conducted on stethoscope contamination worldwide

Setting	Percentage of stethoscopes contaminated	Microorganisms identified	Reference
10 healthcare facilities in the Ebonyi State in south-eastern Nigeria	78.5% (n=107)	*S. aureus* (53.6%) *Ps. aeruginosa* (19.0%) *Enterococcus faecalis* (14.3%) *E. coli* (13.1%).	(Uneke *et al.* 2010)
Tertiary hospital in Greece	90.0% (n=45)	CoNS (87.6%) *Acinetobacter lowffi,* *Streptococcus faecalis* *Streptococcus sanguinis*	(Leontsini, Papapetropoulos and Vantarakis 2013)
Tertiary hospital in Ujjain district in India	86.0% (n=80)	*Bacillus* spp. CoNS *S. aureus* *Ps. aeruginosa*	(Jain *et al.* 2013)
Jimma University Specialized Hospital in Ethiopia	85.8% (n=176)	CoNS (40.2%) *S. aureus* (30.9%) *Bacillus* spp. (5.5%) *Klebsiella* spp. (4.7%) *Salmonella* spp. (3.5%) *Ps. aeruginosa* (1.2%) *E. coli* (0.8%)	(Shiferaw *et al.* 2013)
General Regional Hospital of Leon, Mexico	95.0% (n=112)	*S. aureus* *Enterococcus faecalis* *Klebsiella* spp. MRSA	(Campos-Murguia *et al.* 2014)

Table 2: Summary of results of various studies conducted on stethoscope contamination worldwide (continued)

Setting	Percentage of stethoscopes contaminated	Microorganisms identified	Reference
Tertiary care hospital in Pune, India	100.0% (n=50)	CoNS (77%) *Acinetobacter* spp. (5%) *S. aureus* (5%) *Bacillus* spp. (4%) *Aspergillus* spp. (4%) *Pseudomonas* spp. (2%) *Citrobacter* spp. (2%)	(Gupta *et al.* 2014)
The Royal Infirmary of Edinburgh	98.0% (n=52)	CoNS (82%) *Micrococcus* spp. (32%) *Bacillus* spp. (22%) *α*-hemolytic *Streptococci* (8.7%) *S. aureus* (5.8%) Coliforms (2%)	(James and Young 2014)
Tertiary hospital in rural Bengal	52.0% (n=100)	*Bacillus* spp. (36.8%) *Acinetobacter* spp. (17.1%) CoNS (14.5%) *Micrococcus* spp. (10.5%) *S. aureus* (6.6%) *Ps. aeruginosa* (6.6%) Diphtheroids (6.6%) *E. coli* (1.32%)	(Pal *et al.* 2015)
Tertiary hospital in India	74.0% (n=50) of which 60% showed significant bacterial colonization	CoNS MRSA *Klebsiella* spp. *S. aureus*	(Jeyakumari *et al.* 2017)

n= sample size, %= percentage, spp.= species and CoNS= coagulase-negative staphylococci

A high percentage of stethoscopes in the above eight studies was contaminated with potential pathogenic and non-pathogenic microorganisms. Of further concern is the quantity and degree of antibiotic resistance to the bacteria identified in some of these studies (Table 3).

Table 3: Summary of various studies' findings relating to the antibacterial sensitivity patterns of bacteria isolated from contaminated stethoscopes

Bacterial isolates tested	Antibiotics used	Overall resistance of all isolates to antibiotic %	Findings	Reference
S. aureus *Ps. aeruginosa* *E. faecalis* *E. coli*	Rifampicin 10 µg	100	The results indicated that the majority of the bacterial isolates was resistant to all the antibiotics tested. The *E. coli* isolates displayed the highest level of resistance as they were susceptible only to ciprofloxacin and streptomycin. In contrast, *S. aureus* were the most susceptible to the different antibiotics. Ciprofloxacin and streptomycin were the most effective antibiotics.	(Uneke *et al*. 2010)
	Flucloxacillin 30 µg	100		
	Erythromycin 30 µg	100		
	Chloramphenicol 20 µg	100		
	Ampicillin-cloxacillin 30 µg	100		
	Cephalexin 10 µg	100		
	Ampicillin 30 µg	100		
	Trimethoprim 30 µg	100		
	Nofloxacin 30 µg	83.3		
	Gentamicin 10 µg	83.3		
	Ofloxacin 10 µg	83.3		
	Pefloxacin 10 µg	66.7		
	Lincomycin 30 µg	66.7		
	Streptomycin 30 µg	58.3		
	Ciprofloxacin 10 µg	58.3		
CoNS	Ampicillin	74.6	Of the CoNS tested, 16.9% was multi-resistant strains. These were resistant to at least four of the antibiotic classes tested. No resistance was found to linezolid, teichoplanin, daptomycin, vancomycin, chloramphenicol and fucidic acid.	(Leontsini, Papapetropoulos and Vantarakis 2013)
	Penicillin	74.6		
	Erythromycin	60.5		
	Amoxicillin- clavulanic acid	39.4		
	Clindamycin	39.4		
	Oxacillin	30.9		
	Cephalothin	30.9		
	Gentamicin	22.5		
	Levofloxacin	14		
	Co-trimoxazole	14		
	Tetracycline	8.4		
	Rifampin	2.8		

Table 3: Summary of various studies' findings relating to the antibacterial sensitivity patterns of bacteria isolated from contaminated stethoscopes (continued)

Bacterial isolates tested	Antibiotics used	Overall resistance of all isolates to antibiotic %	Findings	Reference
CoNS *S. aureus* *Bacillus* spp. *Klebsiella* spp. *Salmonella* spp. *Ps. aeruginosa* *E.coli*	Penicillin 10 µg Chloramphenicol 30 µg Tetracycline 30 µg Co-trimoxazole 25 µg Gentamicin 10 µg Cefoxitin 30 µg Erythromycin 15 µg Cefotaxime 30 µg Ampicillin 10 µg Ciprofloxacin 5 µg Clindamycin 2 µg Nalidixic acid 30 µg Norfloxacin 10 µg Vancomycin 30 µg	63.6 54.2 37.3 32.2 30.1 22.0 20.8 19.9 19.5 13.1 7.2 3.0 1.7 0.0	26.6% of the *S. aureus* and 30.1% of the CoNS isolates were identified as Methicillin resistant strains. The antibiogram indicated that 17.7% of *S. aureus* and 8.7% of the CoNS isolates had resistance to eight different classes of antibiotics. The Gram-negative isolates showed multidrug resistance to two to eight of the classes tested. Vancomycin was the only antibiotic which showed no resistance.	(Shiferaw *et al*. 2013)
CoNS *S. aureus*	Erythromycin Amoxicillin Clindamycin Cefuroxime Cefoxitin	70.8 70.8 52.1 48.3 40.5	All the resistant *S. aureus* were susceptible to vancomycin.	(Gupta *et al.* 2014)
Acinetobacter spp. CoNS *S. aureus* *Ps. aeruginosa* *E.coli*	Ampicillin 10 µg Co-trimoxazole 25 µg Cefotaxime 30 µg Ceftriaxone 30 µg Ciprofloxacin 5 µg Levofloxacin 5 µg Gentamicin 10 µg Amoxicillin – clavulanic acid 30 µg Chloramphenicol 30 µg Amikacin 30 µg Cefoxitin 30 µg Vancomycin 30 µg	88.6 71.4 60.0 54.3 42.8 40.0 37.1 37.1 34.3 25.7 11.4 0.0	All isolates were susceptible to vancomycin.	(Pal *et al.* 2015)

%= percentage, spp.= species and CoNS= coagulase-negative staphylococci

Table 3 on the previous page illustrates that resistance to various classes of antibiotics has been identified. This is worrisome as these resistant bacteria are not only capable of initiating a HCAIs, but their resistance also complicates the treatment options available. For this reason it is essential that HCWs disinfect their stethoscopes before and after each patient has been examined as advised by Shiferaw *et al.* (2013), Campos-Murguia *et al.* (2014) and Jain *et al.* (2013) to prevent the spread of bacteria between patients.

2.4.5.2 Sphygmomanometers

de Gialluly *et al.* (2006) collected microbial samples from the inner and outer sides of the cuffs of sphygmomanometers. They established that the inner sides of the cuffs had the highest level of contamination. This finding was later affirmed by Grewal *et al.* (2013). The majority of the microbial isolates corresponded to the saprophytes associated with normal skin. Nonetheless, potential pathogenic isolates were detected on 13% of the cuffs and were identified as *S. aureus* (nine of which were MRSA), *Ps. aeruginosa, S. marcescens* and *E. coli.* In five cases identified as HCAIs, molecular typing indicated a genetic relationship between the bacteria isolated from the patient and the isolates collected from the cuff used on the patient.

Walker, Gupta and Cheesbrough (2006) were able to isolate viable bacteria from all the sampled cuffs in their study, and found that 58.3% of the cuffs harboured pathogenic bacteria that were identified either as MRSA or *C. difficile.* They propounded the need for measures to be put in place in order to decrease the risks posed by sphygmomanometers, which is a concern that was also voiced by de Gialluly *et al.* (2006).

In a study that was conducted by Davis (2009), 86.6% of the cuffs of the sphygmomanometers that had been sampled resulted in positive microbial growth. Even though the findings were largely similar to those of de Gialluly *et al.* (2006), it could be established that most of the isolates were constituents of skin microflora.

The literature review clearly established that both stethoscopes and sphygmomanometers can act as potential reservoirs for pathogenic and non-pathogenic microorganisms. This highlights the need for the implementation and practice of strict infection control protocols.

2.4.5.3 Healthcare workers' hands

Cross-contamination of microorganisms by the hands of HCWs is considered the main route for the spread of nosocomial infections. Pittet *et al.* (1999) established that bacterial contamination of the hands of HCWs increased proportionally with the time spent caring for patients. Activities which were associated with higher contamination levels by hands were physical contact with patients, respiratory care and handling of body fluid secretions.

Paul *et al.* (2011) conducted a study that investigated doctors' hands upon entry and exit of a ward. It was found that 59.1% of the doctors' hands was contaminated at entry while 90.9% was contaminated upon leaving the ward. Pathogenic bacteria isolated from the doctors' hands included staphylococci, *Klebsiella* spp., *Enterococci, Pseudomonas* spp. and *E. coli.* Paul et al. (2011) argued that the contamination of doctors' hands plays a role in the transmission of HCAIs, especially if inadequate hand hygiene is practised. This finding is supported by a handful of studies that suggest adherence to good hygiene practices with regard to regular disinfection of diagnostic equipment and hands in order to limit and possibly eradicate the spread of HCAIs between patients and HCWs (Uneke *et al.* 2010; Leontsini, Papapetropoulos and Vantarakis 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014).

2.4.5.4 Chiropractic tables

Table 4: Summary of the results of different studies regarding chiropractic table contamination in either private or public chiropractic settings

Setting	Areas of table tested	Microorganisms identified	Additional information	Reference
National University outpatient clinic - Illinois	Headrests, armrests and thorax pieces	CoNS *S. aureus* MRSA *Ps. aeruginosa* *E.coli* *Bacillus* spp. *S. marcescens* *Micrococcus* spp.	All samples collected had positive bacterial growth. Highest level of contamination were found on armrests and uncovered part of headrest.	(Bifero, Prakash and Bergin 2006)
Teaching clinic at a chiropractic college - USA	Headrests and armrests of vinyl-covered tables only	*S. aureus* Staphylococci	All samples obtained from the tables indicated the presence of bacterial contamination.	(Evans *et al.* 2007)
Teaching clinic at a chiropractic college - USA	Headrests and armrests of cloth-covered tables only	*S. aureus* Staphylococci *Micrococcus* spp. *Candida* spp. *Bacillus* spp.	Bacterial counts were not quantified. Evans *et al.* (2008) suggest that healthcare professionals should abandon the use of cloth-covered tables as they are porous and difficult to disinfect.	(Evans *et al.* 2008)
Western States Chiropractic College - Portland	Headrests	*S. aureus* MRSA *Ps. aeruginosa* *Moraxella* spp. *Bacillus* spp. Staphylococci		(Burnham *et al.* 2009)

Table 4: Summary of the results of different studies regarding chiropractic table contamination in either private or public chiropractic settings (continued)

Setting	Areas of table tested	Microorganisms identified	Additional information	Reference
Durban University of Technology Chiropractic Day Clinic	Headrests and armrests	*Bacillus* spp. *S. marcescens* *Micrococcus* spp. Staphylococci	Bacterial growth was present on 89.4% of the beds sampled. Logtenberg (2009) reported that the bacterial build-up on tables significantly increased during the day. It was thus recommended that the tables be disinfected between patients.	(Logtenberg 2009)
14 private clinics in Alberta - Canada	Headrests and armrests	CoNS *S. aureus* MRSA *Acinetobacter* spp. *Streptococcus* spp. *Corynebacterium* spp. *Neisseria* spp. *Bacillus* spp. *S. marcescens* *Micrococcus* spp. *Moraxella* spp.	Puhl *et al.* (2011) emphasise that despite face paper acting as an effective barrier to bacterial transmission, more needs to be done with regards to regular disinfection of tables.	(Puhl *et al.* 2011)

*CoNS = coagulase-negative staphylococci, spp. = species

Bifero, Prakash and Bergin (2006), Burnham *et al.* (2009) and Puhl *et al.* (2011) emphasised that chiropractic tables that are found at facilities that do not follow strict infection control procedures may become reservoirs of potential pathogens and that transmission to patients can occur. For this reason, both Evans *et al.* (2007) and Evans *et al.* (2008) urged the immediate development of infection control protocols that should be implemented at all schools and colleges that utilize manual therapy.

2.4.5.5 Other fomites

Bhanot *et al.* (2011) gathered bacterial samples from keyboards, curtains, cell phones and clothing used or worn by HCWs. Of all the inanimate objects sampled, 98.7% was contaminated with bacteria. The percentages of potentially clinically relevant pathogens found on each of the items were as follows: keyboards (56.4%), curtains (79.6%), cell phones (88%), white coats (75%) and ties (66.6%). The potential pathogens were MRSA, Methicillin-resistant *S. epidermis, E. faecalis, Acinetobacter* spp., vancomycin-resistant *Enterococcus faecium* and *Pseudomonas* spp. Bhanot *et al.* (2011) cautioned that the above contaminated inanimate objects can result in HCAIs.

Studies that corroborate the conclusion by Bhanot *et al.* (2011) are:

- Cell phones: Heyba *et al.* (2015), Cuhna (2016), Chang *et al.* (2017), and Sadat-Ali *et al.* (2010).
- Curtains: Ghani *et al.* (2016), Winter (2014), and Klakus, Vaughan and Boswell (2008).
- Keyboards: Schultz *et al.* (2003).
- Clothing of HCWs: Qaday *et al.* (2015), Munoz-Price *et al.* (2012), Lenski and Scherer (2016), and Treakle *et al.* (2009).

2.4.6 Exposure of healthcare workers to HCAIs

Not only patients, but also HCWs are at risk of contracting HCAIs (Rothe, Schlaich and Thompson 2013). Haagsma *et al.* (2012) determined that HCWs have an increased risk of developing certain infectious diseases. Most of the pathogens were found to spread via environmental contamination or by human-to-human contact. This suggests that good hygiene practices are not only vital to protect the patient from disease, but also HCWs themselves. The most common pathogens that may be acquired by HCWs in their work setting are listed in Table 5 below.

Table 5: Work-related pathogens encountered by healthcare workers in their working environment

Site of entry	Pathogen
Skin and mucous membrane	Hepatitis B virus Hepatitis C virus Human immunodeficiency virus Human herpes virus MRSA Vancomycin-resistant enterococci *S. pyogenes* Cytomegalovirus
Respiratory system	Bordetella pertussis *S. pyogenes* Varicella zoster virus Measles virus *Mycobacterium tuberculosis* Human parvovirus SARS Coronavirus Monkey pox virus Mumps virus Influenza virus
Gastrointestinal tract	*Helicobacter pylori* *Salmonella* spp.

spp. = species

Source: (Haagsma *et al.* 2012).

2.5. Microbial resistance to antibiotics

2.5.1 Overview

Antimicrobial resistance refers to the capacity of microorganisms to render an antibiotic ineffective in spite of previous sensitivity to the drug. Antibiotic resistant microorganisms (ARM) are a growing threat worldwide, as common infectious diseases have become more challenging, or in some cases even impossible, to treat (World Health Organization 2016). Even more alarmingly, separate reports by the United States Department of Health: Centers for Disease Control and Prevention (2013) (USDH: CDCP) and World Health Organization (2014), stated that more and more microorganisms are becoming resistant to multiple kinds of antibiotics.

Dr Frieden, Director of the United States Department of Health: Centers for Disease Control and Prevention, stated that ARM could lead to the depletion of first-line and second-line antibiotic treatment options, which will result in the use of antibiotics that are more expensive, less effective and potentially more toxic to the patient (United States Department of Health: Centers for Disease Control and Prevention 2013). For that reason the focus needs to be placed on preserving the efficacy of existing effective antibiotics by minimising the development and spread of ARM while developing new treatment options (World Health Organization 2014).

In 2017, great strides were made in the development of new antibiotic treatment options. Dibrov *et al.* (2017) developed PEG 2S, a new class of antibiotic, which ended a three decade long void in the production of a new class of antibiotic. At the same time Okano, Isley and Boger (2017) developed a modified version of vancomycin, which utilises three different mechanisms of actions to destroy bacteria. Dr Boger, one of the researchers, claimed that this new version was 25 000 times more effective against vancomycin-resistant *enterococci* and vancomycin-resistant *S. aureus* compared to the current vancomycin. He also stated that, due to it having multiple mechanisms of actions, it would be more difficult for bacteria to develop full resistance. Unfortunately, this drug will not be available to the public soon (Service 2017).

2.5.2 Antibiotics classification

An antibiotic is a substance produced either synthetically or by a microorganism that, at a low concentration, can kill or prevent the growth of other microorganisms. Some antibiotics are modified chemically to improve their spectrum of effectiveness (Dreyer *et al.* 2013). An antibiotic class consists of different drugs with similar chemical and pharmacological properties. As such, antibiotics in the same class demonstrate similar outcomes in terms of antibacterial activity, their mechanism of action, adverse effects, and effectiveness (Anderson 2016) (see Table 6 on following page).

Table 6: Overview of various antibiotic classes: Mechanisms of action and examples of antibiotics in each specific class

Class	Examples of antibiotics in class	Mechanism of action	Additional information
Penicillins	Ampicillin Amoxicillin Piperacillin Amoxicillin-Clavulanic acid Cloxacillin	Prevent the formation of a cell wall by inhibiting the synthesis of intact peptidoglycans.	Can be safely used during pregnancy. Cross-hypersensitivity exists between penicillins and cephalosporins as they have a similar basic chemical structure.
Cephalosporins	Cefuroxime Cefixime Cefepime Ceftriaxone	During peptidoglycan synthesis, cephalosporins inhibit the transpeptidation reaction.	Can be safely used during pregnancy. Taken orally for the treatment of UTI, pneumonia and otitis media. Parenteral cephalosporins are used for surgical prophylaxis and for post-operation infections.
Carbapenems	Meropenem Imipenem Ertapenem	Prevent the formation of a cell wall by inhibiting the synthesis of intact peptidoglycans.	Of all the beta-lactam antibiotics, carbapenems are said to have the broadest spectrum of activity as they are effective against both aerobic and anaerobic bacteria.
Macrolides	Erythromycin Azithromycin Clarithromycin Roxithromycin	Binds irreversibly to the bacterial ribosome resulting in the inhibition of the translocation steps of protein synthesis.	Commonly used when patients are allergic to penicillins. Safe to be used during pregnancy and by children. Compared to the other three macrolides, Erythromycin has a shorter half-life, more side-effects and a narrower spectrum of activity. Erythromycin is prescribed for the treatment of atypical pneumonia, pertussis, diphtheria and uncomplicated skin infections.
Tetracyclines	Tetracycline Doxycycline Minocycline Lymecycline	Interfere in the protein synthesis process.	Drugs of first choice in treatment of brucellosis, acne, mycoplasma, rickettsial and chlamydial infections. Should not be prescribed for pregnant women or for children.
Aminoglycosides	Streptomycin Gentamycin Amikacin	Inhibit protein synthesis by directly preventing the synthesis process by attaching to the ribosomal subunit; also result in the misinterpretation of the mRNA.	Are very toxic and can result in ototoxicity, nephrotoxicity and striped-muscle paralysis. They are not water-soluble and are thus administered parenterally. For these reasons, they are reserved for life-threatening infections such as septicaemia and tuberculosis.

Table 6: Overview of various antibiotic classes: Mechanisms of action and examples of antibiotics in each specific class (continued)

Class	Examples of antibiotics in class	Mechanism of action	Additional information
Do not belong to a class	Chloramphenicol	Prevent protein synthesis in sensitive bacteria.	A broad-spectrum antibiotic which is seldom used as a first-line treatment as it is potentially toxic and can cause aplastic anaemia. Mostly limited to ophthalmic and otic dosage forms.
Fluoroquinolones	Ciprofloxacin Norfloxacin Moxifloxacin	Destroy bacteria by selectively inhibiting DNA gyrase which is needed in the process of DNA replication.	Effective against various Gram-positive and Gram-negative microorganisms. Ciprofloxacin is used in cystic fibrosis, typhoid, *Salmonella*, prostatitis and serious or resistant infections. Are contraindicated in pregnancy, lactating mothers or growing children.
Lincosamides	Clindamycin Lincomycin	Prevent bacterial replication by binding to the ribosomal subunit and interfering with the protein synthesis process.	Clindamycin is active against Gram-positive cocci including penicillin resistant staphylococci and anaerobes. It is used in the treatment of chronic osteomyelitis, severe soft tissue infections and intra-abdominal sepsis. Due to clindamycin being structurally related to erythromycin, cross-resistance is common.
Glycopeptide antibiotics	Vancomycin Telavancin Oritavancin Teicoplanin	Inhibit the bacterial cell wall synthesis process.	Vancomycin is a most important reserve drug against life-threatening infections caused by MRSA such as endocarditis and septicaemia. As a result of its narrow therapeutic index, vancomycin can result in ototoxicity and nephrotoxicity. Teicoplanin compared to vancomycin has fewer side effects and a longer half-life which increase patient compliance.

UTI = urinary tract infections, DNA = deoxyribonucleic acid and MRSA = methicillin-resistant *S. aureus*

Sources: (Longmore *et al.* 2010; Dreyer *et al.* 2013; Anderson 2016; Swalaha 2016).

With so many antibiotic classes to choose from, doctors need to take various factors into consideration when selecting the most effective antibiotic to prescribe to the patient (Anderson 2016): These factors are:

- The causative microorganism for the infection.
- Likelihood of antibiotic resistance being present.
- Presence of contraindications towards specific antibiotic.
- Ability of antibiotic to penetrate target tissue.
- Spectrum of activity of antibiotic.

2.5.3 Bacteria and antibiotics most commonly implicated in antibacterial resistance

Table 7: Bacteria and antibiotics most commonly implicated in antibacterial resistance

Bacteria	Antibiotic resistance shown towards	Example of disease caused
Escherichia coli	Cephalosporins Fluoroquinolones Carbapenem	UTI Blood stream infections (BSI)
Klebsiella spp.	Cephalosporins Carbapenems	Pneumonia BSI UTI
Methicillin-resistant *Staphylococcus aureus*	Methicillin Nafcillin Oxacillin Cephalosporins Tetracycline Erythromycin	Wound infections BSI Pneumonia
Drug-resistant *Streptococcus pneumoniae*	Penicillin Amoxicillin Azithromycin	Pneumonia Meningitis Otitis BSI
Drug-resistant *Nontyphoidal Salmonella*	Fluoroquinolones Ceftriaxone Ciprofloxacin	Foodborne diarrhoea BSI
Shigella spp.	Fluoroquinolones	Bacillary dysenteria
Neisseria gonorrhoeae	Cephalosporins Tetracycline	Gonorrhoea
Clostridium difficile	Fluoroquinolones Cephalosporins Carbapenems Clindamycin	Life-threatening diarrhoea
Carbapenem-resistant *Klebsiella* spp.	Nearly all antibiotics including carbapenem which is considered the last resort.	BSI
Multidrug-resistant *Acinetobacter*	Fluoroquinolones Cephalosporins Aminoglycosides	Pneumonia BSI
Drug-resistant *Campylobacter*	Ciprofloxacin Azithromycin	Diarrhoea
Extended spectrum β- lactamase producing *Enterobacteriaceae*	Penicillins Cephalosporins	BSI

Table 7: Bacteria and antibiotics most commonly implicated in antibacterial resistance (continued)

Bacteria	Antibiotic resistance shown towards	Example of disease caused
Vancomycin-resistant *Enterococcus*	Vancomycin	UTI BSI SSI
Multidrug-resistant *Pseudomonas aeruginosa*	Fluoroquinolones Cephalosporins Aminoglycosides Carbapenems	Pneumonia BSI SSI UTI
Drug-resistant *Salmonella typhi*	Ceftriaxone Azithromycin Ciprofloxacin	Typhoid fever
Drug-resistant *Shigella*	Ampicillin Trimethrprim-sulfamethoxazole Ciprofloxacin Azithromycin	Diarrhoea
Vancomycin-resistant *Staphylococcus aureus*	Vancomycin Methicillin Cephalosporins	Wound infections BSI Pneumonia
Erythromycin-resistant Group A *Streptococcus*	Clindamycin Erythromycin Azithromycin Clarithromycin	Pharyngitis Streptococcal shock syndrome Necrotizing fasciitis Scarlet fever Skin infections Rheumatic fever
Clindamycin-resistant Group B *Streptococcus*	Clindamycin Erythromycin Vancomycin Azithromycin	BSI Pneumonia Meningitis Skin infections

UTI = urinary tract infections, BSI = blood stream infections, spp. = species and SSI = surgical site infections

Sources: (United States Department of Health: Centers for Disease Control and Prevention 2013; World Health Organization 2014)

Ramifications of resistance to specific antibiotics by a specific bacterium (World Health Organization 2014) may be the following:

- Cephalosporin resistance by *E.coli* and *K. pneumoniae* is likely to result in the usage of other appropriate, more expensive antibiotics (i.e. carbapenems), which could result in an increase of carbapenem-resistant strains.
- Quinolones are the most common antibiotics used for UTI. As they are taken orally, they are easily and economically administered to patients. If resistance to all oral alternative antibiotics occurs, treatment by injection will be necessary resulting in additional costs and limitation of treatment to poorly resourced areas.

- Carbapenem resistant bacteria will need to be treated with tigecycline or colistin. Not only are these drugs the last resort, but they are less effective and not widely available.
- Resistance to penicillinase-stable antibacterial drugs, such as in the case of MRSA infections, will result in the usage of second-line drugs. These drugs are expensive and constant monitoring is advised throughout treatment as a result of their adverse effects.

As shown in Table 7 on the previous two pages, various bacteria are resistant to multiple antibiotics and, in some instances, to just certain antibiotics. For this reason, the implications for a patient who contracts an antibiotic resistant bacteria (ARB) are far worse than for a patient who is infected with a non-resistant bacteria (World Health Organization 2014).

2.5.4 Prevalence and impact

In 2014, the World Health Organization explained that, due to a paucity of information relating to the extent of ARM, it was impossible to estimate the exact global prevalence and impact of infections. The health implications of ARM are thus far reaching and include higher medical costs, prolonged hospital stays, admission to ICUs, increased mortality and morbidity rates.

2.5.4.1 Europe

It has been estimated that ARM in Europe result in 25 000 deaths, 2,5 million additional days in hospital and a minimum of €1.5 billion in healthcare costs and productivity losses annually (National Health Service choices 2012; European Commission 2017). In an article by the European Commission (2017), it was stated that, according to the World Bank, if nothing was done regarding ARM drug-resistant infections, it could cause global economic damage by 2050 to the same degree as the 2008 financial crisis.

Data obtained from the European Antimicrobial Resistance Surveillance Network indicate that the prevalence of ARM is low in Scandinavian countries and in the Netherlands; however, it is high in Southern Europe. The data also suggest that countries with lower resistance rates normally have lower usage of antibiotics. Conversely, countries that use copious amounts of antibiotics have higher rates of ARM (European Centre for Disease Prevention and Control 2017).

2.5.4.2 USA

The USDH: CDCP stated that, according to conservative calculations, a minimum of two million people are infected with ARM in the USA annually and that at least 23 000 people die each year as a direct consequence of these infections (United States Department of Health: Centers for Disease Control and Prevention 2017a).

Roberts *et al.* (2009) executed a study in a Chicago teaching hospital in 2008 to determine the financial burden associated with ARM. Their sample comprised of 188 patients. It was found that the costs of an infection due to ARM ranged between US$18 588 and US$29 069 per patient, while the societal costs were US$10,7 to US$15,0 million per year. The patients stayed an extra 6.4 to 12.7 days in hospital with the infections contributing to a mortality rate of 6.5%. Mauldin *et al.* (2010) found that patients infected with a resistant Gram-negative bacteria incurred average hospital costs of US$38 121 higher compared to patients infected with susceptible bacteria.

The USDH: CDCP used the above information to suggest that ARM cost the healthcare system an additional US$20 billion, while the societal cost as a result of lost productivity is an estimated US$35 billion each year (United States Department of Health: Centers for Disease Control and Prevention 2013).

2.5.4.3 South Africa

Only a handful of studies have been conducted in South Africa to determine the prevalence of ARM and a select few will be discussed below:

- Perovic *et al.* (2015) established that the frequency of MRSA detection in South Africa was high after conducting their research in 13 academic centres. They found that 46% of the *S. aureus* included in the study was resistant to methicillin, with MRSA more common in Gauteng than the other provinces sampled.
- It was found that 68.3% of the *K. pneumonia* isolates were extended spectrum β- lactamase positive, thus indicating resistance towards cefotaxime, ceftazidime and cefepime. Likewise, 46.5% of all isolates tested was resistant to ciprofloxacin and 33.1% was resistant to piperacillin-tazobactam (Perovic *et al.* 2014).
- Singh-Moodley and Perovic (2016) reported that 68% of the carbapenem-resistant Enterobacteriaceae isolates tested possessed a carbapenemase-producing gene. They also concluded that carbapenemase-producing Enterobacteriaceae was increasing in South Africa.

2.5.5 Mechanisms that result in antimicrobial resistant microorganisms

The unnecessary or inappropriate use of antibiotics leads to the development of ARM, as incorrect use supports genetic modifications by way of horizontal gene transfer or mutagenesis (United States Department of Health: Centers for Disease Control and Prevention 2013; Viswanathan 2014). Fleming-Dutra, Hersh and Shapiro (2016) reported that there was an estimated annual antibiotic prescription rate of 506 per a 1 000 people in 2010–2011 in America. They determined that roughly 30% of the antibiotics that were prescribed was unnecessary.

According to the European Centre for Disease Prevention and Control (2017), unnecessary or inappropriate usage of antibiotics includes:

- Prescription of antibiotics when not indicated, such as in the case of viral infections;
- Poor patient compliance with regards to completing the antibiotic course and not taking the antibiotic at the frequency indicated by the doctor;
- Prescription of insufficient dosages or treatment durations.

2.5.5.1 Manner in which bacteria obtain the ability to become resistant

A bacterium is capable of adapting to an environment that is exposed to an antibiotic. This adaptation occurs in the form of a spontaneous gene mutation as a result of the pressure applied to it by the antibiotic (Salmond and Welch 2008). Bacteria may then share these gene mutations via horizontal gene transfer (Burmeister 2015). Normally, bacteria obtain external genetic material either through transformation or conjugation (Munita and Arias 2016).

Transformation in turn results in the incorporation of DNA fragments or plasmids into the recipient cells' DNA structure after a number of cells have broken up and released their genetic materials (Swalaha 2016).

In a hospital environment, the development of ARB normally involves conjugation. During conjugation, two living cells make contact via a pili. Mobile genetic elements, either in the form of plasmids or transposons, are then transferred from the donor cell to the recipient cell (Munita and Arias 2016). In this manner the antibacterial genes are transferred from one bacterium to the other.

2.5.5.2 Mechanisms used by bacteria to avoid the effects of antibiotics

Gram-negative bacteria have a specialised outer cell membrane which can selectively resist the entry of antibiotics by changing the size of the porins (Swalaha 2016). Antibiotics which are affected the most by this mechanism are β-lactams, tetracyclines, certain fluoroquinolones and vancomycin (Winterhalter, James and Pagès 2008). Gram-positive bacteria are unable to use this mechanism as a result of the structure of their cell wall.

Bacteria make use of efflux pumps located in their cell wall to eliminate the antibiotic from their cytoplasm before it can reach an effective concentration (United States Department of Health: Centers for Disease Control and Prevention 2017c). These pumps are activated by a specific substrate that is associated with an antibiotic, such as *mef* genes for macrolides and *tet* genes for tetracycline in pneumococci (Poole 2005). This mechanism is very effective against protein synthesis inhibitors, *β*-lactams, fluoroquinolones, polymyxins and carbapenems (Singh, Weinstock and Murray 2002).

Certain bacteria, such as *K. pneumonia*, possess the ability to produce carbapenemases, which is an enzyme that breaks down carbapenem, while other bacteria can produce *β*-lactamases which destroy *β*-lactam antibiotics and render them ineffective (Bush 2013; United States Department of Health: Centers for Disease Control and Prevention 2017c).

Therefore, instead of using an enzyme to break down the antibiotic, Gram-positive or negative bacteria modify the antibiotic by making chemical changes to it and thus changing its function. The antibiotics which are affected the most by these enzymatic modifications are those whose mechanism of action is to prevent protein synthesis (Wilson 2014). These modifying enzymes catalyse the following biochemical reactions: acetylation of aminoglycosides, chloramphenicol and streptogramins, phosphorylation of aminoglycosides and chloramphenicol and adenylation of aminoglycosides and lincosamides (Munita and Arias 2016).

Resistant bacteria can alter or protect the target receptors located at the site of protein synthesis, which will result in the antibiotics not recognizing the receptor or not being able to attach to it. An example of target protection is resistant *E. coli* which possess the mcr-1 gene that changes the target receptor by adding an acetyl or phosphate compound to it, resulting in colistin not being able to attach to it (United States Department of Health: Centers for Disease Control and Prevention 2017c). Modification of the target site is very effective against almost all types of antibiotics. It occurs as a result of mutation in the genes that encode the target site, enzymatic alterations of the target site, and the replacement or avoidance of the original binding target (Munita and Arias 2016).

Sometimes resistant bacteria alter their metabolic pathways. This is done by either producing more of the target metabolite or by making use of an alternative pathway to avoid the sequence inhibited by the antibiotic (Swalaha 2016; United States Department of Health: Centers for Disease Control and Prevention 2017c).

2.6 Intervention strategies

Contamination of medical equipment refers to the presence of organic or inorganic substances and microorganisms on the surface of the equipment. According to the Spalding classification, equipment is classified into three different categories, namely critical, semi-critical and non-critical equipment. These categories refer to the degree of risk involved for the contraction of an infection when using that specific piece of equipment. Both the World Health Organization (2009) and the United States Department of Health: Centers for Disease Control and Prevention (2016) make use of the Spalding classification, which is as follows:

- Critical equipment has a high risk for infection if contaminated with microorganisms. These equipment must be sterile or must be sterilized before use as they are used to enter sterile body tissues, cavities or the bloodstream. Examples of critical equipment are surgical instruments, cardiac and urinary catheters and surgical implants.
- Semi-critical pieces of equipment require a high-level of disinfection as their surfaces come into contact with mucous membranes or non-intact skin, such as respiratory therapy and anaesthesia equipment, endoscopes, laryngoscope blades and cystoscopes. High-level disinfection refers to removal of all microorganisms from the instruments' surfaces, except for a few bacterial spores. High-level disinfectants are for example glutaraldehyde and hydrogen peroxide.
- Non-critical pieces of equipment, such as stethoscopes, sphygmomanometers, computers, crutches and bed rails only come into contact with intact skin. These equipment must be disinfected with a low- or intermediate-level disinfectants directly after use. This study evaluated the contamination levels of stethoscopes and sphygmomanometers used by CSs in the DUT CDC.

2.6.1 Cleaning

Cleaning refers to the removal of foreign, organic or inorganic materials from a surface or an object through manual or mechanical means. Cleaning must be performed prior to high-level disinfection and sterilization, because the presence of inorganic or organic materials on the surfaces of instruments will hinder the effectiveness of these processes (United States Department of Health: Centers for Disease Control and Prevention 2016).

Manual cleaning is performed when mechanical units are unavailable or when the instruments are fragile or difficult to clean. Two important components of manual cleaning are friction and fluidics. Friction refers to the scrubbing of the soiled surface with a brush and fluidics refers to the usage of pressurized fluid to remove debris from surfaces not accessible with the brush (United States Department of Health: Centers for Disease Control and Prevention 2016).

2.6.2 Sterilization and disinfection

Sterilization refers to the procedures that remove or eradicate all living cells, spores and acellular entities from an object, fluid or habitat. Sterility is an absolute state as a sterile item is totally free from any viable microorganisms, spores and other pathogenic agents, thus preventing any disease transmission associated with the usage of that item. Critical items must be sterile before they can be used and this can be achieved by either physical or chemical methods (Willey *et al.* 2014). The principal sterilizing agents used in the healthcare setting are dry heat, pressurized steam, hydrogen peroxide gas plasma, ethylene oxide gas and liquid chemicals (United States Department of Health: Centers for Disease Control and Prevention 2016).

Disinfection is the process of destruction and elimination of all vegetative pathogens, except bacterial spores on inanimate objects (Willey *et al.* 2014). In the healthcare setting, inanimate objects or surfaces are usually disinfected by either liquid chemicals or wet pasteurization. High-level disinfectants are capable of killing all microorganisms, expect a large number of bacterial spores. Aldehydes, peracetic acid and chlorine dioxide are all examples of high-level disinfectants. Intermediate-level disinfectants, such as alcohols, phenols, sodium hypochlorite and idophors are effective against mycobacteria, vegetative bacteria, the majority of viruses and fungi, but are unable to kill bacterial spores. Low-level disinfectants normally have an exposure time of less than ten minutes and are lethal for most vegetative bacteria and some fungi and viruses. Disinfectants that fall in this category are alcohols and quaternary ammonium compounds (Juwarkar 2013; United States Department of Health: Centers for Disease Control and Prevention 2016).

2.6.2.1 Factors affecting efficacy of disinfection and sterilization

The effectiveness of germicides depends on a number of factors, some which are intrinsic characteristics of the microorganisms and others which relate to the chemical and external physical environment (United States Department of Health: Centers for Disease Control and Prevention 2016). These factors will be briefly discussed below with reference to Willey *et al.* (2014) and United States Department of Health: Centers for Disease Control and Prevention (2016):

- Presence of organic matter: The presence of organic matter such as saliva, pus, faeces or blood on the surface of an object protects microorganisms against heating and chemical disinfectants by acting as a physical barrier. Thus it is important to clean the object before it is disinfected or sterilized.
- Population size of microorganism: An equal number of microorganisms are killed during each interval; therefore, a larger population will take a longer time to eliminate compared to a small population.

- Temperature: Some chemical agents' activity can be enhanced by increasing the temperature to a certain point at which it acts optimally.
- Population composition: Different microorganisms have different levels of susceptibility and bacterial endospores are more resistant than vegetative bacteria. This is largely due to the fact that their coat and cortex act as a barrier, while mycobacteria have a waxy cell wall that constrains the entry of disinfectants. Gram-negative bacteria also possess an innate resistance to disinfection due to their outer membrane composition.
- Location of microorganisms: Certain medical instruments such as endoscopes consist of multiple pieces and must be disassembled before being disinfected. Objects that are smooth in nature are easier to disinfect compared to those that are porous, as only the surfaces that are in direct contact with the germicide for the entire exposure time will be disinfected.
- Concentration of disinfectant: The effectiveness of a disinfectant is not always directly related to its concentration. For example, 70% ethanol has been found to be more effective that 95% ethanol as its activity is improved by the presence of water.
- Contact/Exposure time: The longer a microbial population is exposed to a microbiocidal agent, the more microorganisms are killed.
- Presence of biofilm: A biofilm is a microbial community that attaches to a surface and cannot be removed easily. It acts as a barrier and impairs the exposure of the chemical sterilant to the microbial cell.
- pH: By altering the pH of the disinfectant, its efficacy can either be increased, such as in the case of glutaraldehyde, or decreased, in for example iodine and phenols.

These factors need to be taken into account when deciding on the appropriate method and contact time in order to achieve disinfection or sterilization (Willey *et al.* 2014).

2.6.2.2 Options available for the disinfection of stethoscopes and sphygmomanometers in healthcare settings

<u>Alcohol</u>

Alcohols such as ethanol (ethyl alcohol) and isopropanol (isopropyl alcohol) are the most commonly used disinfectants as they are bactericidal and fungicidal. The optimum bactericidal concentration is a 60%–90% solution, as the presence of water improves the microbicidal activity of these substances (Davis 2009). The mode of action of alcohol is the denaturation of the microorganism's proteins. Both ethanol and isopropanol at the optimum bactericidal concentration are effective against *E. coli, S. aureus, Ps. aeruginosa, S. pyogenes* and *S. marcescens.* Alcohols are recommended for the disinfection of non-critical items such as stethoscopes and sphygmomanometers (Davis 2009). The disadvantages of alcohols include fast evaporation rates, the fact that they are not EPA-registered disinfectants, are affected by organic matter and they can damage certain instruments (Willey *et al.* 2014).

Gupta *et al.* (2014) found that the disinfection of stethoscopes with the use of 70% ethanol resulted in a significant decrease (86.7%) in the bacterial count. Lecat *et al.* (2009) compared the efficacy of ethanol-based cleanser to isopropyl alcohol pads when disinfecting contaminated stethoscopes. The diaphragms were cleaned with the selected disinfectant and then allowed to air dry for 60 s. The use of both disinfectants resulted in a significant reduction of the CFU counts, with the ethanol-based cleanser decreasing the counts by 92.8% and the isopropyl alcohol pads by 92.5%. It was found that 32% of the stethoscopes that had been cleaned with the isopropyl alcohol pads were growth-free afterwards, compared to 24.5% that had been cleaned using the ethanol-based cleanser. In a study conducted by Jeyakumari *et al.* (2017), all the contaminated stethoscopes that had been disinfected with 70% isopropyl alcohol were growth-free. Núñez *et al.* (2000) evaluated the efficacy of 96% ethanol and isopropyl alcohol-based disinfectants. Isopropyl alcohol was found to be the most effective, reducing the bacterial count by 99.8% while the ethanol resulted in a reduction of 98.2%.

<u>Chlorhexidine</u>

Chlorhexidine is used in the healthcare setting to clean skin prior to surgery, wash hands with and to disinfect non-critical equipment. Compared to alcohol, chlorhexidine has a much longer duration of action. For this reason, the addition of low concentrations of chlorhexidine to an alcohol-based preparation causes a significantly greater duration of action. Its antimicrobial activity is not affected by the presence of organic materials (World Health Organization 2009). Álvarez *et al.* (2016) reported that 1% chlorhexidine prevented the recontamination of stethoscopes for a period of four hours after disinfection.

<u>UV radiation</u>

The ultraviolet (UV) spectrum includes the following types of rays: UVA, UVB and UVC. UVA has a long wavelength (315‒400 nm) and is capable of penetrating the skin's dermis, unlike UVB which has a shorter wavelength (280‒315 nm) and can only reach the outer epidermis of the skin. Prolonged exposure to both UVA and UVC can result in skin damage which could present as premature aging or development of skin cancer (United States Food and Drug Administration 2013).

UVC has the shortest wavelength (240−280 nm) of the three types and has been found to be bactericidal. However, all the UVC are absorbed by the ozone layer surrounding the earth (United States Food and Drug Administration 2013). UV germicidal irradiation devices produce UVC rays which are used for sterilization purposes. The effectiveness of UVC radiation is influenced by the presence of organic material, type of suspension, type of microorganism, setting of wavelength and distance (Willey *et al.* 2014). Microorganisms are inactivated by UVC radiation as it intercalates nucleic acid through induction of thymine dimers. To date, UVC radiation has been primarily used in the healthcare setting to destroy airborne organisms or for the inactivation of microorganisms on specific surfaces such as titanium implants (United States Department of Health: Centers for Disease Control and Prevention 2016).

Messina *et al.* (2015) investigated the efficacy of a UVC light-emitting diode (LED) used for the disinfection of stethoscope membranes. The UVC LED was set to a wavelength of 260 nm and at an irradiation angle of 120˚. The contaminated stethoscope membranes were illuminated with the UVC LED for one minute at a distance of 11,5 mm. The UV treatment resulted in a significant reduction in CFU counts of *E. faecalis* (85.5%), *S. aureus* (87.5%), *E. coli* (94.3%) and *Ps. aeruginosa* (94.9%). Messina *et al.* (2016) established that UVC LED has the ability to maintain high levels of disinfection over a period of 240 hours if the stethoscope membranes were disinfected over a five minute cycle.

2.6.3 Disposable equipment and covers

Alpert and Cohen (1996) conclude that the Cas Papercuff, a disposable sphygmomanometer cuff, should provide accurate readings while at the same time limit the potential spread of infections. However, the cost implications make the use of disposable cuffs an unlikely solution, especially in resource-limited regions, at a cost of between US$12−90 per cuff (School Health 2017). Notwithstanding the above, the global disposable sphygmomanometer cuff market is projected to reach US$356,8 million by 2025, according to Markets Insider (2017). Countries that make use of disposable cuffs include the USA, Canada, the United Kingdom, Germany, Japan, South Africa and China (Markets Insider 2017).

A more cost-effective option might be the use of disposable cuff covers which cost between US$2−20 per cover (Grewal *et al.* 2013). Webb (2002) claimed that the use of single-use barrier sleeves covering the cuff of the sphygmomanometers significantly reduced the number of microorganisms found on the cuffs. Conversely, Bhanot *et al.* (2011) argued that the usage of such disposable covers will make no difference to the level of bacterial contamination found on the sphygmomanometer cuffs. The solution is therefore not obvious, as Wood, Lund and Stevenson (2007) reported that the use of antimicrobial diaphragm covers does not necessarily offer any advantage, as all 37 stethoscopes that they had tested for this application were still contaminated.

2.6.4 Recommended cleaning protocols

2.6.4.1 Hand washing

The United States Department of Health: Centers for Disease Control and Prevention (2017g) and WHO (2009) advise the application of the following protocol:

Protocol for the use of alcohol-based hand sanitizer

- Put an adequate amount of hand sanitizer in a cupped hand, covering the entire palm surface.
- Rub the palm surfaces against each other, followed by the rubbing of the dorsum surfaces of the hands before proceeding to clean areas in-between fingers and the thumbs.
- Finish with rubbing the left palm with clasped fingers of the right hand in a rotational movement, before switching over.
- Keep rubbing until all surfaces feel dry.
- Do not wipe excess hand sanitizer off with a towel.
- The duration of the entire procedure should take about 30 s.

Protocol for the use of soap and water

- Rinse hands with cold water.
- Apply a sufficient amount of soap to the hands.
- Rub the palm surfaces against each other, then rub the dorsum surfaces of the hands before proceeding to clean areas in-between fingers and the thumbs. This step should take about 20s.
- Finish with rubbing the right palm with clasped fingers of the left hand in a rotational movement, before switching over.
- Rinse the soap off with water and dry the hands with a disposable paper towel.
- Make use of the paper towel to close the tap.
- The duration of the entire procedure should take about 1 minute.

2.6.4.2 Stethoscopes and sphygmomanometers

The United States Department of Health: Centers for Disease Control and Prevention (2016) endorses the disinfection of noncritical equipment, such as sphygmomanometers and stethoscopes, with low- or intermediate-level disinfectants registered with the United States Environmental Protection Agency (EPA). The appropriate disinfectant must be applied to the surfaces of the stethoscope and sphygmomanometer for the time specified by the manufacturer and left to air dry. It is the policy of the National Health Service (2012) that a stethoscope and a sphygmomanometer must be cleaned between patients.

However, it is recommended that stethoscopes be disinfected before and after examining each patient (Jain *et al.* 2013; Shiferaw *et al.* 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014).

2.6.4.3 Disinfection of chiropractic tables

Ramcharan, Evans Jr and Burnham (2008) urged every chiropractic facility to implement adequate hand hygiene and table-disinfecting protocols in order to reduce the risk of infection, especially in immune-compromised patients. Evans *et al.* (2009a) argued that chiropractic tables must be considered as a critical surface with regards to their potential for high level of contamination due to the large surface area that is in contact with the exposed skin of the patient. Due to the lack of an established table disinfection policy, Evans *et al.* (2009a) proposed the following protocol:

- Remove used face paper from the headrest.
- Apply a disinfectant to the entire surface of the headrest, face paper bar, armrests and chest piece of table.
- Allow the table surface to air dry completely.
- Place a new face paper onto the headrest for each patient.
- Repeat this procedure after each patient.

The face paper must be changed between patients, as it acts as a physical barrier between the table and body fluids and make-up. However, the face paper does not completely prevent the transmission of bacteria between the patient and the table, but it does decrease the total number that is transferred (Evans *et al.* 2007; Puhl *et al.* 2011).

Hand washing between patients has been found to be the simplest and most effective way of decreasing the risk of bacterial transmission in a healthcare setting (Evans and Breshears 2007). Chiropractic is a form of manual therapy and thus requires a lot of physical contact with the hands. Chiropractors, whether professionals or students, must therefore wash their hands before and after each patient with a disinfectant with an active ingredient of at least 60% alcohol, as described in Section 2.6.2.2 (Evans *et al.* 2009a).

With regard to the material used to cover chiropractic tables, Evans *et al.* (2009a) suggested that cloth-covered chiropractic tables cannot be adequately cleaned as they are very porous and could easily house dirt, body secretions and dust. Hence they stated that the use of such tables must be discontinued immediately and that table surfaces should be covered with material such as vinyl, which is non-porous and can effectively be cleaned by disinfectants (Evans *et al.* (2007). An alternative option is the placement of vinyl slip covers over cloth covered tables in the event of replacement not being an option (Evans *et al.* 2009a).

2.6.5 Knowledge, attitude and practices of healthcare professionals with regards to disinfection practices

The knowledge-attitude-practice model is frequently used to explain the relationship between these three aspects. As a person gains knowledge with regards to a health related practice, changes in attitude are initiated. Over time, this accumulation of attitude changes results in positive changes in the person's practices (Baranowski *et al.* 2003). This model thus suggests that knowledge comes before attitude and then, together, knowledge and attitude predict and precede practice (Aboyeji, Ijaiya and Jimoh 2004).

2.6.5.1 Hand washing

Nair *et al.* (2014) evaluated the knowledge, attitude and practice of nursing and medical students with regards to hand hygiene at a tertiary healthcare centre in India. A significant difference was identified between the two groups with regards to having received prior education relating to hand washing. Only 74.2% of medical students indicated receiving prior training as opposed to 95.4% of the nursing students. Both groups stated that they knew the correct technique of hand washing. It was noted that nursing students had significantly better knowledge, attitudes (52.1% vs 12.9%) and practice (62.1% vs 19.6%) compared to medical students. This corresponds with the findings of Van De Mortel *et al.* (2012).

Nabavi *et al.* (2015) assessed 256 medical residents working in the Imam Hossein Hospital with respect to their knowledge, attitudes and practices of good hand hygiene. The majority of residents (67%) had poor knowledge about hand hygiene, while 26.9% and 4.3% had a moderate and good level of knowledge respectively. The majority of these residents had poor attitudes towards good hand hygiene. Only 3.1% were able to demonstrate the correct hand washing technique and only 0.8% was compliant with the practice of good hand hygiene.

2.6.5.2 Stethoscopes and sphygmomanometers

This section discusses HCWs' attitudes towards the disinfection of stethoscopes (Table 8).

Table 8: Summary of results of studies with regards to actual and perceived adequate frequency of stethoscope disinfection, reasons for lack of adequate disinfection and methods used when conducting stethoscope disinfection

Frequency of stethoscope disinfection	Reasons identified for lack of stethoscope disinfection	Perceived adequate frequency of stethoscope disinfection	Methods used for disinfection	Reference and sample size
8.0% Before and after every patient. 35.0% more than once daily. 16.0% once daily 21.0% weekly 10.0% monthly 10.0% never	Lack of time or too busy. Forgetfulness Lack of access to supplies.	98.0% daily	Ethyl alcohol wipes Propyl alcohol wipes Hand sanitizer	(Tang *et al.* 2011) *n* = 103
24.2% after each use 32.3% more than once daily. 8.8% daily 19.7% weekly 11.0% after seeing patients on contact precautions. 3.8% never	Lack of access to disinfectants. Lack of visual reminders.	78.6% after every use 12.3% more than once daily. 3.8% daily 1.0% weekly 4.1% after seeing a patient on contact precautions. 0.3% never	Alcohol swipe 67.0% Disinfectant wipes 18.0%. Hand sanitizer 14.0%	(Muniz *et al.* 2012) *n*= 1401
4.7% daily 9.9% weekly 15.7% monthly 4.6% yearly 2.9% once every few years.			Dry cloth 53.2% Alcohol wipes 34.4% Water only 5.2% Soapy water 3.9% Other 3.2%	(Hyder 2012) *n*= 408
3.9% after each patient 14.6% daily 17.9% weekly 23.4% monthly 17.9% yearly 22.4% never	Insufficient education Lack of access to disinfectants. Poor role-modelling			(Saunders, Hryhorskyj and Skinner 2013) *n*= 308

Table 8: Summary of results of studies with regards to actual and perceived adequate frequency of stethoscope disinfection, reasons for lack of adequate disinfection and methods used when conducting stethoscope disinfection (continued)

Frequency of stethoscope disinfection	Reasons identified for lack of stethoscope disinfection	Perceived adequate frequency of stethoscope disinfection	Methods used for disinfection	Reference and sample size
32.5% weekly 17.5% monthly 18.8% never		100% indicated it should be done regularly.	Ethyl alcohol 27.5% Dry cloth 7.5%	(Jain *et al.* 2013) *n*= 80
1.0% daily 9.0% weekly 6.0% monthly 84.0% never	Concern over damaging the stethoscope. Lack of time Lack of knowledge	9.0% before and after each patient. 57.0% weekly 34.0% did not know adequate frequency.	Ethyl alcohol Hand sanitizer	(Pal *et al.* 2015) *n*= 100

%= percentage and *n*= sample size

Almost fifty percent of the above studies identified lack of access to disinfectants as an obstacle in regular stethoscope disinfection practices (Tang *et al.* 2011; Muniz *et al.* 2012; Saunders, Hryhorskyj and Skinner 2013). Zaghi *et al.* (2013) determined that the placement of disinfection supplies and visible reminders at strategic locations could significantly increase the rates of stethoscope disinfection by HCWs.

Even though the majority of the participating HCWs was of the opinion that contaminated stethoscopes could result in HCAIs, only 16% of them disinfected theirs while the rest cited a lack of education as a reason for low compliance (Pal *et al.* 2015). Saunders, Hryhorskyj and Skinner (2013) found that only 2.9% of the medical students in their study had received education on stethoscope disinfection. This is alarming, as Hyder (2012) found a strong correlation between regular disinfection of one's stethoscope and having received information regarding the importance of disinfection. In contrast, Jain *et al.* (2013) established that 97% of the HCWs in his study had received information regarding stethoscopes' role in the spread of HCAIs; however, this did not reflect in their stethoscope disinfection practices.

Additional factors identified by Saunders, Hryhorskyj and Skinner (2013) that have a significant correlation with the frequency of stethoscope disinfection are: self-confidence of students in knowing how to disinfect their stethoscope adequately, observing fellow students disinfecting their stethoscopes, and perceiving stethoscope disinfection as important. Muniz *et al.* (2012) suggested that people who believed that stethoscopes were involved in the transmission of infections were more likely to clean their stethoscopes after every use than those who did not harbour this belief.

The researcher could not trace any information relating to the knowledge, attitudes and practices of HCWs with regards to disinfection practices of sphygmomanometers.

2.6.5.3 The disinfection of chiropractic tables

Evans and Breshears (2007) conducted a survey on 484 CSs at a chiropractic college in the USA regarding hand sanitizing and the disinfection of chiropractic tables. Even though 79.6% of the CSs deemed washing of hands between treating patients as important or very important, only 68.8% washed their hands on a regular basis, with 10% of students confessing that they never or rarely washed their hands between patients. It was found that 28% of the CSs carried around their own personal hand sanitizers due to the absence of a sink in the consultation rooms where they worked. If hand-sanitizing dispensers were located at convenient and appropriate places, 86.8% of the students indicated that they would make use of this facility frequently or always. With regards to chiropractic table disinfection, 95% of the students affirmed that they always changed the face paper between patients, while the majority (80%) never or rarely disinfected their chiropractic tables. The students cited the lack of disinfectants as the primary reason for this.

In contrast, all the CSs evaluated by Puhl *et al.* (2011) reported replacing the face paper after each patient. In general, these CSs also had a better compliance rate for the disinfection of chiropractic tables compared to the students assessed by Evans and Breshears (2007). Ten percent of the CSs disinfected their tables after each patient, 37% disinfected their tables more than once daily, 27% disinfected the tables at least once daily, and 27% disinfected them only weekly or when deemed necessary. This suggests that the majority (81%) of the students agreed that chiropractic tables could serve as a source of cross-contamination of pathogens. The fact that 84% reported that the disinfection of tables was either important or very important could also suggest that the training of this group of students had been supported by a better designed and more holistic curriculum than that of their counterparts mentioned in the previous study.

2.6.6 Impact of educational programs on hygiene habits

2.6.6.1 Hand washing

Monistrol *et al.* (2012) determined the impact of an educational campaign on hand hygiene compliance of HCWs by making use of the WHO hand hygiene observation method. The study consisted of four phases of 10 weeks each, namely a pre-intervention, intervention and post-intervention period, with a follow-up evaluation after one year (table 9). The educational program consisted of seminars discussing WHO guidelines for hand hygiene, teaching of proper hand washing techniques, distribution of hand hygiene pamphlets, visual reminders of the correct hand washing technique at every basin, and placement of motivational posters throughout the study area.

Table 9: Summary of results of hand hygiene compliance over three periods as determined by Monistrol *et al.* (2012)

Aspect evaluated	Pre-intervention period	Post-intervention period	Follow-up evaluation after one year
HCWs' hand hygiene compliance	54.3% (*n*= 751)	75.8% (*n*= 780)	75.8% (*n*= 450)
Quantities of alcohol-based hand rub used	10.5 L/1 000 patient-days	27.2 L/1 000 patient-days	31.5 L/1 000 patient-days
Selection of alcohol-based hand rub to sanitize hands	63.7% (*n*= 408)	86.1% (*n*= 591)	Not stated
Disinfection of hands before patient contact	22.7% (*n*= 163)	55% (*n*= 209)	53.5% (*n*= 114)
Disinfection of hands immediately before aseptic procedure	44.1% (*n*= 59)	78.4% (*n*= 51)	71.8% (*n*= 39)
Disinfection of hands immediately after risk of body fluid exposure	77% (*n*= 100)	89.2% (*n*= 93)	82.8% (*n*= 58)
Disinfection of hands after patient contact	69.7% (*n*= 294)	85.9% (*n*= 311)	89.8% (*n*= 187)

%= percentage and *n*= sample size

The compliance of HCWs with good hand hygiene practices improved markedly after the educational campaign. Furthermore, the compliance after a one year period decreased only slightly compared to the results of the post-intervention period, but the rates were still much higher than the results obtained from the pre-intervention period. This suggests than an educational intervention not only has a short-term effect, but also impacts HCWs' practices in the long term. The researchers acknowledged that the Hawthorne effect could not be eliminated entirely. However, by monitoring the quantities of alcohol-based hand rub that were used, the researchers established that hand hygiene remained high even though HCWs were not being observed.

Hagel *et al.* (2015) quantified the Hawthorne effect on hand hygiene performance among HCWs when direct observation and electronic alcohol-based hand rub dispensers were used. They determined that the Hawthorne effect did have a significant effect on good hand hygiene practices.

Other studies which used a similar methodology to the one used by Monistrol *et al.* (2012) also established a significant increase in compliance of hand hygiene following an educational intervention. For example, Mahfouz *et al.* (2014) established an increase of 25.6% over three months, Midturi *et al.* (2015) found an increase of 20.5% over 22 months, and Arntz *et al.* (2016) found an increase of 32% over 2 weeks.

Studies also determined that the rate of HCAIs decreased, in some cases significantly, over their respective study periods (Salama *et al.* 2013; Chen *et al.* 2016; Mu *et al.* 2016). Chen *et al.* (2016) estimated that the decrease in the HCAI rate saved the hospital where their study was based an estimated US$940 000 and 3 564 admission patient days for that year alone. The study thus indicated that an increase in hand hygiene compliance could result in decreasing the occurrence of HCAIs as well as other burdens associated with it.

2.6.6.2 Stethoscopes and sphygmomanometers

Grecia, Malanyaon and Aquirre (2008) conducted a quasi-experimental before and after study in order to determine the effects of educational intervention on the contamination levels of stethoscopes and on the knowledge, attitudes and practices (KAP) of HCWs regarding stethoscope disinfection. The intervention period was four weeks long and consisted of a 30-minute lecture, performance feedback and information dissemination in the form of flyers, posters and stethoscope tags. The percentage of stethoscope contamination was reduced significantly from 68.9% to 27.6% and an overall improvement in the KAP answers was found after the educational intervention.

A stethoscope disinfection campaign was also led by Uneke *et al.* (2014), which resulted in a favourable outcome. The campaign consisted of an education and training session on stethoscope disinfection as well as the placement of 70% isopropyl alcohol dispensers at strategic locations. After the intervention campaign, compliance with regular disinfection of stethoscope regimes increased by 85% and the percentage of contaminated stethoscopes was reduced by 58.3%. Both these results were determined to be statistically significant.

The researcher could not trace any information relating to the impact of educational programs on compliance relating to sphygmomanometer disinfection.

2.6.6.3 Disinfection of chiropractic tables

Evans *et al.* (2009g) evaluated the compliance rates of CSs in terms of chiropractic table sanitization and the practice of good hand hygiene before and after an educational intervention. These researchers analysed a total of 1 403 surveys; pre-education (n=773) and post-education (n=630). The education campaign consisted of a PowerPoint presentation to the students and in-service staff members highlighting the dangers of pathogens and how to reduce infection risks. Posters, hand sanitizing gel dispensers and disinfectant wipes were also placed strategically in the clinics and a presentation was given to staff and students on existing disinfectant protocols and how to properly disinfect the tables and wash their hands.

The significant findings were as follows:

- A 35% increased odds of good hand hygiene practice was reported after the education campaign, compared with the practice before.
- A 30% increased odds was noted with regards to table sanitizing after the campaign.
- Before the campaign, students who reported that they frequently sanitized the tables were 2.8 times more likely to also report good hand hygiene practices. After the educational campaign, this rate increased to a higher likelihood of 5.6 times.
- The data indicated that CSs were nearly five times more likely to practise good hand hygiene when they perceived fellow students to practise good hand hygiene as well.

Evans *et al.* (2009g) concluded that an educational interventional can indeed have a short-term impact on increasing the compliance of good hand hygiene practices and table sanitization. They suggested that the long-term effects still needed to be determined.

2.7 Conclusion

The literature review that was presented in this chapter revealed that both stethoscopes and sphygmomanometers can become contaminated with bacteria, which included antibiotic-resistant bacteria, when used by HCWs during consultations with patients in healthcare settings (de Gialluly *et al.* 2006; Walker, Gupta and Cheesbrough 2006; Davis 2009; Uneke *et al.* 2010; Jain *et al.* 2013; Leontsini, Papapetropoulos and Vantarakis 2013; Shiferaw *et al.* 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014; James and Young 2014; Pal *et al.* 2015). The use of contaminated equipment as well as the practice of inadequate equipment disinfection by HCWs has been associated with the spread of HCAIs (World Health Organization 2011).

CSs make use of both stethoscopes and sphygmomanometers during their consultations with new patients at the DUT CDC. To the researcher’s knowledge, no published work relating to bacterial sampling of these diagnostic equipment or CSs’ practices regarding equipment disinfection in a chiropractic clinic existed before this study. This gap prompted the need for the current study as it would establish the extent of equipment contamination and shed light on the current hygiene practices of CSs during consultations at the DUT CDC. The outcomes were expected to contribute to scholarly discourse on the importance of adequate/good hygiene practices in healthcare settings and inform best practices to ensure the prevention of potential bacterial cross-contamination between a patient and a CS should the necessary disinfection protocols be implemented. In addition, it was envisaged that the outcomes would assist in changing the disinfection practices of CSs through amended protocols in the DUT CDC.

CHAPTER 3: MATERIALS AND METHODS

3.1 Research design

The study employed a cross-sectional investigation within a quantitative paradigm in two phases. Phase one utilised a pre-test post-test design and phase two utilised a descriptive questionnaire design.

Phase one: A pre-test post-test design is the favoured method when measuring the degree of change occurring in participants due to treatments or interventions (Shuttleworth 2009). However, the current research study did not measure intervention or treatment, but the extent of the occurrence of bacterial contamination on surfaces of stethoscopes and sphygmomanometers prior to and post consultation. Phase one also involved an assessment of the efficacy of disinfectants used by CSs as well as the identification and enumeration of antibiotic-resistant bacteria that were isolated from the stethoscopes and sphygmomanometers used by CSs.

Phase two: Using a closed-ended descriptive questionnaire, the researcher collected quantifiable data from the study population. These data were used for statistical interpretations by means of statistical data analysis processes (Penwarden 2014).

3.2 Research setting

Both phases of the research study were conducted in the DUT CDC (Appendix A). During phase one, the samples obtained from the CSs' stethoscopes and sphygmomanometers that had been used during consultations with new patients, were incubated and analysed in the Microbiology laboratory located at the Steve Biko Campus of the DUT in Durban (Appendix B).

3.3 Research population

The research population consisted of CSs who were registered for Clinical Chiropractic V and who were required to consult with new patients in the DUT CDC. The population also comprised of new patients who visited the DUT CDC for chiropractic treatment.

3.4 Sample size

After consultation with a biostatistician and according to results obtained from Raosoft Statistical Software, it was determined that, with a total population of 31 eligible Masters CSs, a minimum sample of 29 would be required for statistical significance (i.e., a confidence level of 95% and a margin of error of 5%). CSs who did not meet the inclusion and exclusion criteria were not included in the sample size calculation.

Each of the 29 sampled CSs were required to conduct two separate consultations with a new patient, thus resulting in 58 opportunities for bacterial data collection. Sampling the instruments used by each CS on two separate occasions allowed for better insight into their hygiene practices.

The sample also comprised of 58 new patients (two per CS) who agreed to participate in the study.

3.5 Sample recruitment

3.5.1 Chiropractic students

At the start of the data collection phase, the researcher approached all the eligible CSs working in the DUT CDC. They were briefly informed about the study and asked whether they would consider participating.

Upon showing interest to participate in the study, a CS was handed documentation containing a letter of information and a consent form (Appendix C) to read and to complete. If the CS had any questions regarding the study, the researcher was on hand to clarify any uncertainties.

The research study made use of convenience sampling by including the first 29 CSs who were scheduled to consult with a new patient and who met the inclusion and exclusion criteria. In an attempt to minimize the Hawthorne effect, data collection took place on randomly selected week days over 21 weeks.

3.5.2 Chiropractic patients

After written consent from the eligible CSs had been obtained, the researcher started recruiting new chiropractic patients. This occurred on the selected days of data collection, when the researcher studied the clinic roster and identified eligible chiropractic patients.

Each eligible patient, who agreed to participate, received a specifically designed patient letter of information and a consent form (Appendix D) for completion upon arrival for their consultation. The researcher was also on hand to clarify any enquiries the patients might have had about the study. A total of 58 new patients, two per CS, was identified and recruited for the study.

3.6 Criteria for the selection of participants and bacteriological samples

3.6.1 Chiropractic students

- The inclusion criteria for CSs were:
    - Registered for Clinical Chiropractic V;
    - Signed the informed consent form (Appendix C);
    - Scheduled to consult a new patient at the DUT CDC;
    - Own and use their own stethoscopes and sphygmomanometers.
- Exclusion criterion:
    - Pilot study and focus group participants.

3.6.2 Chiropractic patients

- Inclusion criteria for patients required that they had to:
    - Sign the informed consent form (Appendix D)
    - Be examined by a 5^{th} or 6^{th} year CS.
    - The examination had to include a case history, a physical examination and a regional examination.
    - Only patients who visited the clinic and were examined by a CS for the first time, or after a period of six months from their last consultation, were selected for inclusion in the study.
- Exclusion criterion:
    - Patients who visited the clinic for follow-up consultations were excluded.

3.6.3 Bacteriological samples

- Inclusion criteria for bacteriological samples:
    - Bacteriological samples were obtained from consultations taking place on Mondays, Tuesdays, Wednesdays, Thursdays and Fridays.
    - Bacteriological samples obtained from stethoscopes and sphygmomanometers.
- Exclusion criterion:
    - Bacteriological samples that might obviously have been cross-contaminated.

3.7 Research procedure

3.7.1 Permissions obtained

The researcher had to obtain permission from the following gatekeepers in order to conduct the research study:

- Dr C. Korporaal, Director of the DUT CDC (Appendix A).
- Prof. K. Perumal, Head of the Biotechnology Department, DUT (Appendix B).
- Dr A. Puhl, Dr C. Saunders and Dr W. Evans (Appendix F).
- Dr C. Hall, Head of the Department of Homeopathy, DUT (Appendix J).
- The Institutional Research and Ethics Committee (IREC), DUT (Appendix Q).
- Prof. S. Moyo, Director of Research and Postgraduate Support, DUT (Appendix T).

3.7.2 Pre-focus group questionnaire

The questionnaire aided in establishing the CSs' knowledge, attitudes and practices with regards to disinfecting their stethoscopes and sphygmomanometers. The pre-focus group questionnaire (Appendix E) was developed by the researcher by selecting various components from three different questionnaires which had been used in similar contexts. Permission to make use of these established questionnaires was obtained (Appendix F) from the following authors: Evans *et al.* (2009a), Puhl *et al.* (2011) and Saunders, Hryhorskyj and Skinner (2013).

3.7.3 Focus group

A focus group typically consists of five to eight participants who partake in a guided discussion with regards to a specific topic (Krueger and Casey 2015). The pre-focus group questionnaire (Appendix E) was subjected to a focus group where the content was discussed with regards to each question's relevance and importance to the study and its objective. The questions that would best address the objectives of the study were finally selected and incorporated into the questionnaire. In this manner the questionnaire's face and content validity was established (Bernard 2013).

The focus group consisted of the following participants:

- Two M.Tech Homeopathy students who actively consulted with patients in the Durban University of Technology Homeopathic Clinic and who complied with relatively similar inclusion criteria as the CS study population. Due to the study population being limited, the researcher did not want to include any CSs in the pilot study in order to minimise the effect on the study's sample size.

- A qualified chiropractor who could provide insight into hygiene habits in private practice and when consulting at the DUT CDC.
- A newly qualified chiropractor who could provide insight into her hygiene habits when consulting in the DUT CDC. As a student this participant had conducted a questionnaire-based research and therefore had experience in how to validate a questionnaire.
- The research co-supervisor, who had experience in participating in a focus group and in validating a questionnaire.
- The researcher.

The main supervisor could not attend the meeting due to unforeseeable circumstances on the day.

All candidates were contacted via e-mail inquiring whether they would be interested in participating in a focus group. Attached to the e-mail was a letter of information (Appendix G) to provide the candidate with background information about the research study. A suitable date, time and venue were determined.

At the start of the meeting, the researcher welcomed and introduced each participant, provided a short introduction to the study, and explained the purpose of the focus group and the procedure that would be followed during the meeting. Each participant was then asked to complete the consent form (Appendix H) and the confidentiality agreement (Appendix I). Each participant was given a copy of the pre-focus questionnaire (Appendix E) and some time to familiarize themselves with it. The researcher, who chaired the meeting, read out each question in chronological order after which the participants were given an opportunity to contribute their insights and opinions. In order for any suggestions or amendments to be implemented with regards to a question, a unanimous vote needed to be reached (Morgan 1998; Adamson 2006; Bernard 2013). The changes that were agreed upon (Appendix U) were implemented and led to the development of the post-focus group questionnaire (Appendix V). The meeting was audio recorded to allow the researcher to re-examine all the suggestions and amendments made (Bernard 2013). The recording was stored in a safe location out of the public domain.

3.7.4 Pilot study

A pilot study is a small-scale trial study in order to evaluate the measurement tool and the research procedure before committing to the full-scale research project (Crossman 2017). The researcher made the decision to conduct the pilot study on M.Tech Homeopathy students as they also use diagnostic equipment during the initial examination of a patient at the Durban University of Technology Homeopathic Clinic, which mimics the procedures followed by CSs in the DUT CDC. At the same time this decision aided in minimising the effect on the sample size of the study.

The researcher obtained permission from the Head of Department of Homeopathy (Appendix J) to approach the students for the purpose of recruiting participants for the pilot study. The researcher recruited four M.Tech Homeopathy students via e-mail and supplied each participant with a letter of information (Appendix K). Prior to the commencement of the pilot study, each participant was required to complete the consent form (Appendix L) and the confidentiality agreement (Appendix M).

The participants were asked to complete the post-focus group questionnaire (Appendix V), while the researcher recorded the time it took them to complete the questionnaire. Afterwards, each participant was given an opportunity to provide feedback regarding their experience and what changes they would recommend. All the participants were satisfied with the questionnaire, resulting in no amendments being made. This resulted in the post-focus group questionnaire becoming the research questionnaire (Appendix V) which was to be used as a data collection tool. Final IREC approval (Appendix Q) was obtained after submitting the final research questionnaire (Appendix V) as well as the list containing the amendments (Appendix U) suggested by the focus group.

3.7.5 Data collection procedure

3.7.5.1 Recruitment and obtaining informed consent from participants

The CSs who fulfilled the inclusion criteria were approached by the researcher and briefly informed about the research study. Upon showing willingness to participate, the CSs were given a letter of information and a consent form (Appendix C) to complete. After establishing a list of eligible CSs who gave their informed consent, the clinic roster was studied. Only new patients who would consult with CSs and who had already given their consent were approached by the researcher. Upon arrival for their consultation, the new patient was given a letter of information and a consent form (Appendix D) to complete if they decided to participate in the study. The researcher was on hand to answer any of the patients' questions with regards to the study.

Note:

- In order to minimize the Hawthorne effect, the CSs were not informed beforehand of the days on which data collection would occur.
- Data were twice collected from each CS; i.e., their instruments were examined after consultation with two different new patients on two different days.
- The CSs were encouraged to follow the same procedures they would under normal circumstances.

3.7.5.2 Collection of bacteriological samples

Phase 1 of the research study incorporated the first 29 CSs that consulted with new patients and who had given their consent for inclusion in the study. The researcher made sure that all the equipment that was necessary for collecting the samples was in her clinic room on the days of data collection.

The researcher received the diagnostic kit bag from each CS containing all his/her diagnostic equipment before the consultation with the new patient commenced. All the testing was done in the researcher's room which had been cleaned and sanitized as far as was possible. Wearing latex gloves to prevent cross-contamination, the researcher collected the pre-test samples from each respective sphygmomanometer's cuff and the stethoscope's diaphragm.

When sampling the stethoscope, the diaphragm was pressed against the surface of the Tryptic Soy agar (TSA) (Merck) for five seconds, as Campos-Murguia *et al.* (2014) concluded from their pilot study that this technique was quicker, simpler and more efficient than other techniques. The imprint technique was also utilised by Jones, Hoerle and Riekse (1995), Wood, Lund and Stevenson (2007) and Gupta *et al.* (2014). The same procedure was used during the collection of the bacterial samples from the sphygmomanometers. To ensure that the samples that were collected would be consistent in size, a disinfected plastic stencil was placed on the surface of the sphygmomanometer's cuff before being pressed against the TSA surface for five seconds. The plastic stencil was disinfected with 70% ethanol before and after each usage. After obtaining the pre-test samples, the cuff of each sphygmomanometer and the stethoscope's diaphragm were disinfected with 70% ethanol. All the necessary information was written on the base of the Petri dish and then the TSA plate was parafilmed to prevent any cross-contamination. Once the above process had been finalised, the diagnostic kit bag was returned to the CS.

After completion of the vitals and a full physical examination of each patient, the CSs handed their kit bag to the researcher. Wearing a new set of gloves, the researcher followed the same procedure as explained above to collect the post-test samples. The kit bag was then returned to the CS. The pre- and post-test samples collected on the day were taken to the Microbiology laboratory and placed in the incubator room in an inverted position at $37^{o}C$ for 24 to 48 hours (Logtenberg 2009; Dabsu, Woldeamanuel and Asrat 2014; Gupta *et al.* 2014).

3.7.5.3 Confirmation of presence, enumeration and identification of bacteria

Objective 1 was to determine the presence, quantities and identification of bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC before and after 58 new patient consultations using conventional bacterial plate count techniques and identification tests.

TSA was used as it allows for the cultivation of a wide variety of fastidious and non-fastidious bacteria (Atlas 2010). The TSA plates were analysed following 24 to 48 hours of incubation.

Confirmation of the presence of bacteria

The formation and existence of bacterial colonies observed on the incubated TSA plates containing the pre- and post-test samples served as evidence of bacteria being present on the selected diagnostic equipment at the time of sampling. Thus, if bacterial colonies were observed on the TSA plate after a specific sample, it indicated that the sampled piece of equipment was contaminated with bacteria at the time of sampling. This served to demonstrate that the stethoscopes and sphygmomanometers that were examined were reservoirs for bacterial growth.

Enumeration of viable bacteria

A colony counter machine (P-Selecta: Digital S) was used to enumerate the CFUs located on the surface of the TSA plates and the findings were recorded on data collection sheet one (Appendix N) (Biology online 2008). Campos-Murguia *et al.* (2014), Lecat *et al.* (2009), Rehman, Razzaq and Owais (2011) and Jones, Hoerle and Riekse (1995) made use of this method when they conducted their studies.

The post-test counts indicated the extent of the bacterial transfer from the chiropractic patient onto the stethoscope and the sphygmomanometer, and these data served to determine whether this equipment served as sources of bacterial transfer.

Identification of bacteria to genus level

Representative colonies were selected from the enumerated CFUs on the basis of their macroscopic morphology, their location and prevalence on the TSA plate. Pure cultures were made from the representative CFUs through sub-culturing them onto new TSA plates by using the streak plate technique (Appendix W). The appropriate identification information was written on the base of the Petri dish and the TSA plate was then parafilmed. After the TSA plates were incubated at 37^{o}C for 24 to 48 hours (Pollack *et al.* 2002; Logtenberg 2009), the bacterium of each pure culture was identified to genus level by assessing its macroscopic colony morphology, its Gram-staining characteristics and its bacterial cellular morphology.

Macroscopic colony morphology characteristics refer to the features of the colony when observing it with the naked eye. The following characteristics were assessed as explained by Tille (2014) and (Swalaha 2013):

- Size − either measured in millimetres or defined as pinpoint, small, medium or large.
- Colour.
- Colour changes to the agar media caused by the bacterial growth.
- Shape (form) − for example pinpoint (punctiform), round, rhizoid, irregular, curled or filamentous.
- Margin − for example entire (smooth), undulate (irregular), lobate, curled or filiform (filamentous).
- Surface appearance of colony − for example smooth and glistening, rough, wrinkled or dry and powdery.
- Elevation − for example flat, raised, convex, umbonate, growth into media and crateriform.
- Odour.

The Gram staining technique was used in the identification process as it indicates a bacterium's Gram staining characteristic and facilitates the assessment of its cellular morphology. The cells staining blue/purple were categorised as Gram-positive bacteria, while cells staining pink were classified as Gram-negative bacteria (Tille 2014; Bruckner 2016). For more information regarding the Gram stain procedure, see Appendix W.

A light microscope (Nikon eclipse E 200) was used to observe bacterial cellular morphology at 1 000x magnification. The bacteria were categorised on the basis of their shape, arrangement and the presence of spores. Bacilli (rod) and cocci (circular) were the two shapes that were most commonly identified. Cocci could be arranged either as single, diplococci, streptococci, sarcinae, tetrad or staphylococci. On the other hand, bacilli could be arranged as single, palisades, streptobacilli and Chinese letter patterned (Swalaha 2013; Tille 2014).

Certain bacteria are difficult to differentiate from each other, solely on the basis of their Gram staining and their macroscopic and light microscopic morphology. For this reason, the researcher made use of the catalase, oxidase and coagulase tests. The oxidase test helped to tell apart the *Micrococcus* spp. and the *Staphylococcus* spp. *Micrococcus* spp. had a positive result for the oxidase test. The catalase test aided in differentiating between catalase negative streptococci and enterococci and catalase positive micrococci and staphylococci (Tille 2014). The coagulase test on the other hand helped to distinguish between *S. aureus* and the CoNS (Sigma-Aldrich 2013). All the data regarding the identification of bacteria to genus level were recorded on data collection sheet two (Appendix O). The procedures regarding the oxidase and catalase tests are explained in detail in Appendix W.

The above identification process was also utilised in the following studies in order to address their various outcomes and objectives: (Bifero, Prakash and Bergin 2006; Uneke *et al.* 2010; Puhl *et al.* 2011; Shiferaw *et al.* 2013; James and Young 2014).

Identification of bacteria to species level

Certain potential pathogenic bacteria of specific interest were identified up to species level using specialized media that are selective or differential in nature. Gram-positive cocci were subcultured onto Mannitol Salt agar (MSA) (Merck), as it is selective for staphylococci and micrococci. At the same time, it is a differential medium as it can distinguish between *S. aureus* and CoNS on the basis of the bacteria's ability to ferment the mannitol. Baird-Parker agar (Biolab) was used to confirm the identification of *S. aureus.* Gram-negative bacilli were inoculated onto MacConkey agar (MAC) (Merck) as it aids in the isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacilli. Eosin Methylene-Blue agar (EMB) (Merck) was used to confirm the identification of *E. coli* (Atlas 2010; Tille 2014). The MSA, EMB, MAC and Baird-Parker agar plates were incubated in the inverted position at 37^{o}C for 24 to 48 hours (Leontsini, Papapetropoulos and Vantarakis 2013; Dabsu, Woldeamanuel and Asrat 2014). The data generated by the procedure above were recorded on data collection sheet three (Appendix P).

3.7.5.4 Evaluation of the efficacy of disinfectants

Objective 2 was to assess the efficacy of disinfectants used by CSs in the DUT CDC for cleaning stethoscopes and sphygmomanometers using a modified AOAC use dilution method.

The AOAC use dilution method is considered a standard method for evaluating liquid disinfectants for registration with regulatory agencies such as United States Environmental Protection Agency, Health Canada and United States Food and Drug Administration (Microchem Laboratory 2015).

The disinfectants selected for evaluation were:

- Water.
- 70% isopropyl alcohol.
- 70% ethanol.
- Hand sanitizer (62% ethanol).

CoNS, *S. aureus,* micrococci, *E. coli* and coliforms identified in the process pertaining to objective 1 were selected for testing due to their high degree of clinical importance. For the purpose of this study and due to financial limitations, the AOAC use dilution method as described by Microchem

Laboratory (2015) and the United States Environmental Protection Agency Office of Pesticide Programs (2009) was modified. For each bacterium, the procedure was repeated four times so as to test the efficacy of all four disinfectants. The data generated by the procedure above were recorded on data collection sheet four (Appendix R). The protocol followed in the execution of the modified AOAC use dilution method can be reviewed in Appendix W.

Note:

- Water is not a disinfectant. However for the duration of this research project water was categorized under disinfectants, as it was theorized that uneducated individuals might incorrectly consider water to have antimicrobial properties.
- Initially the efficacy of the disinfectants would have been determined with the use of a modified Kirby-Bauer disk diffusion technique (Logtenberg 2009). However, due to the high alcohol content of the various disinfectants, the technique was not deemed appropriate due to the quick evaporation rate of the disinfectants when placed inside the incubator at 37ºC. The researcher also tested the agar well diffusion method, but even though a greater volume of each disinfectant was used, the same problem was encountered. To eliminate this obstacle, the researcher decided to rather make use of a modified AOAC use dilution method which is a method that is approved by the IREC (Appendix Z).

3.7.5.5 Confirmation of the presence and enumeration of antibiotic-resistant bacteria

Objective 3 was to determine the presence and quantities of antibiotic-resistant bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC using therapeutic antibiotics with the disk diffusion assay to determine bacterial susceptibility to antibiotics.

The Kirby-Bauer disk diffusion test was used to determine the sensitivity of bacteria to various antibiotics and, in this manner, to identify any antibiotic-resistant bacteria (Uneke *et al.* 2010; Shiferaw *et al.* 2013; Dabsu, Woldeamanuel and Asrat 2014).

The bacteria selected for antibiotic sensitivity testing were CoNS, *S. aureus,* coliforms, micrococci *and E. coli.* The following Oxoid antimicrobial susceptibility test discs were used: Chloramphenicol (C) (30 µg), Ciprofloxacin (CIP) (5 µg), Erythromycin (E) (15 µg), Vancomycin (VA) (30 µg), and Amoxicillin (AMO) (25 µg). The concentrations for the antibiotics were carefully selected in accordance with WHO requirements (Sigma-Aldrich 2014).

Pure cultures of each bacterium were sub-cultured into a Tryptic Soy broth (TSB) (Merck) and incubated at 37ºC for 18 to 24 hours as indicated by Merck (2017). The spread-plate technique (see Appendix W) was utilized in order to evenly inoculate the surface of a Mueller-Hinton agar (MHA) (Merck) plate. Within five minutes of completing the inoculation, one disc of each antibiotic was placed onto the MHA surface. Each MHA plate test was duplicated in order to improve the test's validity. The plates were inverted and incubated at 35±2ºC for 16-24 hours according to the manufacturer's specifications, after which they were analysed. The diameter of the zones of inhibition were measured in millimetres and compared to the tables supplied by the Clinical and Laboratory Standards Institute (2014). When values were not available for specific bacteria, the researcher, as instructed by her main supervisor, used other values instead, as shown in Appendix AA. This gave an indication of the degree of sensitivity of each bacterium to the various antibiotics (Swalaha 2013; Tille 2014). The data generated were recorded on data collection sheet five (Appendix S).

3.7.5.6 Evaluation of hygiene practices

Objective 4 was to use a structured questionnaire in order to evaluate the hygiene practices of 29 chiropractic students regarding the disinfection of their stethoscopes and sphygmomanometers.

Phase two of the study started once all the bacteriological samples had been collected from the participating 29 CSs. The researcher personally issued each CS who had participated in phase one with a copy of the research questionnaire (Appendix V) to complete. Each questionnaire was coded for a dual purpose: first, so that no name would be associated with any data, and secondly for identification purposes only known to the researcher, which enabled the researcher to compare the results of the CS questionnaire with the specific participant's bacterial samples.

The questionnaire consisted of questions relating to the CSs' knowledge, attitude and hygiene practices regarding disinfection of stethoscopes and sphygmomanometers.

Objective 5 was to determine if CSs' hygienic practices correlated with the data that were generated from their questionnaire. The researcher therefore compared each CS's bacteriological findings with the data that were generated from his/her questionnaire.

3.7.5.7 Protocols used to prevent cross-contamination

- The researcher wore gloves at all times during the sample collection and analysis processes.
- All work processes, except the Gram staining, was done within the laminar flow (Labtec Bioflow II, South Africa) and in close vicinity of a Bunsen burner.
- The laminar flow was disinfected with 70% ethanol before and after use.
- All test tubes and agar plates were parafilmed after inoculation and after they had been analysed.
- All pipette tips, tweezers and carriers were autoclaved before the start of the testing on the day.

- During testing, the tweezers were sterilized by dipping the tips in 70% ethanol and then flaming the tips with a Bunsen burner.
- The plastic stencil used to demarcate the sampling area of the cuff was disinfected with 70% ethanol before and after each use.

3.8 Data analysis

Data were initially captured on spreadsheets in Microsoft Excel 2013. The data were subsequently exported to the IBM SPSS version 24.0 (SPSS Inc., Chicago, Illinois, USA) for statistical analyses. *p*-values of less than 0.05 were considered statistically significant.

Frequency distributions were conducted for categorical variable and descriptive statistics consisting of mean, standard error mean and the standard deviation was calculated for numerical variables where appropriate. The results also reflect the descriptive statistics in the form of bar charts.

Spearman's rank correlation test was performed to determine the relationship between the answers of the questions from the questionnaire (Objective 4).

Paired *t*-tests were used to determine if there were statistically significant differences in the mean values of the bacteria isolated from the surfaces of the CSs stethoscopes and sphygmomanometers with regards to the pre- and post-test samples. Paired *t*-tests were also used to determine a difference in the distribution of the mean CFUs found on the stethoscopes' edges and diaphragms when comparing the pre- and post-test samples (Objective 1).

No statistical test could be performed on the data generated from the efficacy testing of the various disinfectants (Objective 2).

One-way ANOVA and Tukey post hoc tests were used to determine whether there were any statistically significant differences in the degree of sensitivity to various antibiotics by the bacteria as a whole (Objective 3). One-way ANOVA was also used to determine a difference in the mean CFU counts isolated from the CSs stethoscopes and sphygmomanometers and their reported disinfection practices (Objective 5).

3.9 Ethical considerations

The DUT's IREC approved the research proposal (Appendix Q). Dr C. Korporaal granted the researcher permission to conduct the research at the DUT CDC (Appendix A).

Permission to utilize the Microbiology laboratory for the analysis of the bacteriological samples was obtained from Prof. K. Perumal, Head of the Biotechnology Department (Appendix B).

Written informed consent was obtained from CSs and chiropractic patients before they were included in the study. Each CS was given a coded identity that was only known to the researcher to ensure confidentiality and maintain the anonymity of each participant.

All the research data were stored in a secure location during the research process and could only be accessed by the researcher, supervisor and co-supervisor.

After the conclusion of the research study, all the research data that had been captured and recorded appropriately were securely stored and will be safeguarded in the Department of Chiropractic and Somatology for 15 years, after which they will be destroyed.

CHAPTER 4: RESULTS AND DISCUSSIONS

4. 1 Data

4.1.1 Primary data

Primary data were obtained from the data collection sheets that were generated subsequent to the analyses of the bacteriological samples collected from the CSs' stethoscopes and sphygmomanometers (Appendixes N, O and P), the disinfectants efficacy testing (Appendix R), the antibiotic disk diffusion assay (Appendix S), and the analysis of the research questionnaire (Appendix V) that had been completed by the CSs.

4.1.2 Secondary data

The secondary data were obtained from several journal articles, books and online sources such as websites. The secondary data were compared with the primary data that had been generated by the various test analyses and the findings pertaining to the questionnaire.

4.2 Results and discussion of results presented per objective

In order to facilitate a better understanding of the context of this study, objective 4 is presented first. This enables a better understanding of the CSs' practices and how these practices might differ from HCWs' practices reported in other published studies. These differences are illuminated to allow the researcher to be better able to contextualise and explain the results when presenting objectives 1 to 3. Objective 5 is then achieved by summarising the results and synthesising the findings.

4.2.1 Objective 4

This objective was to evaluate the hygiene practices of 29 CSs regarding the disinfection of their stethoscopes and sphygmomanometers using a structured questionnaire.

The questionnaire was completed by all 29 CSs who participated in phase 1 of the research study, thus reflecting a 100% response rate.

The researcher could not find existing literature relating to the knowledge, attitude and practices of HCWs with regards to disinfection practices of sphygmomanometers. For this reason, no comparisons pertaining to this instrument could be drawn between the findings of this study and similar studies.

The questionnaire consisted of closed-ended questions which were answered by selecting an option or options were applicable, from the Likert scale provided.

4.2.1.1 Demographics of respondents

Question 1 and Question 2 established the demographics of the respondents. The majority (65.5%) of the respondents was female (*n*=19/29), while 34.5% was of the male gender (*n*=10/29). With reference to the respondents' years of study, 62.1% was in their fifth year (*n*=18/29) while 37.9% was in their sixth year of study (*n*=11/29).

A difference in gender distribution between the two years of study was noticeable, as illustrated in Figure 1. Females were dominant in the fifth year group at 77.8% (*n*=14/18), compared to the 22.2% males (*n*= 4/18). The gender of the respondents was more evenly balanced in the sixth year group, where females were in the minority at 45.5% (*n*= 5/11) while males (*n*= 6/11) formed the majority at 54.5%.

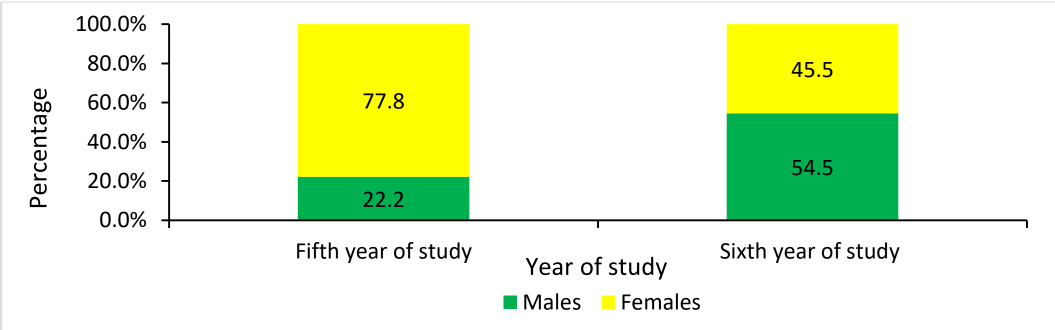


Figure 1: Distribution of respondents according to gender and year of study.

4.2.1.2 Knowledge about disinfection procedures

<u>Question 6: Are you knowledgeable regarding disinfection procedures?</u>

The number of 'neutral' responses recorded was noteworthy, as 41.4% (*n*=12/29) and 48.3% (*n*= 14/29) of the respondents reported a neutral response with regards to being knowledgeable of stethoscope and sphygmomanometer disinfection procedures respectively (Figure 2 on following page).

Only four respondents (13.8%), of which three were females, strongly agreed that they were indeed knowledgeable regarding the disinfection procedures for both pieces of equipment. By selecting 'agreed', 44.8% (*n*= 13/29) of the respondents considered themselves knowledgeable with regards to disinfection procedures of stethoscopes, while 37.9% (*n*= 11/29) said they were knowledgeable about such procedures pertaining to sphygmomanometers. No disagreement or strong disagreement was indicated for either of the two pieces of equipment (Figure 2).

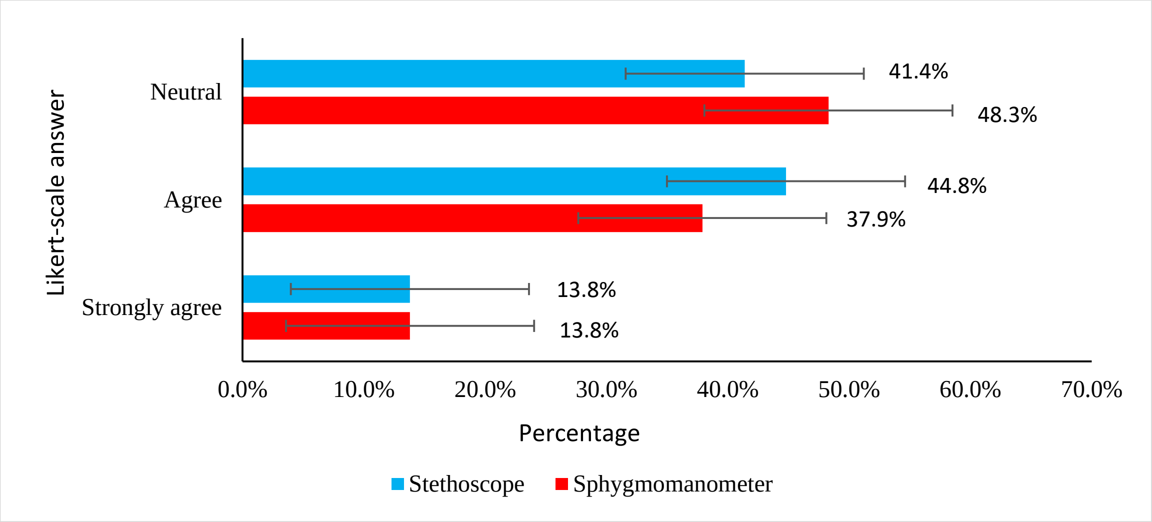


Figure 2: Respondents' level of knowledge pertaining to stethoscope and sphygmomanometer disinfection procedures.

The high percentage of respondents who selected 'neutral' for both pieces of equipment could suggest a degree of uncertainty with regards to what is considered 'knowledgeable'. However, the above results indicate that the majority of the respondents (Stethoscope (S): 58.8% and Sphygmomanometer (B): 51.7%) considered themselves knowledgeable regarding the disinfection procedures for both stethoscopes and sphygmomanometers.

<u>Question 9: How often do you think should your equipment be disinfected?</u>

The respondents recorded identical answers for both pieces of equipment. For that reason, Figure 3 located on the next page, does not specify stethoscope or sphygmomanometer as the distribution of the answers was exactly the same for both.

A combined 75.8% (*n*= 22/29) of the respondents thought that their equipment should be disinfected at least on a daily basis. The majority (44.8%) of these respondents (*n*=13/29) believed that disinfection should be performed after every patient as advised by the United States Department of Health: Centers for Disease Control and Prevention (2016) and Saloojee and Steenhoff (2001). A further 20.7% (*n*=6/29) reported that disinfection should be performed on a weekly basis, while only one female reported that it should be executed on a monthly basis. No respondents selected the 'yearly' or 'never' options.

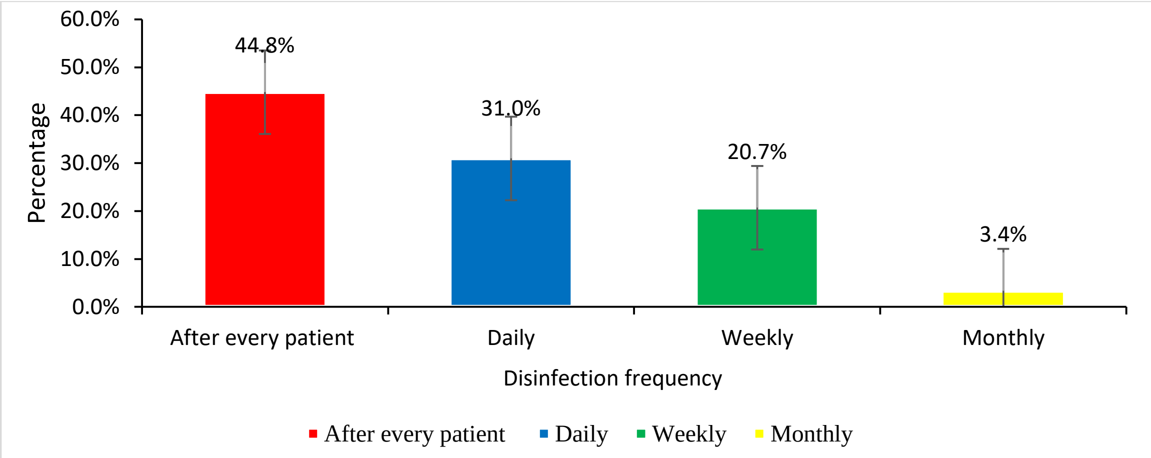


Figure 3: Respondents' knowledge regarding the frequency that stethoscope and sphygmomanometer should be disinfected.

In a study that was performed by Muniz *et al.* (2012) at the Children's Hospital in Boston, 94.7% of the HCWs indicated that stethoscopes should be disinfected after every use. This outcome was considerably higher compared to the 44.8% established by the current study. As the previous study was conducted in America, which is considered a developed country, one would suspect that the HCWs had been exposed to better education relating to the importance of preventing HCAIs through good hygiene practices compared to HCWs in a developing country (Allegranzi *et al.* 2011; World Health Organization 2011). This finding was even more appropriate for HCWs in a children's hospital, as neonates and infants have been found to be susceptible to HCAIs as their immune systems are still developing (Lorenzini, Costa and Silva 2013). It could also be assumed that the best trained and most experienced members of staff were employed at the children's hospital for the latter reason. The difference could also be attributed to the fact that HCWs in the children's hospital, where diseases of microbial origin occurred, were possibly more conscious of the contamination of their stethoscopes compared to the CSs whose patients most commonly presented at the DUT CDC with musculoskeletal complaints (McDonald 2014). The difference that was found in the perceptions of HCWs and CSs could potentially have influenced how often they thought their equipment should be disinfected.

Although the Spearman's rank correlation test failed to establish a positive correlation between Question 9 and Question 6 for both equipment pieces (S- p= 0.76; B- p= 0.91), the percentage of respondents (44.8%) who stated that the equipment should be disinfected after every patient was lower than the 58.6% (44.8% plus 13.8% in Figure 2) and 51.7% (37.9% plus 13.8% in Figure 2) of respondents who reported being knowledgeable in the disinfection procedures concerning stethoscopes and sphygmomanometers respectively (Question 6). However, when combined with the respondents who stated that disinfection should at least be performed daily, this total reached 75.8%, which again was higher than the percentage of respondents who indicated being knowledgeable. This could be attributed to either the respondents guessing how often the equipment should be disinfected rather having this knowledge, or because some of the respondents who selected 'neutral' in Question 6 were indeed more knowledgeable than they perceived themselves to be.

Whether being knowledgeable in the adequate frequency at which equipment disinfection should be performed influenced the respondents' actual disinfection practices, will be established in section 4.2.1.5.

Note: As the questionnaire did not provide the option 'before and after every patient' for disinfection of both equipment pieces, the respondents' answers could not be compared to the proposed frequency of disinfection as advised by (Jain *et al.* 2013; Shiferaw *et al.* 2013; Campos-Murguia *et al.* 2014; Gupta *et al.* 2014).

Question 14: In your opinion, have you received adequate education regarding disinfection of the following equipment during your chiropractic course?

The vast majority (79.3%) of the respondents (*n*= 23/29) was of the opinion that the training they had received pertaining to disinfection was inadequate for stethoscopes, while 86.2% (*n*= 25/29) felt the same regarding sphygmomanometer disinfection. Opinions varied concerning other equipment, with 86.2% (*n*=25/29) and 58.6% (*n*= 7/29) of the respondents reporting that adequate education had been received in terms of thermometer, otoscope and speculum disinfection practices respectively. More than half (55.2%) of the respondents (*n*=16/29) was not satisfied with the level of ophthalmoscope disinfection education (Figure 4).

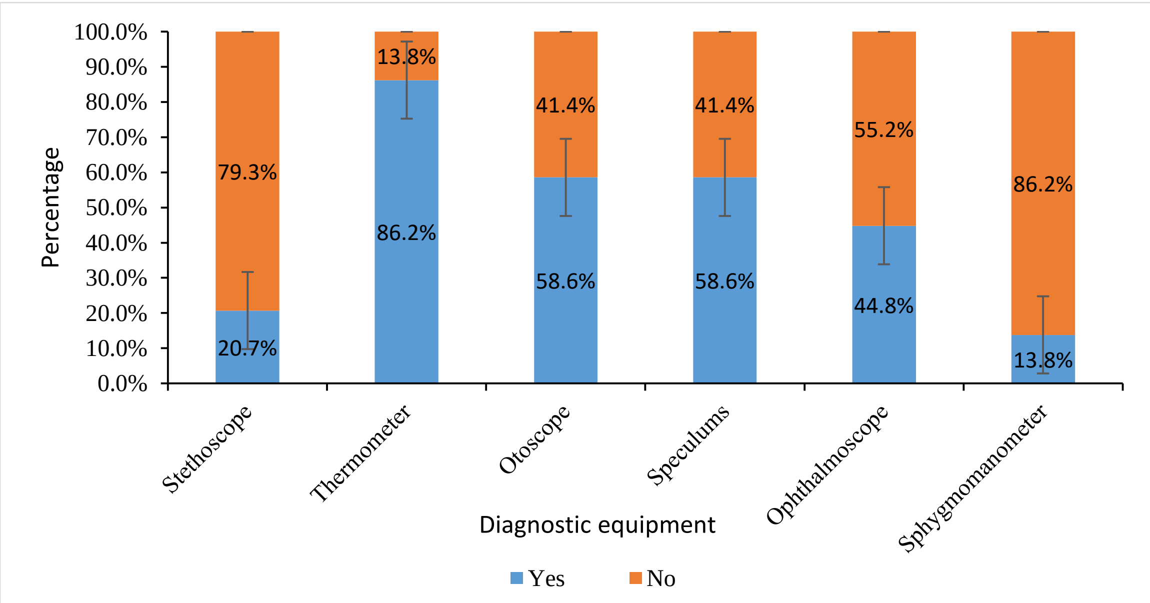


Figure 4: Respondents' opinions pertaining to having received adequate stethoscopes, thermometer, otoscope, speculums, ophthalmoscope and sphygmomanometer disinfection education during their chiropractic course at DUT.

The analysis of the paired *t*-test showed that there were statistically significant differences between the mean values of the thermometer, otoscope, speculums and ophthalmoscope groups when individually compared with both the stethoscope and sphygmomanometer groups, as shown in Figure 4 on the previous page.

Table 10: Results of paired *t*-tests: Comparison of mean values of other equipment with those pertaining to stethoscope and sphygmomanometer

Other equipment	Paired *t*- tests results	
	Stethoscope	**Sphygmomanometer**
Thermometer	$t(28)= 7.294$; $p< 0.01$ *	$t(28)= 7.392$; $p< 0.01$ *
Otoscope	$t(28)= 3.285$; $p< 0.01$ *	$t(28)= 3.822$; $p< 0.01$ *
Speculum	$t(28)= 3.018$; $p< 0.01$ *	$t(28)= 3.822$; $p< 0.01$*
Ophthalmoscope	$t(28)= 2.544$; $p< 0.02$ *	$t(28)= 3.087$; $p< 0.01$ *
Stethoscope	N/A	$t(28)= 1.000$; $p> 0.05$
Sphygmomanometer	$t(28)= 1.000$; $p> 0.05$	N/A

N/A= not applicable, *= statistically significant, *p*= *p*-value, *t*= *t*-value

No significant relationship (both S and B $p> 0.05$) was found between being knowledgeable in disinfection procedures (Question 6) and having received adequate education regarding disinfection during their chiropractic training (Question 14) (table 10).

It could be postulated that disinfection was discussed as part of a subject during the respondents' chiropractic course as the majority ticked 'yes' regarding thermometer, otoscope and speculum disinfection training. However, it seemed that no clear education was provided on stethoscope and sphygmomanometer disinfection in particular.

Even though only a fifth (20.7%) of the respondents indicated that they had received adequate stethoscope disinfection education during their course, it was still higher than the 4.2% established by Hyder (2012) and the 2.9% reported by Saunders, Hryhorskyj and Skinner (2013). This could perhaps be attributed to more advanced education regarding disinfection being provided during the chiropractic course.

The fact that only 20.7% and 13.8% of the respondents reported that they had received adequate education pertaining to stethoscope and sphygmomanometer disinfection respectively either strengthens the suggestion that the respondents guessed the correct answer to Question 9, or that they received disinfection education from external sources.

<u>Question 16: Have your received adequate education on good hand hygiene practice during your chiropractic course?</u>

Nineteen respondents (65.6%) agreed that they had received adequate education regarding good hand hygiene practice. Of these, seven indicated strong agreement while 12 merely agreed. Of the remaining respondents, 24.1% (n=7/29) remained neutral and a further 10.3% (n=3/29) disagreed. No respondent strongly disagreed (Figure 5).

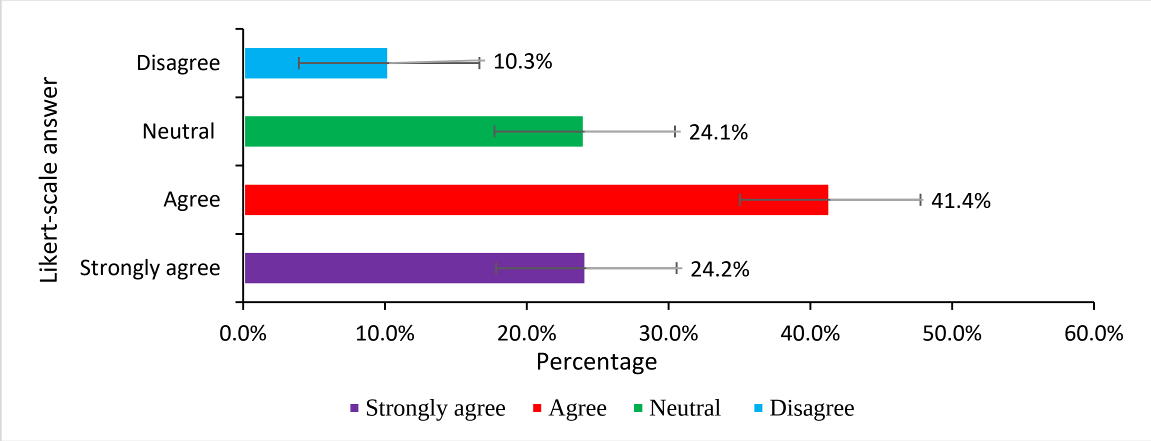


Figure 5: Responses regarding adequate education on good hand hygiene practice during chiropractic course at DUT.

Due to the implementation of a different Likert-scale for Question 14 and Question 16, no direct comparison could be made between the respondents' answers pertaining to these two questions.

If the respondents who stated that they had received adequate education on hand hygiene practices during their chiropractic course (sum of 65.6%) were to be converted hypothetically to a 'yes', it would allow for a direct comparison with Question 14. In such a scenario, it would appear that better education was provided to the respondents with regards to good hand hygiene practices than to stethoscope (20.7%) and sphygmomanometer (13.8%) disinfection practices. As chiropractic means in Latin 'done by hands', it rightfully suggests that, as a form of manual therapy, contact between the chiropractor's hands and the patient's skin is required during the execution of a manipulation or adjustment and/or implementation of traction, massage, exercise and acupuncture therapy (Department of Chiropractic and Somatology 2016). For this reason, more emphasis might have been placed on good hand hygiene practices over the disinfection of diagnostic equipment which are predominantly used during the initial consultation only.

Although the overall findings of Section 4.2.1.2 suggest that the majority of the respondents was knowledgeable regarding disinfection procedures, a need for improvement was identified with regards to supplying CSs with adequate education regarding good hand hygiene practices and disinfection protocols for diagnostic equipment during their chiropractic course. This would potentially prevent CSs from relying on external sources to receive this vital information and could result in improving the frequency of regular stethoscope disinfection as identified by Hyder (2012).

4.2.1.3 Attitudes of respondents towards disinfection practices

Question 4: Do you think that the following equipment can be possible sources of cross-contamination of pathogenic agents?

The instruments that were referred to in this question were stethoscopes and sphygmomanometers. Of the 29 respondents, 24 (82.8%) acknowledged that stethoscopes were possible sources of cross-contamination. Of these 24 respondents, 18 (62.1%) agreed, while a further six (20.7%) strongly agreed. With regards to sphygmomanometers, 58.6% (*n*=17/29) agreed, while a further 13.8% (*n*= 4/29) strongly agreed with the statement. Of the 29 respondents, 13.8% (*n*= 4/29) and 20.7% (*n*= 6/29) were neutral in their assessment of whether stethoscopes and sphygmomanometers respectively were possible sources of contamination. A small percentage of the respondents disagreed with the statement pertaining to both these instruments (Figure 6).

In a similar study, all the participants (*n*=90) believed that stethoscopes posed the potential for causing HCAIs as they could serve as reservoirs for bacteria (Pal *et al.* 2015). In a study conducted by Muniz *et al.* (2012), it was found that the belief that stethoscopes could transmit microorganisms and result in HCAIs significantly increased ($p< 0,01$) the occurrence of disinfection after every use. This would suggest that the perception that stethoscopes act as sources of cross-contamination of pathogenic agents could influence disinfection practices. This statement would either be confirmed or contradicted later.

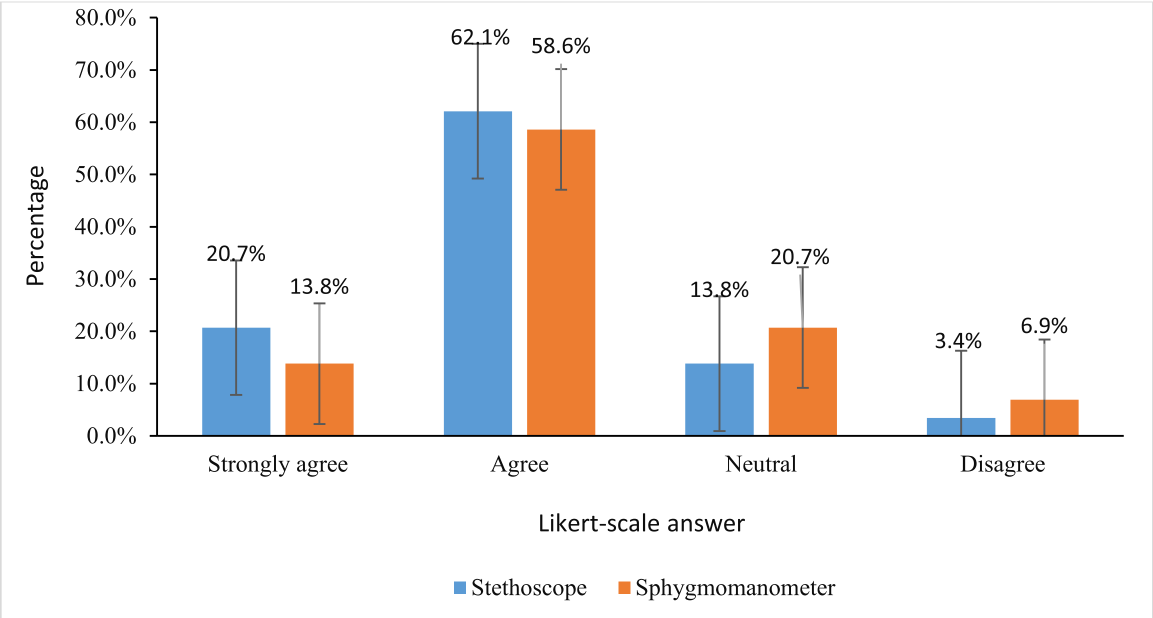


Figure 6: Responses regarding the ability of stethoscopes and sphygmomanometers to act as possible sources of pathogenic cross-contamination.

Question 5: Do you think it is important to disinfect the surfaces of these equipment?

The majority of respondents (96.5% or *n*=28/29 and 89.7% or *n*= 26/29 respectively) thought it was important to disinfect the surfaces of stethoscopes and sphygmomanometers. Only one respondent, a female in her sixth year of study, thought that the disinfection of these instruments was unimportant, while two female fifth year respondents answered 'neutral' with regards to sphygmomanometer disinfection. No respondent indicated that disinfection of either instrument was very unimportant (Figure 7, on following page).

Pertaining to stethoscopes specifically, 58.6% (*n*= 17/29) believed that disinfection was important, with an additional 37.9% (*n*=11/29) believing it to be very important. Regarding the disinfection of the surfaces of sphygmomanometers, these rates were 55.2% (*n*=16/29) and 34.5% (*n*=10/29) respectively (Figure 7, on following page).

Significant correlations for both pieces of equipment (S- r_s= 0.51 p< 0.021, B r_s= 0.60 p< 0.01) were identified between Question 5 and Question 4, which sought to determine whether the respondents thought both pieces of equipment could act as sources of cross-contamination.

In a similar study, Saunders, Hryhorskyj and Skinner (2013) determined that the majority of 308 medical students agreed that stethoscope disinfection were important. Furthermore, Saunders, Hryhorskyj and Skinner (2013) established a significant correlation (p= 0.01) between frequency of stethoscope disinfection and whether the medical students thought stethoscope disinfection were important. Once again, this could possibly be attributed to the medical students having received education highlighting the importance of disinfection, which then influenced their attitude towards disinfection which then ultimately effected their frequency of stethoscope disinfection, as explained by the knowledge-attitude-practice model.

With regards to sphygmomanometers, a significant and moderate strength correlation (r_s= 0.43 p= 0.02) was found between Question 5 and Question 6 which queried the respondents about their knowledge of sphygmomanometer disinfection procedures. Seventy-five percent of the respondents who strongly agreed that they were indeed knowledgeable in disinfection procedures regarding sphygmomanometers, also indicated that it was very important to disinfect this equipment's surfaces. Even though this correlation was with regards to a sphygmomanometer, the foundation of the finding concurred with the above mentioned statement, which suggests that being knowledgeable in sphygmomanometer disinfection procedures did influence the respondents' perception with regards to the importance of disinfecting the surfaces of a sphygmomanometer. A correlation between the same two questions could not be established regarding stethoscopes (p= 0.051).

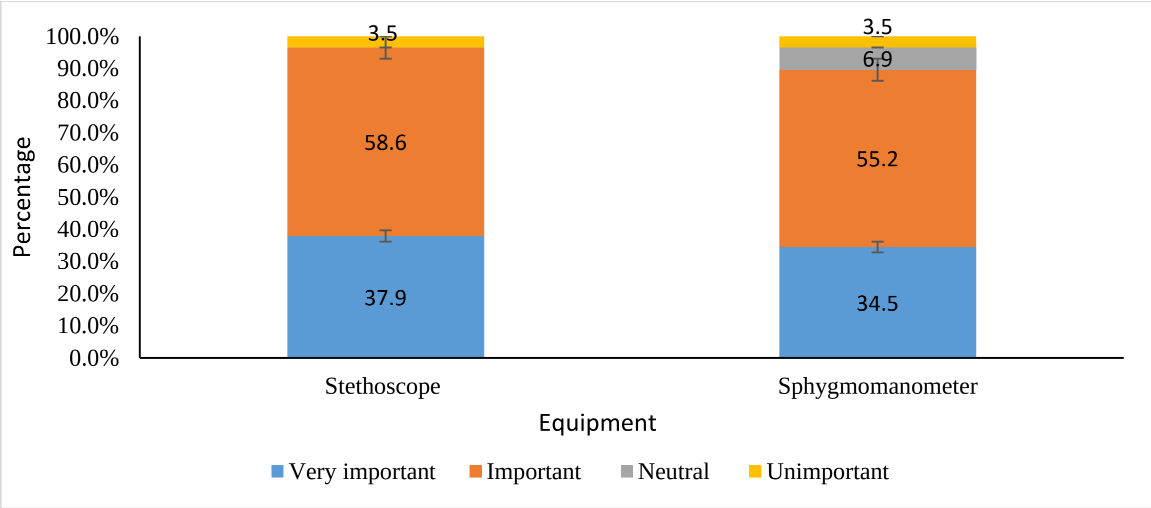


Figure 7: Respondents' thoughts regarding the importance of disinfecting the surfaces of stethoscopes and sphygmomanometers.

Question 8: In your opinion, is it important for students to disinfect their equipment?

The response patterns for stethoscope and sphygmomanometer were identical as each respondent recorded exactly the same answer for both equipment pieces, as shown in Figure 8 on the next page. A majority of 93.1% of respondents (*n*= 27/29) was of the opinion that the disinfection of both equipment pieces was either very important or important (51.7% and 41.4% respectively). Only 6.9% (*n*= 2/29) selected the neutral option. It was noted that these two respondents were different individuals to the two who chose the neutral option to Question 5. No respondent was of the opinion that the disinfection of the equipment was either unimportant or very unimportant.

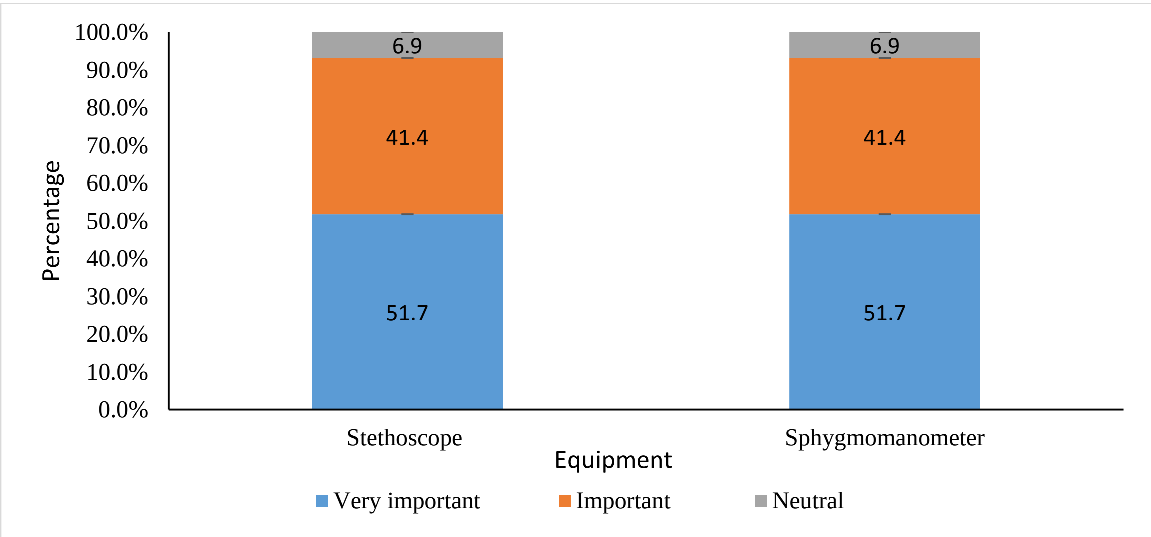


Figure 8: Respondents' opinions regarding the importance of stethoscope and sphygmomanometer disinfection by students.

Question 8 served as a repetition of Question 5 to increase the determination of the reliability of the respondents. A similar result was indeed obtained for the two answers, as 93.1% of the respondents was of the opinion that the disinfection of both pieces of equipment was important compared to the 96.5% and 89.7% of the respondents who respectively indicated in Question 5 that they thought disinfection of the surfaces of stethoscopes and sphygmomanometers was important. This was confirmed by Spearman's rank correlation test that identified a strong positive correlation (S- r_s= 0.77 p< 0.01; B- r_s= 0.65 p< 0.01) between Question 5 and Question 8 for both pieces of equipment.

A significant moderate positive correlation was identified for both stethoscopes (r_s= 0.52 p< 0.01) and sphygmomanometers (r_s= 0.45 p= 0.02), regarding these pieces of equipment being possible sources of pathogenic cross-contamination (Question 4) and the importance for students to disinfect their equipment (Question 8).

Conversely, no correlation (S- p= 0.18; B- p= 0.20) could be established between Question 8 and Question 6 pertaining to whether respondents were indeed knowledgeable in disinfection procedures relating to stethoscopes and sphygmomanometers.

<u>Question 17: Do you think education regarding hand hygiene and equipment disinfection is important?</u>

The majority (69.0%) of respondents (n=20/29) strongly agreed that education regarding hand hygiene and equipment disinfection were important, while the remaining respondents (n= 9/29) agreed. No respondents selected the 'neutral', 'disagree' or 'strongly disagree' options (Figure 9).

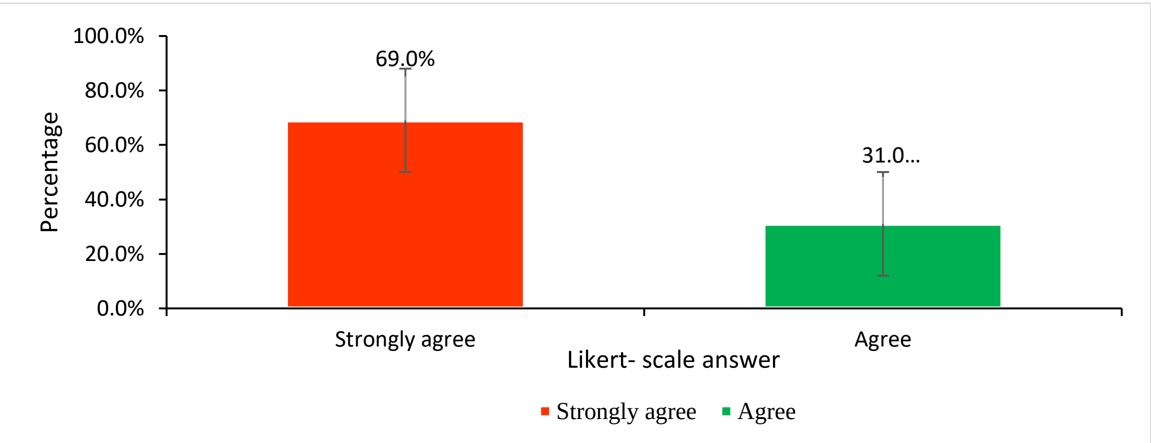


Figure 9: Respondents' responses pertaining to the importance of education in terms of hand hygiene and equipment disinfection.

A significant poor negative correlation (r_s= -0.39 p= 0.04) was established between Question 17 and Question 14. In Question 14, the respondents were asked whether they received adequate education throughout their chiropractic course pertaining to the disinfection of the listed equipment (which included stethoscopes and sphygmomanometers). This correlation thus indicated that the majority of the respondents was of the consensus that education concerning hand hygiene and equipment disinfection was important, even though they might not personally have received adequate disinfection education pertaining to the listed diagnostic equipment themselves. This finding reiterates the need to provide adequate education regarding hand hygiene and equipment disinfection during chiropractic training.

The positive outcomes of educational campaigns regarding stethoscope disinfections have been demonstrated on numerous occasions. For example, after an intervention campaign Uneke *et al.* (2014) established an increase of 85% in compliance with regular stethoscope disinfection, while the percentage of contaminated stethoscopes was reduced by 58.3%. Both these findings were determined to be statistically significant. Grecia, Malanyaon and Aquirre (2008) likewise identified a decrease of 41.3% in the rate of stethoscope contaminated after an educational intervention.

Educational campaigns regarding hand hygiene have also been found to have positive outcomes, as such campaigns have been demonstrated to result in a significant increase in compliance to good hand hygiene practices (Monistrol *et al.* 2012; Mahfouz *et al.* 2014; Midturi *et al.* 2015; Arntz *et al.* 2016). Furthermore, these authors also established that the rate of HCAIs decreased following such educational campaigns.

In the current study, no significant relationship ($p = 0.47$) was identified between Question 17 and having received adequate education on good hand hygiene practice during their chiropractic course (Question 16).

Overall, the respondents indicated a positive attitude towards disinfection, as the majority:

- Thought that both pieces of equipment could be possible sources of cross-contamination of pathogenic agents (Question 4).
- Thought that disinfecting the surfaces of stethoscopes and sphygmomanometers was important (Question 5).
- Was of the opinion that it was important for students to disinfect their stethoscopes and sphygmomanometers (Question 8).
- Thought that education regarding hand hygiene and equipment disinfection was important (Question 17).

4.2.1.4 Perceptions regarding disinfection

Question 7: Do you regularly see other students disinfect their equipment?

As illustrated in Table 11 below, the distribution of response percentages was exactly the same for both pieces of equipment. No respondent reported that they strongly agreed with the question, whereas only 10.3% (*n*=3/29) agreed that they regularly saw other students disinfect their equipment. The vast majority (89.7% or *n*=26/29) replied in the range of 'neutral' to 'strongly disagree'. The 89.7% response rate comprised 34.5% (*n*= 10/29) of the respondents who ticked 'neutral', another 34.5% who selected 'disagree', and 20.7% (*n*=6/29) who strongly disagreed.

Table 11: Rates of observing other students disinfecting their stethoscopes and/ or sphygmomanometers

	Stethoscope *n*=29		**Sphygmomanometer *n*=29**	
Answer	**%**	***N***	**%**	***N***
Strongly agree	0.0%	0	0.0%	0
Agree	10.3%	3	10.3%	3
Neutral	34.5%	10	34.5%	10
Disagree	34.5%	10	34.5%	10
Strongly disagree	20.7%	6	20.7%	6

n= total sample size, *N*= number of respondents selecting that particular answer, %= percentage

This low observance rate of students seeing others disinfecting both pieces of equipment could perhaps be due to the physical layout of the DUT CDC building. At the start of the academic year, each 5th and 6th year CS is assigned his/her own consultation room for each shift, unlike in certain public hospital settings where the ward has an open plan and the HCWs have to consult with their patients in the presence of their colleagues and surrounding patients. Thus students might not have been able to observe other CSs disinfecting their equipment unless they were present in the consultation room or if the student performed this procedure outside the room in the common corridor.

Question 18: Do you think your perception of disinfection protocols would alter between DUT CDC versus private practice?

The respondents seemed to be divided on this issue. Eight respondents (27.6%) remained neutral, another eight respondents indicated that their perception would alter and 44.8% (*n*= 13/29) reported that their perception would not alter (Figure 10, illustrated on next page).

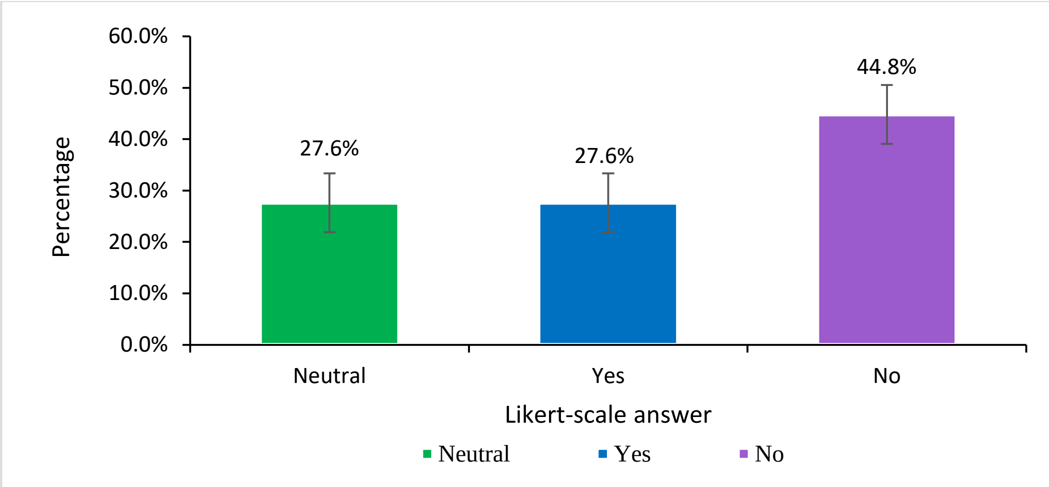


Figure 10: Respondents thoughts on whether their perceptions of disinfection protocols would alter between DUT CDC versus private practice.

Respondents who indicated that their perception would alter, i.e., a 'yes' answer, were asked to specify in the provided space why they would change their behaviour in private practice. One of the eight respondents failed to specify the response, while the other seven respondents provided the following answers:

- "I will be personally responsible for patients, making me more conscious of small details".
- "You would see more patients".
- "Be more cautious".
- "Can work at your own pace".
- "Disinfection wipes for beds".
- "DUT – not as hygienic".
- "There will be no pressure in my practice, unlike at CDC".

A wide range of answers were provided with no clear theme emerging.

The respondents who chose the 'neutral' option could have done so for various reasons. One reason could be that they were uncertain as they had never practised in a private setting before. Another reason might be that they were reluctant to provide a reason should they choose the 'yes' option.

In a scenario where the respondents who selected 'neutral' were excluded from the results temporarily, the results would have indicated that the majority of the respondents thought that their perception regarding disinfection protocols would not alter. This would then have suggested that the majority of the respondents thought that their current disinfection practices were appropriate.

4.2.1.5 Hygiene practices

Question 10: On average, how often do you disinfect your equipment?

The rates for the self-reported average disinfection practices of the respondents concerning stethoscopes and sphygmomanometers were distributed across all the answer options, but showed similar results between the two equipment groups.

It was established that it was common practice for only 13.8% (*n*=4/29) of the respondents to disinfect both pieces of equipment after every patient, while 10.3% (*n*=3/29) disinfected their equipment on a daily basis. On average, 27.6% (*n*=8/29) of the respondents disinfected their stethoscopes on a weekly basis, while 20.7% (*n*= 6/29) did the same pertaining to their sphygmomanometer. A collective 27.6% (*n*=14/29) of the respondents indicated that they disinfected both equipment pieces either on a monthly or yearly basis. An astonishing 20.7% (*n*=6/29) specified that they never disinfected their stethoscopes, while 27.6% (*n*= 8/29) never disinfect their sphygmomanometers (Figure 11).

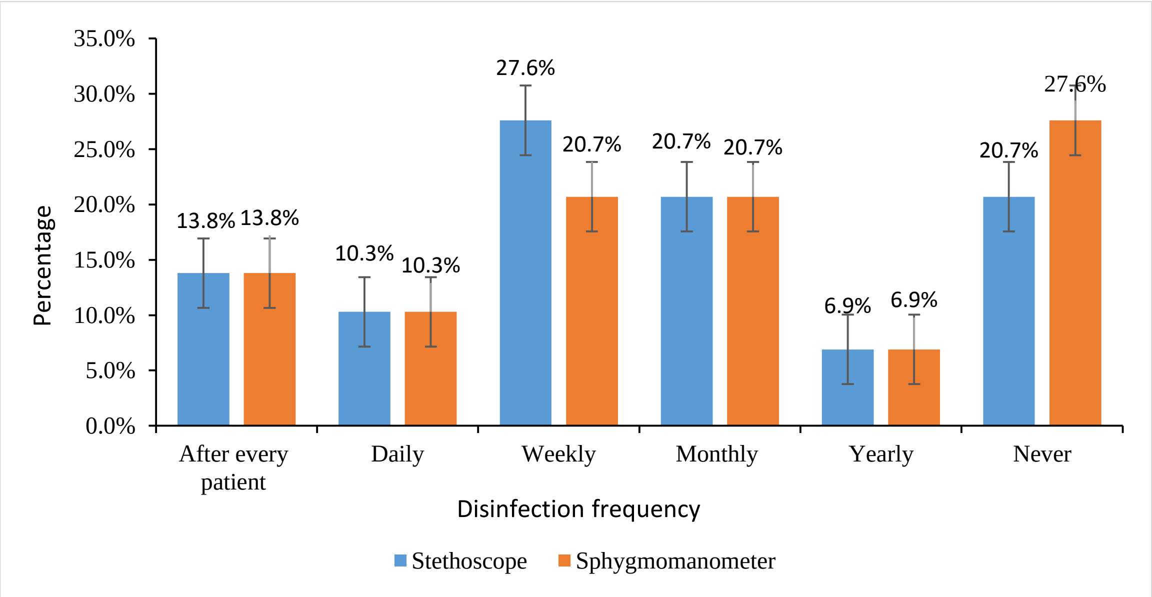


Figure 11: Respondents' average disinfection rates of stethoscopes and sphygmomanometers.

For both stethoscopes and sphygmomanometers, Question 10 was significant and moderately positively correlated with Question 9 (S- r_s= 0.54 p <0.01; B- r_s = 0.42 p= 0.03) pertaining to how often the respondents though the two pieces of equipment should be disinfected. However, where only sphygmomanometers were concerned, a significant moderate positive correlation (r_s= 0.49 p= 0.02) was identified between Question 10 and how knowledgeable they were in disinfection procedures (Question 6).

No correlation (both S- and B- p=0.24) could be identified between Question 10 and Question 14, which pertained to whether the respondents were of the opinion that they had received adequate education on disinfection concerning various pieces of equipment during their chiropractic training.

With reference to both equipment pieces, Question 10 was significantly positively correlated with Question 7 (S- r_s= 0.49 p< 0.01; B- r_s= 0.42 p= 0.03), which pertained to whether the respondents had seen other students disinfect their equipment. Saunders, Hryhorskyj and Skinner (2013) also established a significant correlation (p< 0.01) between the frequency of stethoscope disinfection and whether the respondents had witnessed others disinfecting their stethoscopes. Thus it could be suggested that colleagues who disinfect their diagnostic equipment could serve as role-models for their peers to do likewise.

The findings pertaining to Question 10 were similar to those that had been established by Saunders, Hryhorskyj and Skinner (2013), while they were in contrast to the results obtained by Tang *et al.* (2011) and Muniz *et al.* (2012). In the studies conducted by Tang *et al.* (2011) and Muniz *et al.* (2012), 59.0% and 65.3% of the respondents respectively reported that they disinfected their stethoscopes at least on a daily basis, compared to the 24.1% (13.8% plus 10.3% as reflected in Figure 11) of the current study who followed this practice. Furthermore, the percentages of the respondents in these two studies who reported that they had never disinfected their stethoscopes were also much lower in comparison to the findings of the current study. This difference in the results could potentially be attributed to the HCWs receiving better disinfection education, having more convenient access to disinfectants, and the presence of visual reminders in strategic positions throughout the hospital setting.

The above results indicated that the majority of the respondents failed to disinfect their stethoscopes and sphygmomanometers at least once daily, even though the majority of the respondents:

- Considered both pieces of equipment as potential sources of pathogenic bacterial cross-contamination (Question 4).
- Considered themselves knowledgeable in terms of disinfection practices (Question 6).
- Could provide the correct answer to how often these pieces of equipment should be disinfected (Question 9).
- Stated that the disinfection of these pieces of equipment was important (Questions 5 and 8).

No significant (p< 0.05) correlations were established between Question 10 and Question 4 (S- p= 0.58; B- p= 0.45), Question 5 (S- p= 0.74 and B- p= 0.22), Question 6 (pertaining to only S- p=0.33) and Question 8 (S- p= 0.15 and B- p= 0.39). In contrast to the findings of the current study, Saunders, Hryhorskyj and Skinner (2013) identified a significant correlation (p=0.01) between the frequency of stethoscope disinfection and whether the respondents thought stethoscope disinfection was important. Thus the assumption (section 4.2.1.3) that the respondents would practise adequate disinfection control due to their overall good perception and attitudes towards disinfection was repudiated.

The lack of adequate disinfection practices could possibly be attributed to factors such as forgetfulness, lack of time, regarding the practice as unimportant, pressure and the unavailability of disinfectants rather than inadequate training in disinfection protocols. This statement will either be confirmed or refuted in section 4.2.1.7, which reports on the question where the respondents were asked to provide reasons for failing to comply with the recommended disinfection protocols.

As a consequence of not practising adequate disinfection control, it is proposed that the respondents might have exposed their patients to pathogenic and non-pathogenic bacteria by using contaminated equipment. Whether this was the case is disclosed and discussed in section 4.2.2.3.

Question 11: When was the last time you disinfected your equipment?

Only two respondents (6.9%) ticked the ‘today’ option (i.e., same day on which the questionnaire was completed) for both pieces of equipment. Disinfection within the last week was performed by 37.9% (*n*=11/29) of the respondents with respect to their stethoscopes, while 27.6% (*n*= 8/29) did the same concerning their sphygmomanometers (Figure 12).

It was noted that 24.1% (*n*=7/29) never disinfected their stethoscopes. Of these seven respondents, six were the same respondents who also indicated in Question 10 that they had never disinfected their stethoscopes. Pertaining to sphygmomanometers disinfection, 27.6% (*n*= 8/29) of the respondents reported that they had never disinfected theirs before. These were the exact same respondents who had ticked ‘never’ in Question 10.

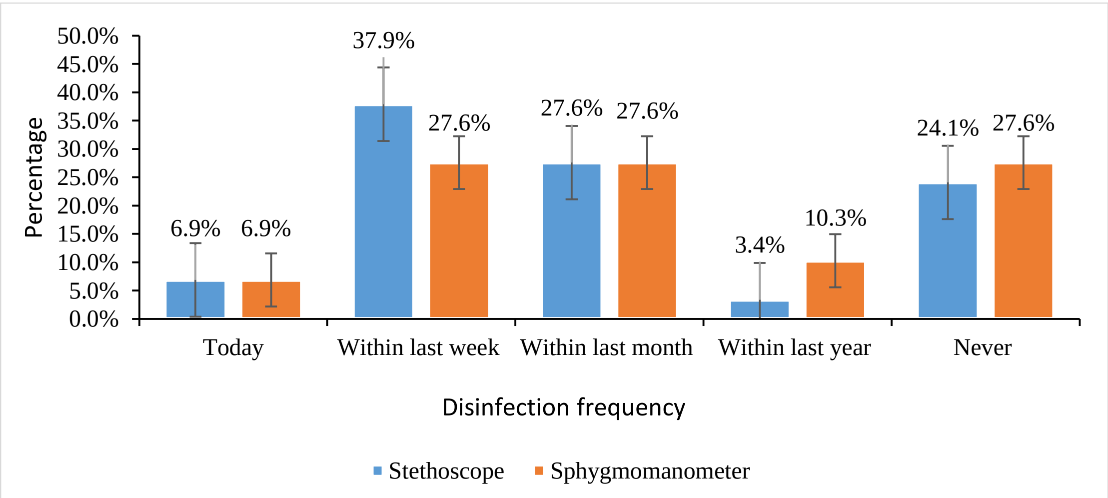


Figure 12: Last occurrences of stethoscope and sphygmomanometer disinfection by respondents.

With regards to both stethoscopes and sphygmomanometers, Question 11 was significantly positively correlated with Question 9 (S- r_s= 0.64 p< 0.01; B- r_s= 0.40 p= 0.03) pertaining to how often the respondents thought their equipment should be disinfected and with Question 10 (S- r_s= 0.90 p< 0.01; B- r_s= 0.88 p< 0.01) where the respondents had to indicate how often they disinfected their equipment on average.

It had been expected that the percentage of respondents who would select 'today' would have been higher, especially when 13.8% and 10.3% indicated in Question 10 that they disinfected their equipment after every patient or daily respectively. The overall discrepancy between the two sets of results could be attributed to a number of factors:

- Even though respondents indicated in Question 10 that they disinfected their equipment after every patient or daily on average, it could have been that on the day of completing the questionnaire the respondents did not consult with a patient which would have resulted in him/her most likely selecting the next option 'within the last week'.
- Some of the respondents might have just returned from leave when completing the questionnaire, which could have resulted in them having to select 'within the last week' or 'within the last year', even though on average they disinfected their equipment at least on a daily basis.

No correlations (S- p= 0.40; B- p= 0.26) were identified between Question 11 and Question 4 which pertained to stethoscopes and sphygmomanometers acting as possible sources of pathogenic cross-contamination.

Question 15: On average, how often do you disinfect your hands while in the clinic?

The vast majority (75.9% or n= 22/29), reported that they disinfected their hands on average after each patient, while a further four respondents (13.8%) stated that they disinfected their hands more than twice daily. Three respondents indicated that they disinfected their hands either twice daily or daily while on clinic duty. No respondent indicated that they never disinfected their hands while in the DUT CDC (Figure 13, on following page).

No correlations were established between Question 15 and whether the respondents had received adequate education on good hand hygiene during their chiropractic course in terms of Question 16 (p> 0.05) and Question 17 (p= 0.74), which pertained to whether the respondents thought that education regarding hand hygiene and equipment disinfection was important.

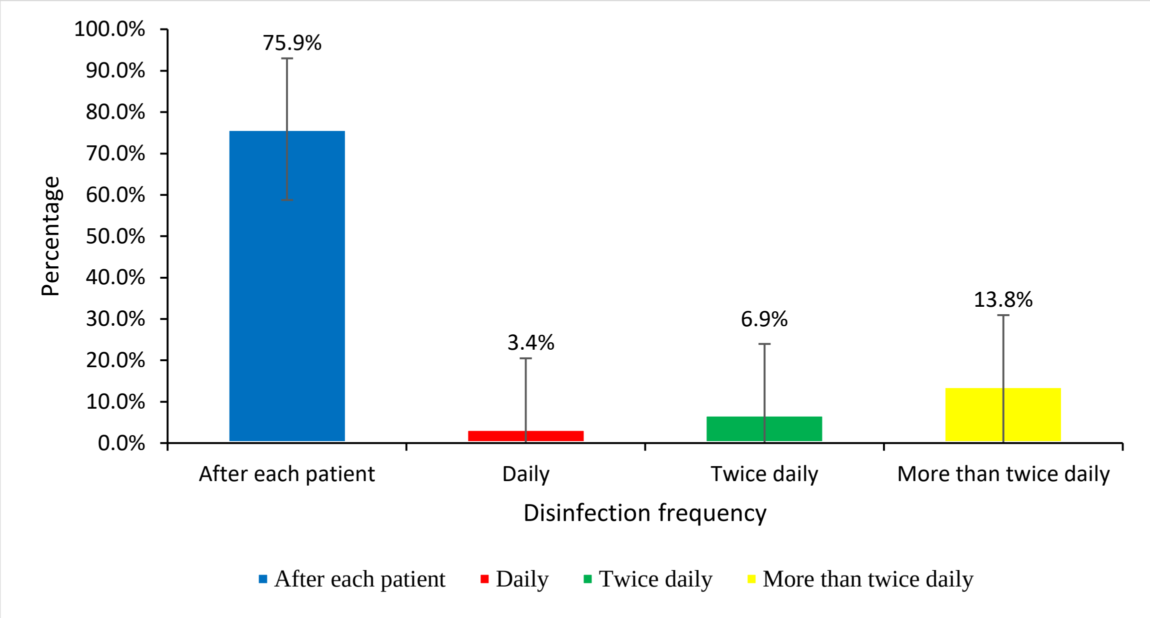


Figure 13: Average frequency of hand disinfection by respondents while working in the DUT CDC.

In a similar study, Evans and Breshears (2007) established that 69.0% of CSs who consulted with patients at the Parker College chiropractic clinic in Texas disinfected their hands after each patient.

The results pertaining to Question 16 and Question 17 indicated that the majority of respondents had received adequate education regarding good hand hygiene and that they perceived that education as important. The knowledge-attitude-practice model suggests that, as a person gains new knowledge on for example hand hygiene, that knowledge will influence their attitude towards hand hygiene. Together the knowledge and alteration in attitude will predict and precede the practice of good hand hygiene (Aboyeji, Ijaiya and Jimoh 2004). Thus the fact that 75.9% of the respondents reported that they disinfected their hands after each patient could be attributed to the fact that they were knowledgeable and had a positive attitude towards the practice of good hand hygiene.

As previously stated, chiropractic is a form of manual therapy which results in physical contact between the patient's skin and the chiropractor's hands. Other factors which may have contributed towards the reported 75.9% disinfecting their hands after each patient might be a preference for maintaining good personal hygiene and/or the removal of residual oil after massage therapy.

The nonexistence of any correlations (S- $p= 0.96$ and B- $p= 0.94$) between Question 15 and Question 10, which pertained to how often on average the respondents disinfected their equipment, confirmed the findings reported in section 4.2.1.5 by indicating that although the majority of respondents implemented good hand hygiene practices, these practices did not extend to the execution of adequate equipment disinfection. This reiterates the need for adequate education pertaining to equipment disinfection.

4.2.1.6 The use of disinfectants

<u>Question 12: What do you use to disinfect your equipment?</u>

The respondents were allowed to select more than one of the options provided. This option was only taken by one male, who selected ‘alcohol based wipes’ and ‘surgical alcohol and cotton wool’ for both pieces of equipment. This resulted in the disinfectant usage percentage being calculated by dividing by 30 and not by the usual 29.

The favoured disinfectant by far for both the stethoscope and sphygmomanometer was alcohol based wipes at 56.7% (*n*=17/30) and 50.0% (*n*= 15/30) respectively. For stethoscopes, this was followed by surgical alcohol and cotton wool at 16.7% (*n*=5/30) and hand sanitizer at 3.3% (*n*= 1/30). The same order of disinfectants was identified for sphygmomanometer disinfection, but the frequencies were different as can be seen in Figure 14 on the next page. No respondents selected water, soapy water or other.

It is noteworthy that 23.3% and 26.7% (*n*=8/30) of the respondents reported that, respectively, they used nothing to disinfect either their stethoscopes or their sphygmomanometers. This reflected the same tendency that was discovered for Question 10 and Question 11. The same respondents who indicated in either Question 10 or Question 11 that they never disinfected either of the pieces of equipment now confirmed the fact by selecting the ‘nothing’ option in Question 12.

Paired *t*-tests did not determine any statistically significant differences in the mean values of disinfectants when comparing their usage between stethoscope and sphygmomanometer disinfection practices ($p > 0.05$).

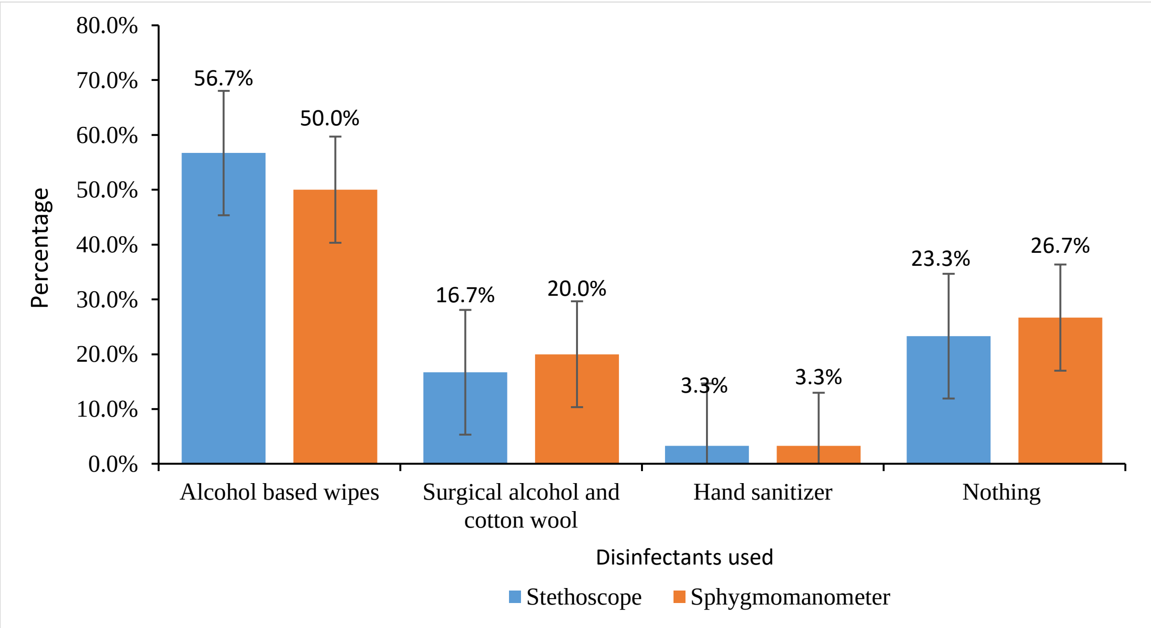


Figure 14: Disinfectants used by respondents to disinfect their stethoscopes and sphygmomanometers.

Results of similar studies concurred with the above findings. Other studies also found that alcohol based wipes/disinfectants and hand sanitizers were the most popular choices by HCWs when performing stethoscope disinfection (Tang *et al.* 2011; Muniz *et al.* 2012; Pal *et al.* 2015).

Results that were in contrast with those of the current study were reported by Hyder (2012), who established that 53.2% of the respondents made use of a dry cloth, 34.4% used alcohol wipes, 5.2% used water only, 3.9% used soapy water and 3.2% selected the ‘other’ option. As the latter study was conducted in Pakistan, the reasons why the majority of the HCWs made use of inadequate disinfecting products and methods might be attributed to a lack of disinfectant supplies, disinfectants not being easily accessible, poor education, and overcrowding of the hospital.

The effectiveness of 70% ethanol, 70% isopropanol, water and hand sanitizer (62% ethanol) against bacteria will be identified and discussed in section 4.2.3.

4.2.1.7 Reasons identified for not disinfecting equipment appropriately

<u>Question 13: If you do not disinfect your equipment, what is the reason/s?</u>
As with the previous question, more than one appropriate answer could be ticked. Three respondents failed to select any reason for both items of equipment. Conversely, eight respondents selected more than one reason for not disinfecting their equipment. In total, 37 reasons were indicated for stethoscopes and 39 for sphygmomanometers not being disinfected appropriately, thus *n*= 37 for stethoscopes and *n*= 39 for sphygmomanometers were considered in the analyses.

Eight respondents (21.6%) selected the ‘not applicable’ option regarding stethoscope disinfection, while five (12.8%) did the same for the sphygmomanometer (Figure 15, illustrated on following page).

The main reasons identified for failure to disinfect stethoscopes were: ‘forgetfulness’ at 27.0% (*n*= 10/37), ‘lack of time’ at 21.6% (*n*= 8/37) and ‘other’ and ‘uneducated about importance’ both at 8.1% (*n*=3/37). With regards to the sphygmomanometer, the same reasons but at different frequencies were noticed: ‘forgetfulness’ at 28.2% (*n*=11/39), ‘lack of time’ at 23.1% (*n*= 9/39), and 7.7% (*n*= 3/39) for each of the following options: ‘unimportant’, ‘other’, ‘uneducated about importance’ and ‘lack of disinfectant [available]’ (Figure 15, illustrated on following page).

Three respondents, all female, selected the ‘other’ option for both pieces of equipment. Unfortunately only two specified their reason in the area provided. One respondent indicated that she did ‘not want to damage the equipment’ while the other stated that she ‘only disinfect[ed] the equipment after the last patient’.

The analysis of the paired *t*-tests did not reveal any statistically significant differences ($p > 0.05$) in the mean values of the reasons identified by the respondents for not disinfecting either the stethoscopes or the sphygmomanometers.

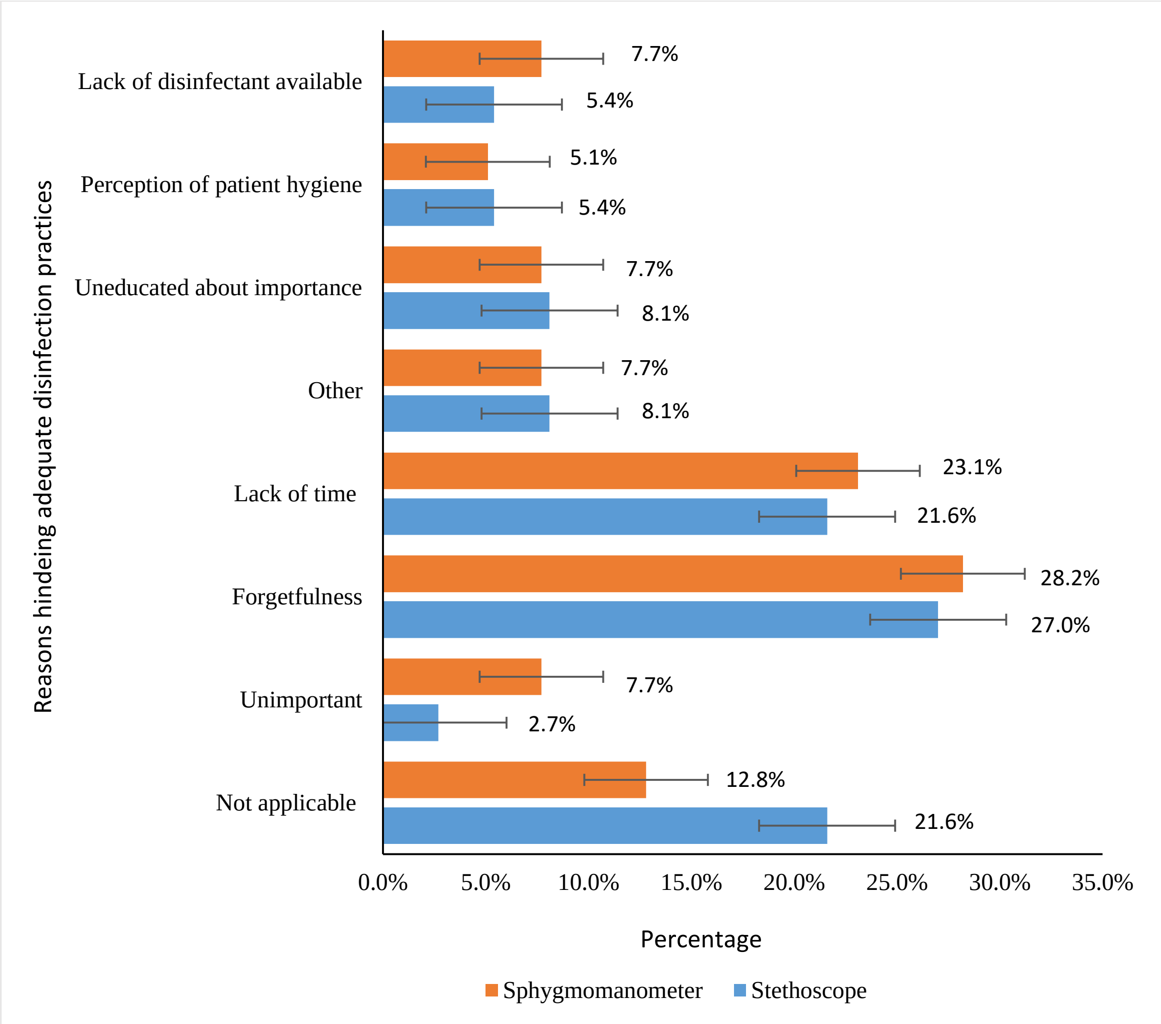


Figure 15: Reasons that hindered adequate stethoscope and sphygmomanometer disinfection practices.

Reasons for failing to practise adequate stethoscope disinfection were also identified by similar studies, such as 'forgetfulness', 'inadequate access to disinfectants', 'insufficient knowledge/ education', 'poor role-modelling' and 'lack of time' and 'lack of visual reminders' (Tang *et al.* 2011; Muniz *et al.* 2012; Saunders, Hryhorskyj and Skinner 2013; Pal *et al.* 2015). No direct percentage comparisons could not be made because these studies did not supplying qualitative information in this regard.

Because the most common reasons that were selected by the respondents were 'forgetfulness' and 'lack of time', the statement made in section 4.2.1.5 pertaining to Question 10 was confirmed. This statement suggests that inadequate education was not likely to be the main reason for respondents failing to practise adequate equipment disinfection. These findings are cause for concern as they suggest that patients might be exposed to potential pathogenic bacteria simply due to respondents not having a spare minute or forgetting (which is even worse) to disinfect their equipment.

The respondents who selected the 'not applicable (N/A)' option might have misinterpreted the question as they disinfected their equipment after every patient. However, Table 12 below shows that this might have been applicable to only two of the respondents. Also, 63.5% of the eight respondents who ticked 'N/A' disinfected their stethoscopes either on a weekly or monthly basis, even though they indicated that they had a fair amount of knowledge regarding appropriate disinfection procedures (Question 6). The results for the sphygmomanometers were similar to the results for the stethoscopes.

Table 12: Respondents indicating 'not applicable' to Question 6, Question 9 and Question 10

	Question 13 answers		Question 6 answers		Question 9 answers		Question 10 answers	
Respondents	**S**	**B**	**S**	**B**	**S**	**B**	**S**	**B**
CS 17	N/A	N/A	Agree	Agree	Weekly	Weekly	Weekly	Weekly
CS 2	N/A	N/A	Agree	Agree	Daily	Daily	Monthly	Monthly
CS 20	N/A	N/A	Neutral	Neutral	After every patient	After every patient	Weekly	Weekly
CS 8	N/A	Lack of time	Agree	Agree	After every patient	After every patient	After every patient	After every patient
CS 28	N/A	Forgetfulness	Neutral	Neutral	After every patient	After every patient	Daily	Never
CS 30	N/A	N/A	Neutral	Neutral	After every patient	After every patient	After every patient	After every patient
CS 15	N/A	N/A	Agree	Agree	After every patient	After every patient	Weekly	Daily
CS 26	N/A	Forgetfulness	Neutral	Neutral	After every patient	After every patient	Monthly	Never

S= stethoscope, B= sphygmomanometer, CS= chiropractic student, N/A= not applicable, Question 6: Are you knowledgeable of the disinfection procedure?; Question 9: How often do you think should your equipment be disinfected?; Question 10: On average, how often do you disinfect your equipment?

4.2.1.8 Material from which sphygmomanometer cuffs are made

Question 3: Of what material is your sphygmomanometer cuff made?

The vast majority (89.7%) of the respondents (*n*= 26/29) reported that their sphygmomanometer cuffs were made of plastic, with the remaining 10.3% (*n*= 3/29) reporting that it was covered by fabric.

The researcher was unable to find published literature on the difference in contamination levels between sphygmomanometer cuffs made of plastic and fabric. This could be investigated in a further study.

Bhanot *et al.* (2011) established that 98.7% of all keyboards, curtains, cell phones and clothing used or worn by HCWs were contaminated with bacteria. This finding suggests that both plastic and fabric fomites could act as reservoirs for bacterial growth.

4.2.1.9 Additional comments offered by respondents

Question 19: Do you have any comments?

The respondents were provided with an opportunity to leave a comment at the end of the questionnaire. Only 10.3% (*n*= 3/29) made use of this opportunity. The following comments were offered:

- "I would like to pay more attention to hygiene details".
- "Disinfecting hands and equipment is very important; however, it is easy to forget to do so".
- "Our skin serves as an incredible barrier to infectious agents that we are expose to 24/7. And our immune system is amazing. [It is] important to remember the immunocompromised though, I suppose!"

4.2.2 Objective 1

Objective 1 was to determine the presence, quantities and identification of bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC before and after 58 new patient consultations using conventional bacterial plate count techniques and identification tests.

4.2.2.1 Confirmation of the presence of bacteria

The formation of one or more CFUs on the TSA plate confirmed the presence of bacteria on the tested equipment at the time of sample collection (Figure 16).

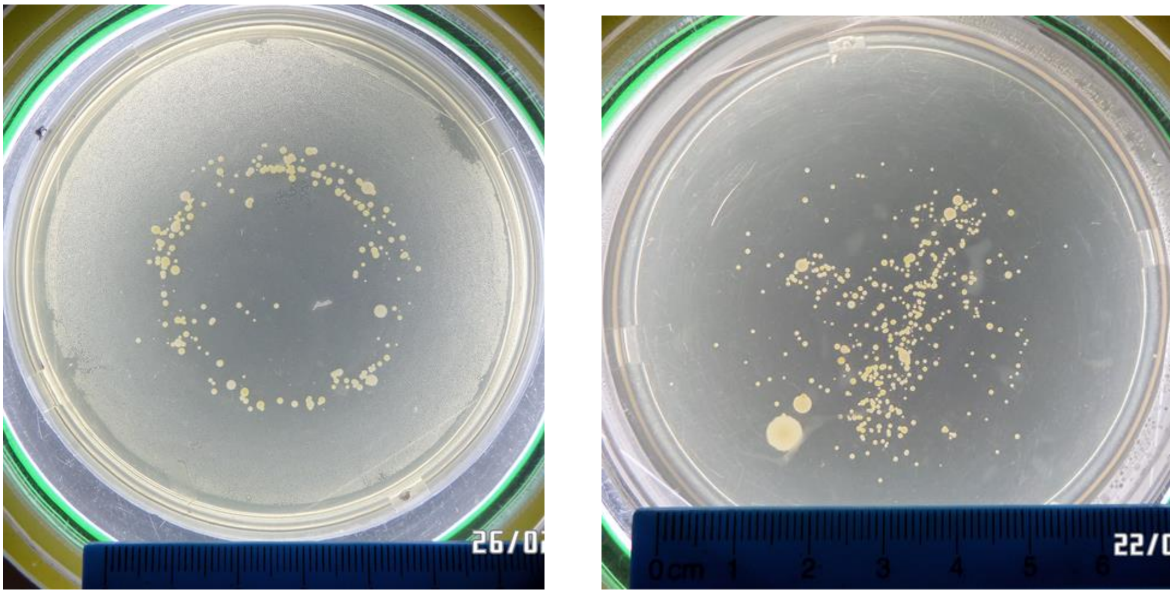


Figure 16: Bacterial growth in the form of CFUs was observed on TSA plates after incubation for 36 hours at 37ºC following sampling of a stethoscope (left) and a sphygmomanometer (right).

The analyses of the pre- and post-test TSA plates revealed that 96.6% of the pre-test stethoscope (*n*= 56/58), 100.0% of the post-test stethoscope (*n*= 58/58), 94.8% of the pre-test sphygmomanometer (*n*= 55/58) and 100.0% of the post-test sphygmomanometer samples (*n*= 58/58) were contaminated with bacteria at the time of sampling (Figure 17). The paired *t*-tests did not establish a significant difference between the mean values of the pre- and post-test samples of the stethoscope ($t(57)= 1.427$; $p= 0.159$) and sphygmomanometer groups ($t(57)= 1.763$; $p= 0.083$).

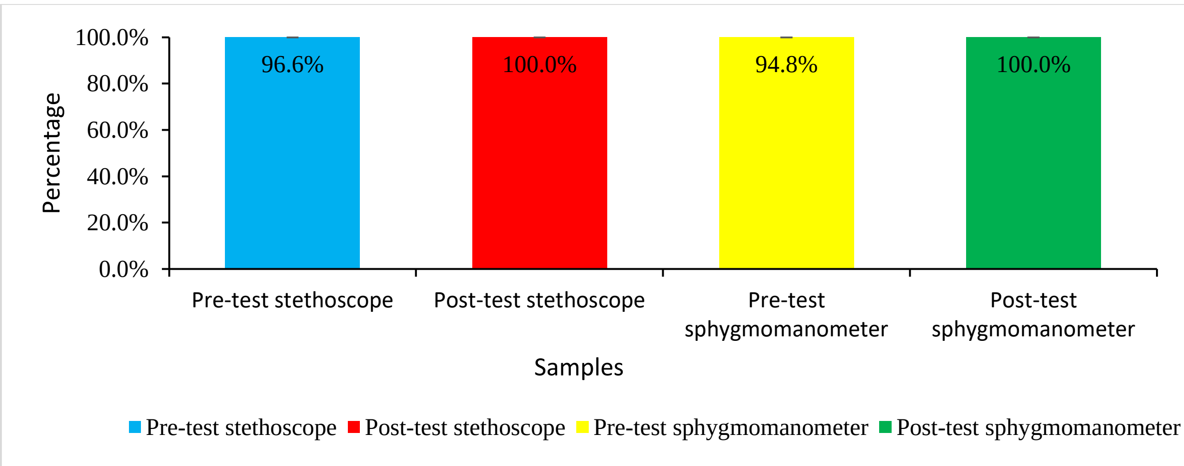


Figure 17: Percentage of stethoscope and sphygmomanometer samples exhibiting bacterial growth after analyses of the incubated pre- and post-test TSA plates.

The pre-test sample findings for both pieces of equipment suggest that, in general, the chiropractic patients were unnecessarily exposed to bacteria when they were examined because the majority of the CSs' equipment was contaminated. The high percentage of pre-test stethoscope and sphygmomanometer samples that exhibited bacterial contamination may directly be attributed to the failure of the majority of the respondents to adhere to adequate equipment disinfection practices. This fact is corroborated by the finding pertaining to Question 10 that was discussed in section 4.2.1.5. However, it is also possible that the contamination of the equipment could have been caused by the disinfectants used by the respondents (as reported in section 4.2.1.6) were ineffective in adequately disinfecting the pieces of equipment under study. This possibility is further explored and discussed in section 4.2.3.

The bacterial contamination (96.6%) of the pre-test stethoscope samples was similar to the findings of Gupta *et al.* (2014) (100.0%), James and Young (2014) (98.0%), Campos-Murguia *et al.* (2014) (95.0%) and Leontsini, Papapetropoulos and Vantarakis (2013) (90.0%). Likewise, the findings pertaining to pre-test sphygmomanometer contamination (94.8%) were in line with the results obtained by Walker, Gupta and Cheesbrough (2006) (100.0%) and Davis (2009) (83.0%).

The studies mentioned above employed a cross-sectional design resulting in only one sample being collected from the specified diagnostic equipment. As the current study had a pre- and post-test design, the findings relating to the bacterial contamination of the post-test samples could not be compared to these studies. However, Paul *et al.* (2011) determined an increase of 31.8% in the percentage of doctors' hands being significantly contaminated on exiting the ward in comparison to upon entry. Similarly, in this study a higher percentage of the post-test samples was contaminated for both pieces of equipment than the percentage that was contaminated in the pre-test samples.

Shiferaw *et al.* (2013), Pal *et al.* (2015) and Jeyakumari *et al.* (2017) considered the presence of greater than or equal ($\geq$) 20 CFUs per sample as significant bacterial contamination. When this definition was taken into consideration, a noticeable drop in the number of contaminated samples was observed. However, the majority of the post-test stethoscope (74.1%) (n= 43/58) and post-test sphygmomanometer (51.7%) (n=30/58) samples exhibited the presence of significant bacterial contamination in comparison to the pre-test stethoscope (36.2%) (n= 21/58) and pre-test sphygmomanometer (39.7%) (n= 23/58) samples (Figure 18 on following page). The paired t-test analyses revealed a significant difference between the mean values of the pre- and post-test stethoscope samples ($t(57) = 4.484$; $p < 0.01$), but not between the pre- and post-test sphygmomanometer samples ($t(57) = 1.308$; $p = 0.196$).

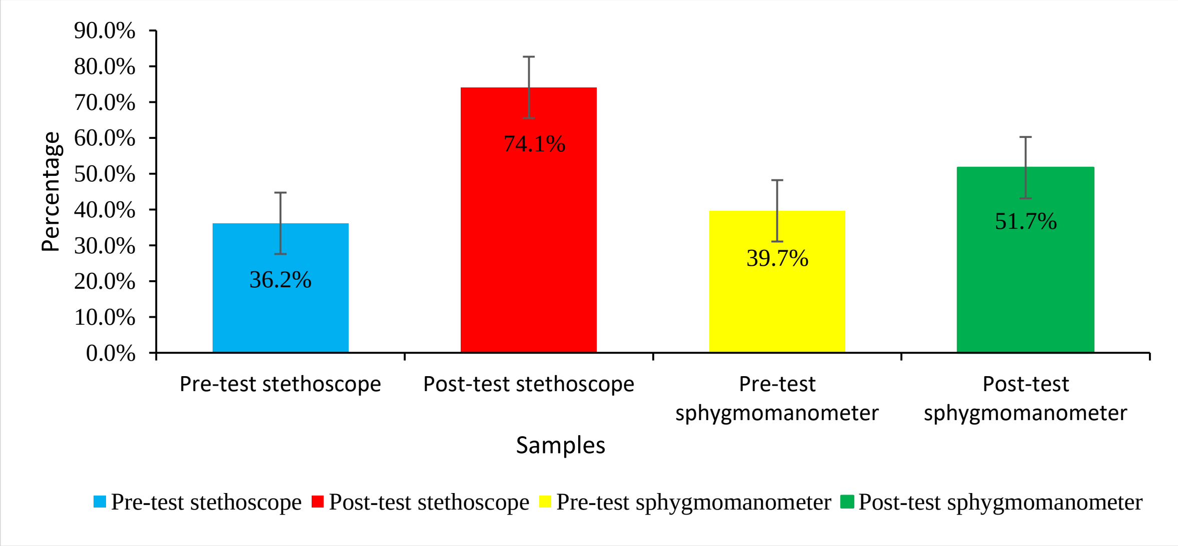


Figure 18: Percentage of stethoscope and sphygmomanometer samples considered to be significantly contaminated with bacteria after the analysis of the incubated pre- and post-test TSA plates.

The finding that only 36.2% of the pre-test stethoscope samples was considered as being significantly contaminated was much lower than the 85.8% by Shiferaw *et al.* (2013), the 74.0% by Jeyakumari *et al.* (2017) and the 52.0% by Pal *et al.* (2015). The difference between this study and the cited literature examples may be because the current study was conducted in a low patient traffic Chiropractic Day Clinic (CDC) compared to the three studies that were performed among HCWs in a high patient traffic public hospital settings (Shiferaw *et al.* 2013; Pal *et al.* 2015; Jeyakumari *et al.* 2017). It may be argued that, in a public hospital setting:

- HCWs are more likely to examine high numbers of patients on a daily basis. In this context, Logtenberg (2009) found that the bacterial load on chiropractic beds in the DUT CDC increased significantly as the day passed. It was believed that the bacterial increase was related to the number of patients treated on the chiropractic beds throughout the day: as more patients came into contact with the bed, the more microflora were transferred from the patients' skin onto the bed. Pittet *et al.* (1999) also established that the bacterial contamination on the hands of HCWs increased proportionally with the time spent caring for patients throughout the day. This supports the argument that HCWs who use their stethoscopes on multiple occasions throughout the day without adequate disinfection will have higher levels of stethoscope bacterial contamination compared to CSs who consult with only one new patient daily. With regards to sphygmomanometer contamination, no studies that used the same definition for significant bacterial contamination could be traced, and therefore no direct comparison could be drawn with this specific finding of the current study. However, it is highly likely that the same principles that apply for stethoscope use will be relevant to the use of a sphygmomanometer.

- Moreover, patients who are examined and treated in hospital settings are more likely to contract a disease of microbial origin compared to patients who visit the DUT CDC, where patients with musculoskeletal complaints are treated (McDonald 2014). The latter patients are unlikely to present with diseases of overt microbial origin.

Whether or not the definition that ≥ 20 CFUs constitute significant contamination was applied, the fact remains that the number of post-test stethoscope and sphygmomanometer samples that were contaminated was higher compared to their relevant pre-test samples. The increase was more evident when the definition of ≥ 20 CFUs signifying significant contamination was applied, as the post-test samples revealed an increase in the percentages of CFUs on the stethoscopes and sphygmomanometers by 37.9% and 12% respectively.

A possible explanation for the fact that fewer pre-test samples were found to be contaminated could be that some CSs had disinfected their equipment either directly before they examined their new patient or after they had examined the previous new patient. Unfortunately, responses from the research questionnaire could not confirm this, as such an option was not available for selection. However, the responses to the questionnaire did reveal that four CSs had disinfected both pieces of equipment directly after every new patient consultation, while a further three CSs had disinfected both pieces of equipment on a daily basis.

Overall, the fact that bacterial contamination was established on some of the pre- and post-test samples serves as evidence that both stethoscopes and sphygmomanometers are reservoirs for bacterial growth.

4.2.2.2 Enumeration of viable bacteria

Table 13 on the following page, illustrates that the total CFU counts for the post-test samples of both the stethoscope (2984 CFUs) and sphygmomanometer (2631 CFUs) groups were higher compared to their respective pre-test samples. Likewise, the mean CFUs were higher for both the post-test stethoscope (51.5 CFUs) and sphygmomanometer (45.4 CFUs) groups compared to their relevant pre-test groups. However, the paired *t*-test showed that only the post-test stethoscope group had a significantly ($t(57) = -4.288$; $p < 0.01$) higher mean value.

Table 13: Bacterial counts of pre- and post-test samples isolated from the surfaces of stethoscopes and sphygmomanometers used by 29 CSs in 58 new patient examinations

Sample	*n*	Total CFU count	Mean CFUs %	Standard deviation %	Standard error mean
Pre-test: stethoscope	58	1 351	23.3	31.48	4.13
Post-test: stethoscope	58	2 984	51.5	41.40	5.44
Pre-test: sphygmomanometer	58	1 877	32.4	72.39	9.50
Post-test: sphygmomanometer	58	2 631	45.4	66.14	8.68

CSs = chiropractic students; *n* = number of samples, CFUs = colony-forming units

<u>Mean CFU values</u>

The mean value of 23.3 CFUs for the pre-test stethoscope samples was substantially lower than the mean value of 89 CFUs that was determined by Longtin *et al.* (2014) and the value of 132 CFUs that was establish by Núñez *et al.* (2000). Again, this difference could be attributed to the fact that this study was performed in a low traffic CDC rather than in a hospital setting. It must be reiterated that the cited studies were cross-sectional in design, which made it impossible to compare the findings of the post-test samples of this study. Moreover, sphygmomanometer contamination studies did not list the mean or total values, which made it impossible to draw any direct comparisons between these studies and the current study (de Gialluly *et al.* 2006; Walker, Gupta and Cheesbrough 2006; Davis 2009; Grewal *et al.* 2013).

One might have expected more pre- and post-test stethoscope and sphygmomanometer samples to be significantly contaminated as their mean CFU counts were above the ≥ 20 CFU mark. However, upon closer analysis, the standard deviations for all four samples were relatively high, thus indicating a wide distribution of the data from the mean values.

<u>Total CFU counts</u>

The total CFU counts for the post-test samples of both pieces of equipment were considerably higher compared to their respective pre-test samples (Table 13). At the risk of being repetitive, it is reiterated that the reason might be that some CSs had disinfected their equipment either directly before they examined their new patient or after they had examined the previous new patient (as discussed previously in section 4.2.2.1).

Kramer, Schwebke and Kampf (2006) established that different bacteria could survive on inanimate objects for various lengths of time. Another explanation could be that the surfaces of the stethoscopes and sphygmomanometers served as an unfavourable environment for bacterial survival, resulting in them entering their death phase prematurely and leading to decreased levels of contamination of the pre-test samples. As the researcher collected the pre-test samples at random and without any prior knowledge about when they had last been used, it was unknown in which phase of bacterial growth the bacteria were at the time of sampling.

The higher total CFU counts for both categories of post-test samples and the subsequent higher contamination levels could be attributed to the transfer of viable bacteria from the patients' skin onto the pieces of equipment. The researcher collected the samples on average one hour after transfer had occurred, and it could therefore be theorised that the majority of the bacteria had not yet entered their death phase, which resulted in an increase in the number of CFUs on the post-test TSA plates.

Also, because the same surface area size was sampled for both pieces of equipment, the differences between the post-test total CFU counts for the stethoscopes and sphygmomanometers could be linked to the number of exposures of each instrument to the patients' skin. As was explained in section 2.2.3.2, during a physical examination the stethoscope makes contact with a patient's skin on multiple occasions during the cardiovascular, respiratory and abdominal examinations. In comparison, the sphygmomanometer's cuff makes contact with the patient's skin only twice when it is used in conjunction with the stethoscope during the assessment of the patient's vital signs. Thus, with each exposure of the stethoscope's diaphragm to the patient's skin, bacterial transfer could occur between the two surfaces. It may be for this reason that a higher total CFU count was found for the stethoscope post-test compared to the total CFU post-test count for the sphygmomanometer.

<u>Stethoscopes and sphygmomanometers as sources of bacterial transfer</u>

Amod (2017) determined that bacterial transfer could occur from a chiropractic patient's skin onto the chiropractor's hands during spinal manipulation, while the converse was also found to be true. Due to the methodology used in this study, the CFU counts for both the post-test stethoscope and sphygmomanometer samples could represent the bacterial transfer from the chiropractic patient onto these equipment. However, the possibility that some of the CFUs could have originated from other sources may not be ignored or eliminated. Possible sources of contamination could have included the diagnostic bag in which the equipment was placed between sampling and the conclusion of the physical examination, airborne bacteria, and contact with surfaces such as clothing or a desk top during the execution of the physical examination.

From the findings established by Amod (2017), it could be argued that in the scenario where a contaminated stethoscope or sphygmomanometer was used on a patient, bacterial transfer in either direction could occur between the patient's skin and the surface of the equipment. Thus both pieces of equipment that were examined in the current study could have been sources of bacterial transfer and could potentially have resulted in the spread of HCAIs (Shiferaw *et al.* 2013). However, the ability of contaminated equipment to cause HCAIs depends on a number of factors.

According to World Health Organization (2011), Willey *et al.* (2014) and Swalaha (2016), these factors are:

- Host factors
    - Age: In general, infants and geriatrics are more susceptible to infections due to lack of immunity.
    - Current health status: Immunocompromised patients, either as a result of chemotherapy, prolonged corticosteroid usage or chronic disease, are commonly more prone to infections. Comorbidities contribute to an altered state of immunity.
    - Lifestyle factors: Habits (smoking and alcohol), nutritional status, emotional wellbeing (stress levels) and socio-economic status also contribute to the functioning (or loss of functioning) of the immune system.
- Microbial factors
    - Number of microorganism present.
    - Pathogenicity and virulence of microorganisms, including factors such as antibiotic resistance.
    - Portal of entry.

Nevertheless, with the implementation of adequate education pertaining to equipment disinfection and strict adherence to these infection prevention protocols, the occurrence of resultant HCAIs could be minimized, at the same time improving patient and staff safety in the healthcare setting.

<u>Distribution of bacteria along the edge and diaphragm surfaces of a stethoscope</u>

Figure 16 illustrates the difference in the distribution of CFUs that were isolated from the surfaces of a stethoscope. When the TSA plates from the pre- and post-test stethoscope samples (n=58 each) were analysed, it was noted that 86.2% (n=1164/1351) and 82.8% (n= 2472/2984) of the total CFUs respectively were located on the edges of the stethoscopes' diaphragm imprint (Table 14 and Figure 19 illustrated on following page).

With regards to the post-test stethoscope samples, the paired t-test determined that the mean CFU counts were significantly higher for both the edge ($t(57)$= -3.993; p <0.01) and diaphragm surfaces ($t(57)$= -3.212; p< 0.05).

Table 14: Bacterial counts of 58 pre- and post-test stethoscope samples: distribution of CFUs on edge versus diaphragm

Samples *n*=58	**Surfaces**	**Total CFU count on surface**	**Percentage of total CFU count on surface**	**Mean CFUs per sample**	**Standard deviation**	**Standard error mean**
Pre-test stethoscope	Edge	1164/ 1351	86.2%	20.7	25.79	3.39
Post-test stethoscope	Edge	2472/ 2984	82.8%	42.6	36.43	4.78
Pre-test stethoscope	Diaphragm	187/ 1351	13.8%	3.2	8.85	1.16
Post-test stethoscope	Diaphragm	512/ 2984	17.2%	8.8	10.34	1.36

n= number of samples, CFUs= colony-forming units

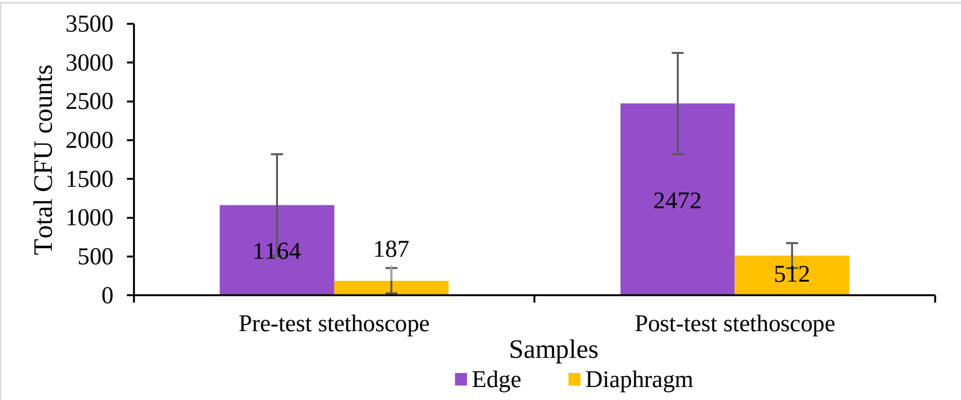


Figure 19: Total distribution of CFUs across the edge and diaphragm surfaces of 58 pre- and post-test stethoscope samples.

The results above determined that a greater distribution of the total CFUs was found in the region of the diaphragms' edges and not on the diaphragms themselves. In an e-mail communication on 25 March 2016, Longtin, one of the authors of the journal article "Contamination of stethoscopes and physicians' hands after a physical examination", mentioned that they also found that the "growth was always sparser in the middle compared to the edge of the diaphragm". Longtin (2016) termed this appearance the "the halo effect".

Closer inspection of a stethoscope surface (Figure 20 on following page) revealed that the edges appeared protruded in comparison with the rest of the diaphragm which appeared concave. This means that when the diaphragm is placed onto a hard, even surface, the edges will make firmer contact with the surface while the rest of the diaphragm will be suspended in the air. This could explain why the diaphragms' edges produced more bacterial isolates, as they had made firmer contact with the patients' skin every time they were used, while the diaphragms in comparison only touched the skin if the users applied enough force to push the inside surfaces of the diaphragms against the patients' malleable skin. Another argument is that the ridge at the edge of the diaphragm is an area where the device may not be properly cleaned, and this area then creates a reservoir for bacterial growth to a greater extent than the rest of the surface of the diaphragm.

Figure 20 illustrates the surface of the diaphragm, showing that the edge protrudes above the rest of the surface.

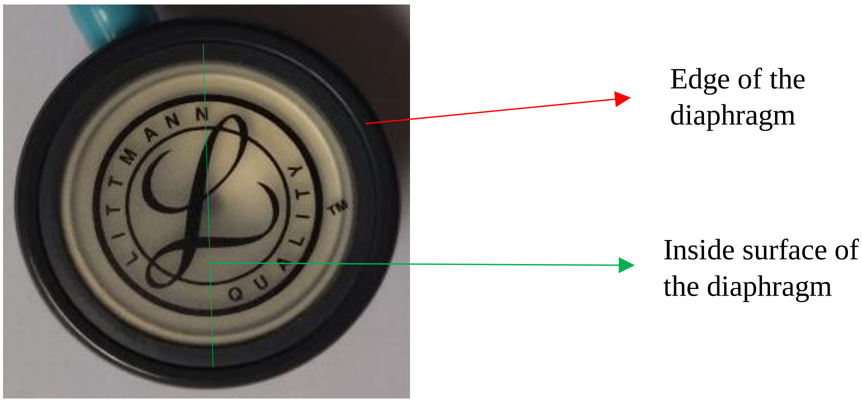


Figure 20: Illustration of the surface of a stethoscope diaphragm.

4.2.2.3 Identification of bacteria

Six genera of bacteria were identified as well as one broad class called coliforms. These bacteria were isolated from the pre- and post-test samples that were collected from the diaphragms of the stethoscopes and the cuffs of the sphygmomanometers.

The macroscopic morphology of the first colony was small, circular, entire, raised, smooth and white in colour. The light microscopic morphology consisted of Gram-positive cocci arranged in cluster formations as illustrated in Figure 21. After inoculated onto a MSA plate, white growth was noted. Taking all of these characteristics into consideration as well as the negative coagulase test, the bacterium was identified as CoNS.

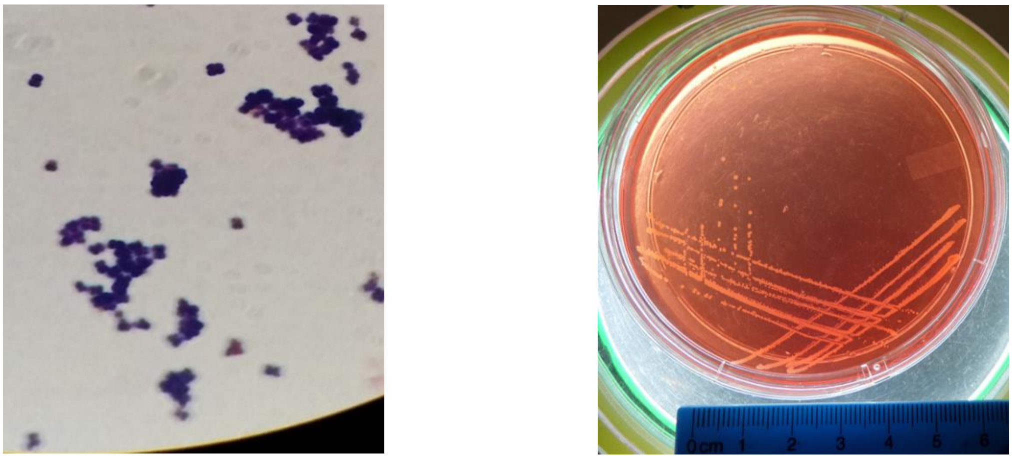


Figure 21: CoNS: Light microscopic morphology consisting of Gram-positive cocci in cluster arrangements (1 000x) (left) and macroscopic appearance on MSA after incubation at 37°C for 24 hours (right).

The second colony was identified as *S. aureus.* Its macroscopic and light microscopic morphologies were similar to those of CoNS, with the exception that its colony was pale yellow in colour. When inoculated onto a MSA plate, yellow growth and mannitol fermentation were observed, as illustrated in Figure 22. The *S. aureus* were further distinguished from CoNS on the basis of a positive coagulase test and the formation of black colonies with a white edge surrounded by a clear zone when streaked onto Baird-Parker agar.

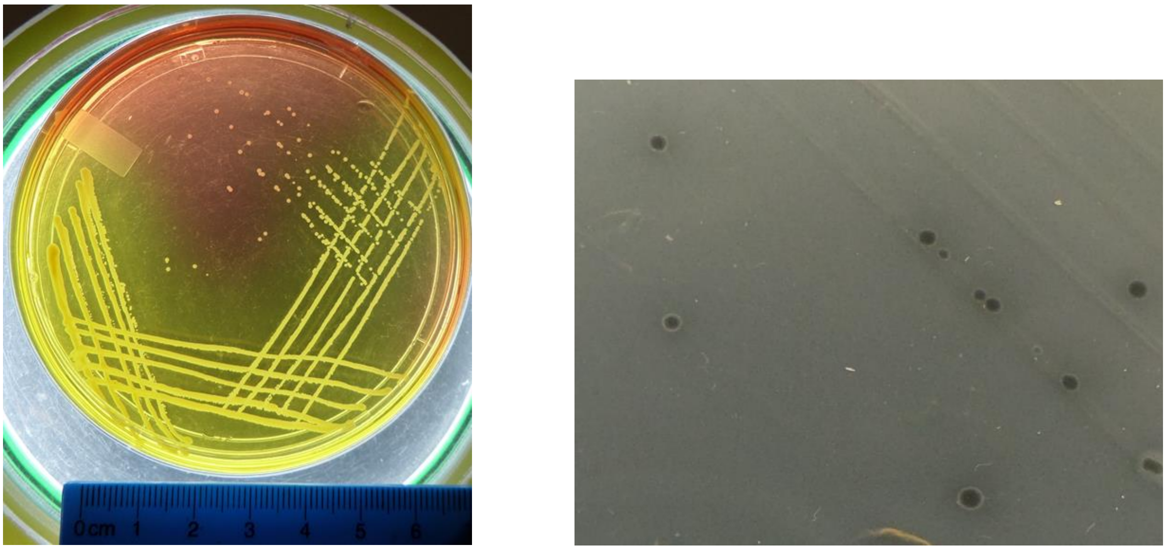


Figure 22: ***S. aureus*****: Macroscopic morphology on MSA showcasing presence of yellow growth and mannitol fermentation (left) and formation of white rimmed black colonies with a surrounded clear zone on Baird-Parker agar following 24 hours of incubation at 37ºC (right).**

The third type of colony was circular, yellow, smooth, convex, entire and ranged between 3 mm and 10 mm in size. Its light microscopic morphology entailed Gram-positive cocci arranged in either pairs or tetrads. On the basis of its macroscopic and microscopic characteristics, the bacterium was identified as *Micrococcus* spp. Due to both micrococci and staphylococci being catalase positive, the oxidase test was performed in order to definitively differentiate between the two species. The oxidase test was positive (refer to Figure 23), thus confirming the bacterium as *Micrococcus* spp.

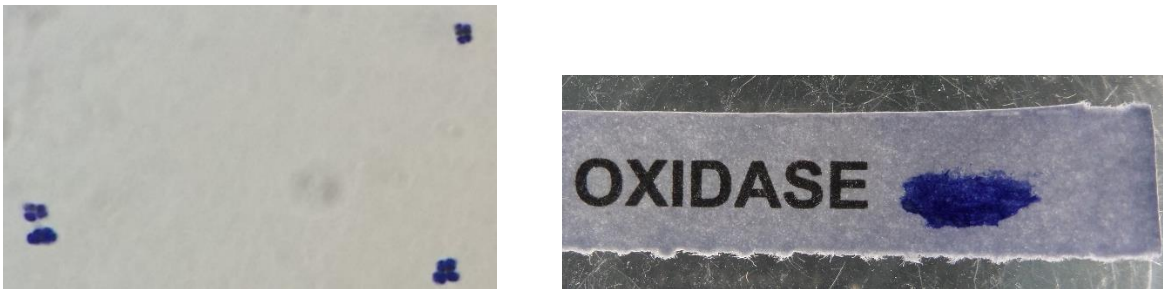
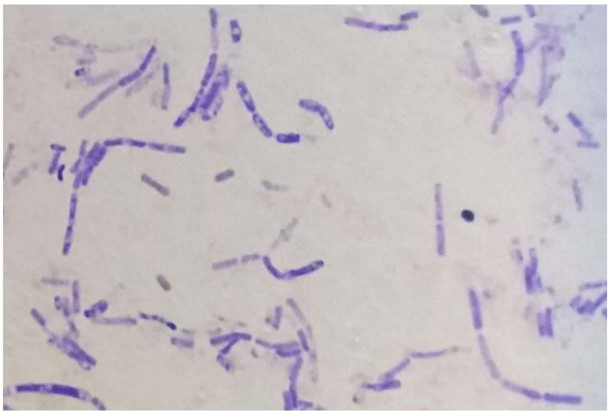


Figure 23: ***Micrococcus*** **spp.: Light microscopic morphology consisting of Gram-positive cocci arranged in pairs or tetrads (1 000x) (left) and a positive oxidase test result due to production of cytochrome c oxidase (right).**

The fourth genus was identified as *Bacillus* spp. The description of the macroscopic morphology is flat, large, irregular tan-white colonies with lobate margins. The microscopic findings included long Gram-positive rod chains with visible endospores as illustrated in Figure 24 below.



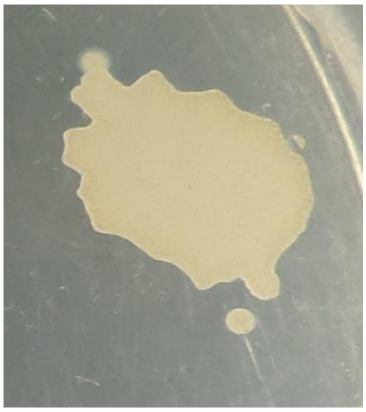


Figure 24: *Bacillus* spp.: Light microscopic morphology showing Gram-positive rod chains with the presence of endospores (1 000x) (left) and macroscopic morphology illustrated on TSA after incubation of 24 hours at 37ºC (right).

Corynebacterium spp. was identified as the fifth genus. It was largely differentiated from *Bacilllus* spp. on the basis of its light microscopic morphology. Under the light microscope, Gram-positive non-sporulating bacilli were observed. The bacilli arrangement included snapping (V-shaped) and clusters resembling Chinese letters (Figure 25). A positive result was achieved during the catalase test.

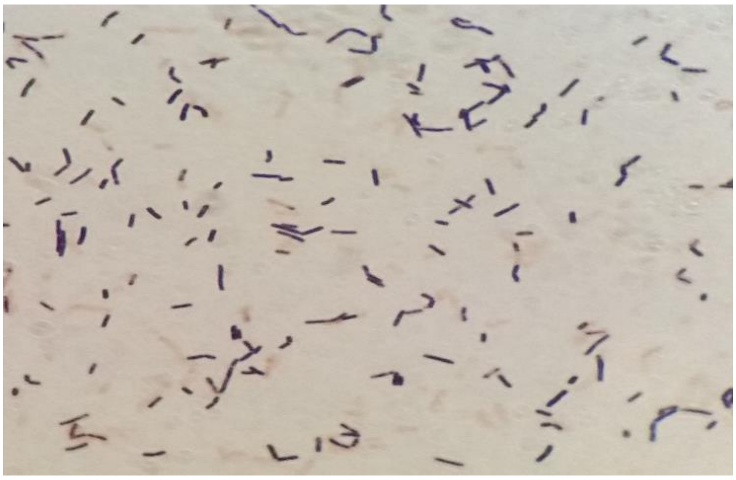


Figure 25: *Corynebacterium* spp.: Light microscopic morphology consisting of Gram-positive non-sporulating bacilli arranged in snapping and cluster formations (1 000x).

The colony of the sixth bacterium was grey-white, small, circular, smooth, entire and convex macroscopically, while the microscopic findings consisted of Gram-negative rods. The catalase test had a positive result. When inoculated onto MAC, pink colonies were observed, thus indicating that the bacterium was a lactose fermenter (Figure 26). The bacterium was identified as *E. coli* after it had been streaked onto EMB agar as it resulted in dark purple colonies exhibiting a green metallic sheen. Bacteria that exhibited the same light microscopic characteristics and growth features on MAC as *E. coli*, but that presented differently on the EMB agar, were classified as coliforms. Thus the Coliform group is completely separate from *E. coli*.

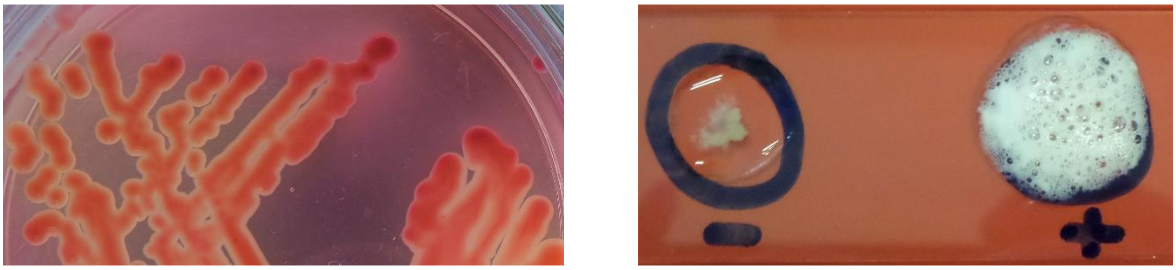


Figure 26: *E. coli:* Macroscopic morphology on MAC after incubation for 24 hours at 37ºC (left) and a positive catalase test result due to production of oxygen bubbles (right).

As illustrated in Table 15 and Figure 27, located on the following two pages, the vast majority of bacteria that were isolated from the pre- and post-test samples from both pieces of equipment consisted of CoNS, *Micrococcus* spp. and *S. aureus,* which are noted constituents of the skin's normal microflora (Brooks *et al.* 2013; Willey *et al.* 2014). It was only with regards to the post-sphygmomanometer category composition that the largest portion of bacteria was identified as *Micrococcus* spp. (45.0%) and not CoNS (41.8%), as was the case in the other three sample categories. The minority on the other hand comprised *Bacillus* spp., *Corynebacterium* spp., coliforms and *E.coli*. *Bacillus* spp. sometimes transiently colonizes on the skin, whereas *Corynebacterium* spp. forms part of the skin's normal microflora. *E.coli* forms part of the normal bowel flora, although it can also be located on the human skin for a temporary period of time (Tille 2014).

With regards to the stethoscope group, the results of the paired *t*-tests showed that the CoNS ($t(57)= -3.295$; $p< 0.01$) and the *Micrococcus* spp. ($t(57)= -2.381$; $p= 0.02$) groups had significantly higher post mean values.

Five of the species that were identified in this study were also isolated from stethoscopes in the studies that were conducted by Pal *et al.* (2015), Núñez *et al.* (2000), Shiferaw *et al.* (2013) and James and Young (2014). The bacterial profile of the bacteria that were isolated from the stethoscopes by Shiferaw *et al.* (2013) consisted of CoNS (40.2%), *S. aureus* (30.9%), *Bacillus* spp. (5.1%), *Klebsiella* spp. (4.7%), *Citrobacter* spp. (4.3), *Salmonella* spp. (3.5%), *Proteus* spp. (3.5%), *Enterobacter* spp. (3.1%), Gram-positive filamentous (2.3%), *Ps. aeruginosa* (1.2%), *E. coli* (0.8%)

and *Micrococcus* spp. (0.4%). The findings pertaining to isolating *Klebsiella* spp., *Salmonella* spp. and *Ps. aeruginosa* could potentially be attributed to this study being conducted in a hospital setting, where the risks of contracting HCAIs are higher compared to a CDC setting.

Davis (2009) reported that that cuffs of the fifteen sphygmomanometers that had been tested were only contaminated with saprophytes consisting of mixed skin flora. Likewise, de Gialluly *et al.* (2006) established that the majority of the bacterial contamination found on both sides of the tested sphygmomanometer cuffs comprised saprophytes which consisted of CoNS and *Corynebacterium* spp. in particular, while a few colonies of *E.coli* and *S. aureus* were also isolated. Overall, the findings pertaining to the sphygmomanometer cuffs of the current study were similar to those of de Gialluly *et al.* (2006).

Table 15: Bacterial profiles of pre- and post-test samples isolated from the surfaces of 58 stethoscopes and sphygmomanometers used by 29 CSs during their consultation with 58 new patients at the DUT CDC

	Pre-test stethoscope samples *n*= 1351		**Post-test stethoscope samples *n*= 2984**		**Pre-test sphygmo manometer samples *n*= 1877**		**Post-test sphygmo-manometer samples *n*= 2631**	
Bacteria isolated	**Total CFUs of isolate**	**% of samples' total CFUs count**	**Total CFUs of isolate**	**% of samples' total CFUs count**	**Total CFUs of isolate**	**% of samples' total CFUs count**	**Total CFUs of isolate**	**% of samples' total CFUs count**
CoNS	633	46.9%	1637	54.9%	959	51.1%	1100	41.8%
Micrococcus spp.	406	30.1%	801	26.8%	596	31.8%	1183	45.0%
S. aureus	198	14.7%	343	11.5%	199	10.6%	158	6.0%
Bacillus spp.	77	5.7%	97	3.3%	84	4.5%	124	4.7%
Corynebacterium spp.	26	1.9%	42	1.4%	20	1.1%	35	1.3%
Coliforms	9	0.7%	37	1.2%	11	0.6%	24	0.9%
E. coli	2	0.1%	27	0.9%	8	0.4%	7	0.3%
Totals	1351	100.0%	2984	100.0%	1877	100.0%	2631	100.0%

n=total CFU count for samples, CFUs= colony-forming units, %= percentage, spp.= species,
CoNS= coagulase-negative Staphylococci

Figure 27 on the succeeding page, provides a graphic presentation of the bacterial profiles of the 58 pre-test stethoscope, post-test stethoscope, pre-test sphygmomanometer and post-test sphygmomanometer samples. The figure facilitates a visual comparison of the distribution of the various bacteria between the four sample groups.

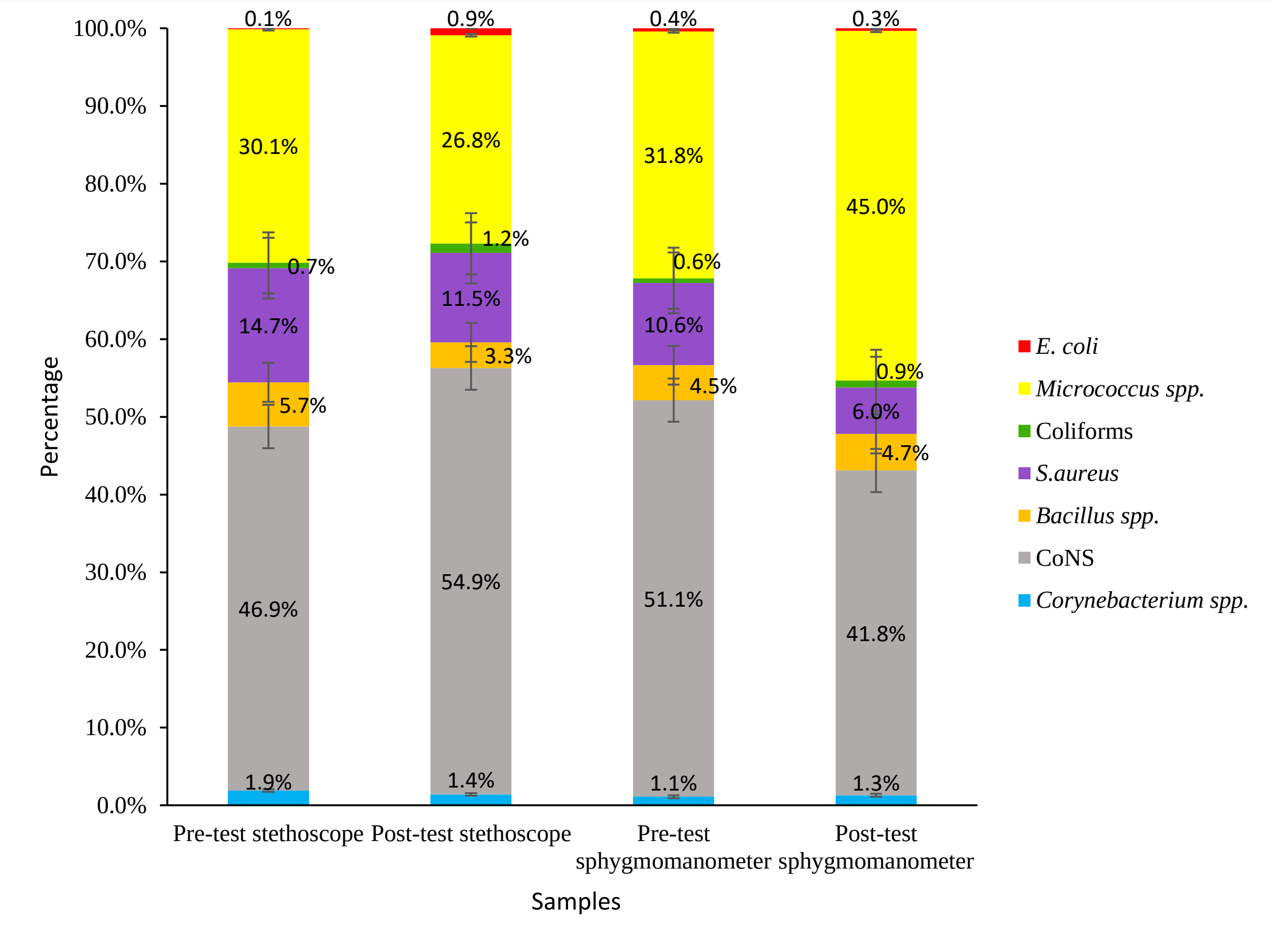


Figure 27: The complete bacterial profile of the 58 pre- and post-test stethoscope and sphygmomanometer samples expressed in percentages.

CoNS, *S. aureus*, coliforms and *E. coli* were allocated to the potentially pathogenic bacterial group on the basis of their potential to cause HCAIs, while *Micrococcus* spp., *Bacillus* spp. and C*orynebacterium* spp. formed the non-pathogenic bacterial group (World Health Organization 2011; United States Department of Health: Centers for Disease Control and Prevention 2013; Tille 2014). The majority of the bacteria that were identified in the pre-test stethoscope (62.3%), post-test stethoscope (68.5%) and pre-test sphygmomanometer (62.7%) sample groups were deemed potentially pathogenic. Conversely, the post-test sphygmomanometer sample group was determined to have been contaminated with non-pathogenic (51.0%) rather than with potentially pathogenic microbes (49.0%) (Table 16 and Figure 28).

Analyses using the paired *t*-test revealed that the potentially pathogenic ($t(57) = -3.623$; $p < 0.01$) and the non-pathogenic ($t(57) = -2.542$; $p < 0.02$) bacterial groups from the stethoscope samples had significantly higher post mean values. The paired *t*-test results for the sphygmomanometer samples were not statistically significant and were as follows: ($t(57) = -0.200$; $p = 0.84$) for the potentially pathogenic bacterial group and ($t(57) = -1.912$; $p = 0.06$) for the non-pathogenic bacterial group.

The finding that 62.3% of the total bacterial isolates on the pre-test stethoscope samples consisted of potentially pathogenic bacteria was slightly higher than the 52.0% that had been determined by Shiferaw *et al.* (2013). Studies that could be traced that investigated sphygmomanometers did not list the mean or total values for the different bacteria that had been isolated, thus the bacteria could not be grouped accordingly in order to make a direct comparison regarding the distribution of potential pathogenic and non-pathogenic bacteria on this piece of equipment.

The above findings confirm the statement (section 4.2.1.5) that CSs who fail to adhere to adequate equipment disinfection could expose their patients to potentially pathogenic bacteria. However, it must be reiterated (see section 4.2.2.2) that although these pieces of equipment may serve as sources of bacterial transfer, various other factors also contribute to the fact that patients may contract HCAIs.

Table 16: Degree of pathogenicity and distribution of the bacteria isolated from the pre- and post-test stethoscope and sphygmomanometer samples

Samples *n*=58	Bacterial group	Total CFU count for sample	Percentage of total CFU count for sample	Mean CFUs per sample	Standard deviation	Standard error mean
Pre-test stethoscope *N*= 1351	Potentially pathogenic bacteria	842	62.3%	14,5	25.28	3.32
	Non-pathogenic bacteria	509	37.7%	8,8	12.23	1.61
Post-test stethoscope *N*= 2984	Potentially pathogenic bacteria	2044	68.5%	35,2	37.33	4.90
	Non-pathogenic bacteria	940	31.5%	16,2	19.12	2.52
Pre-test sphygmomanometer *N*= 1877	Potentially pathogenic bacteria	1177	62.7%	20,3	68.73	9.02
	Non-pathogenic bacteria	700	37.3%	12,1	13.48	1.77
Post-test sphygmomanometer *N*= 2631	Potentially pathogenic bacteria	1289	49.0%	22,2	46.57	6.12
	Non-pathogenic bacteria	1342	51.0%	23,1	42.39	5.57

n= number of samples, CFUs= colony-forming units, *N*= total CFU count for sample, %= percentage

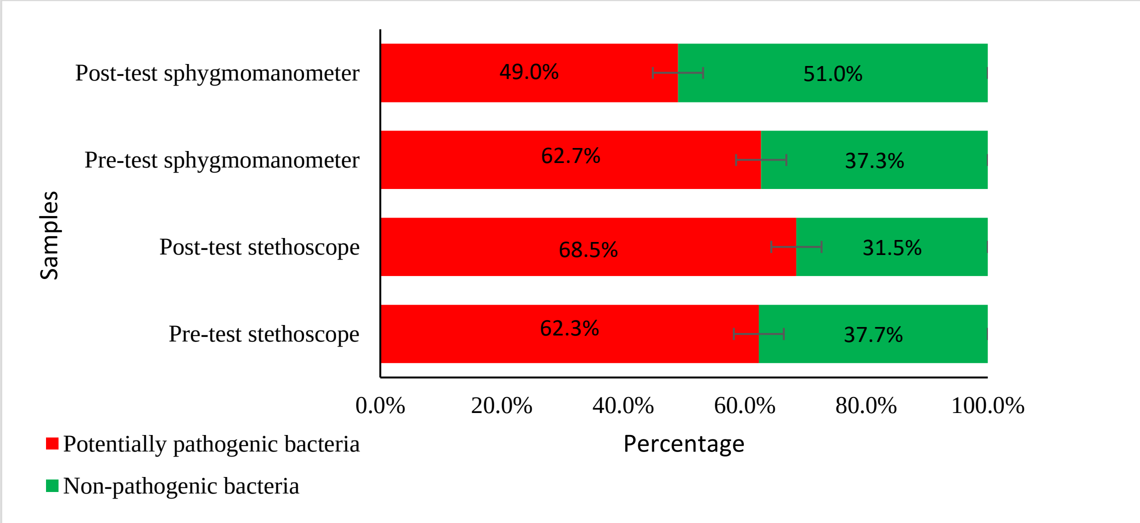


Figure 28: Distribution of bacteria isolated from pre- and post-test stethoscope and sphygmomanometer samples according to their degree of pathogenicity.

4.2.3 Objective 2

Objective 2 was to assess the efficacy of disinfectants used by CSs in the DUT CDC for cleaning stethoscopes and sphygmomanometers using a modified AOAC use dilution method.

The results of the efficacy testing of water, 70% isopropyl alcohol, 70% ethanol and hand sanitizer (62% ethanol) indicated that all four disinfectants failed the efficacy test at a rate of 100% (Table 17). This means that none of the disinfectants were able to kill even one out of 443 bacterial isolates used during testing. Thus all of the tests resulted in the TSA broth presenting with growth as illustrated in Figure 29, on the subsequent page. No statistical analyses could be performed on these data as all the values were exactly the same.

Table 17: Results of the efficacy testing of water, 70% isopropyl alcohol, hand sanitizer and 70% ethanol against the five pre-specified bacteria with the use of the AOAC use dilution method

Disinfectants	**Isolates showing growth after the application of various disinfectants**				
	CoNS *n*= 156	***S. aureus*** *n*=99	***Micrococcus* spp.** *n*=156	**Coliforms** *n*=19	***E. coli*** *n*=13
Water	100.0%	100.0%	100.0%	100.0%	100.0%
70% isopropyl alcohol	100.0%	100.0%	100.0%	100.0%	100.0%
70% ethanol	100.0%	100.0%	100.0%	100.0%	100.0%
hand sanitizer (62% ethanol)	100.0%	100.0%	100.0%	100.0%	100.0%

n= number of isolates tested, %= percentage

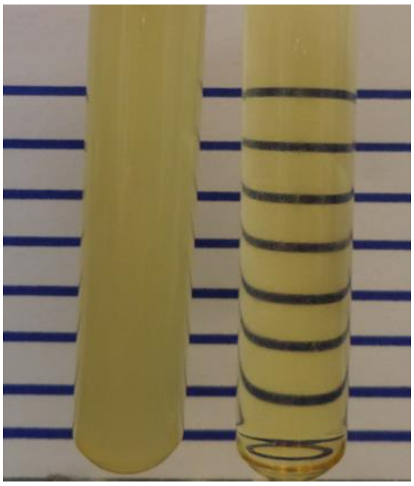


Figure 29: Comparison between the control tube which exhibits no bacterial growth as it is clear (right) and a test tube which shows bacterial growth as a result of an increase in turbidity of the TSA broth (left).

The findings pertaining to the efficacy of disinfectants that were tested in this study were in complete contrast when compared to findings in the published literature. Gupta *et al.* (2014), using a pre- and post-test study design, established that the use of 70% ethanol for stethoscope disinfection resulted in a significant decrease of 86.7% in the bacterial CFU count. The two bacteria on which Gupta *et al.* (2014) tested the efficacy of 70% ethanol were CoNS and *S. aureus*. Lecat *et al.* (2009) also examined the efficacy rates for disinfecting stethoscopes, and found a significant reduction of 92.8% and 92.5% in the number of bacterial isolates with the use of an ethanol-based cleanser (62% ethanol) and 70% isopropyl alcohol pads respectively. Once again, CoNS and *S. aureus* were some of the bacteria on which these disinfectants were tested. In a study that was conducted by Jeyakumari *et al.* (2017), all the stethoscopes that had been contaminated with isolates ranging from coliforms, MRSA, and CoNS to *S. aureus* were growth-free after disinfection with 70% isopropyl alcohol. It is noteworthy that Paul *et al.* (2011) determined that when doctors washed their contaminated hands with tap water, it resulted in a 76% decrease in the carriage rates. The above studies were cited as they tested the same disinfectants at the same concentrations as the current study.

The literature thus indicated that none of the disinfectants were 100% effective. This suggests that some of the CFUs in the post-test sample CFU counts (see section 4.2.2.2) could possibly have been residual pre-test CFUs. However, in such a scenario, one would not expect the number of CFUs that 'survive' to be high and to have a significant influence on the post-test CFU counts.

Both ethanol and isopropanol, at the optimum bactericidal concentration consisting of a 60-90% solution, are effective against bacteria such as *E. coli, S. aureus and Ps. aeruginosa* (Davis 2009). This statement, coupled with the results of the cited research studies, thus implies that the findings of the current study were anomalous. One explanation is that the findings could have been due to a flawed methodology with regards to the execution or selection of the technique that was used. This is discussed below in section 4.2.3.1.

4.2.3.1 Shortcomings of the AOAC use dilution method and its implementation

- The stock solution of the inoculated broth was prepared to a 0.5 McFarland standard which equated roughly to 1 x 10^8 CFU/mL (Donay *et al.* 2007). For this study, the highest CFU count for a single sample was 548 CFUs. This means that the stock solution failed to represent the actual level of contamination found on the equipment of the CSs.
- Insufficient contact time could have contributed to the disinfectants failing the test. The longer a bacterial population is exposed to an effective disinfectant, the more bacteria are killed (Willey *et al.* 2014). However, this was unlikely due to the concentration of the stock solution being used. An increase in the contact time by a couple of minutes would probably not have made any difference to the outcome.
- The outcome of the AOAC use dilution method could be either the presence of growth or the absence of growth. It does not calculate the log reduction of the bacterial population caused by the disinfectant (United States Environmental Protection Agency Office of Pesticide Programs 2009). This means that even if the disinfectant had killed 99% of the bacteria on the carrier, it would still have failed the test as the Nutrient broth (NB) would have supplied the necessary nutrients to the surviving 1% of bacteria to produce an increase in the turbidity of the NB.

The implementation of a pre- and post-test design when testing the efficacy of disinfectants, as used by Gupta *et al.* (2014) and Lecat *et al.* (2009), would have been more appropriate. This design would have allowed the disinfectants to be tested in the same environment they would normally be used. The pre-test would have allowed for the enumeration of the bacteria present on the surface of the stethoscopes' diaphragms and sphygmomanometers' cuffs. Thereafter, the sampled surfaces would have been disinfected and the post-test sample collected. By comparing the pre- and post-test CFU counts, the efficacy of the disinfectant could have been calculated and reported.

If this design had been applied in the existing design of the study, the bacterial sample collection from the surfaces of the stethoscopes and sphygmomanometers would have been as follows:

- Collection of the first sample would have occurred before the consultation with the new patient. This sample would have allowed for the enumeration of the bacterial contamination on the surface tested, hence giving insight into the disinfection practices of each CS. It would also have served as the pre-test for the efficacy testing of the disinfectants.
- The second sample would have been collected after the sampled surface had been disinfected with the specified disinfectant, thus acting as the post-test for the efficacy testing.

- Collection of the third sample would have occurred after the CS had concluded the physical examination on the new patient. The CFU count of this reading would have indicated the bacterial load transfer from the patient's skin onto the surface of the tested equipment.

Overall, the literature indicates that ethanol and isopropyl alcohol, at the optimum bactericidal concentrations, are effective when implemented for disinfection purposes of equipment (Ramesh *et al.* 2004; Lecat *et al.* 2009; Paul *et al.* 2011; Graziano *et al.* 2013; Gupta *et al.* 2014; Casey *et al.* 2015; Raghubanshi *et al.* 2017).

4.2.4 Objective 3

This objective was to determine the presence and quantities of antibiotic-resistant bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC using therapeutic antibiotics with the disk diffusion assay to determine bacterial susceptibility to antibiotics.

In total, 443 individual bacterial isolates were tested against five antibiotics, each of which was from a different antibiotic class. The results are presented in Table 18 on the following page. The most effective antibiotic in relation to inhibiting all species isolated was C with an overall bacterial susceptibility rate of 95.7%, followed by CIP (93.2%), VA (80.8%), AMO (69.5%) and E (57.7%). With regards to the antibacterial sensitivity patterns of CoNS, *S. aureus* and *Micrococcus* spp., the results indicated that the most effective antibiotic was C, trailed by CIP, VA, AMO and lastly E. No CoNS (*n*= 156) were resistant to C, with only 4.5% indicating resistance to CIP. However, the resistance to VA, E and AMO was much higher at rates of 19.2%, 35.9% and 34.6% respectively. Figure 30, on page 106, illustrates the resistance of a CoNS isolate to E, as the size of the zone of inhibition is smaller than 13 mm (Appendix AA). More than a quarter of the *S. aureus* isolates (27.3%, *n*=27/99) was resistant to the AMO, with 15,2% being resistant to VA. The least effective antibiotic against *Micrococcus* spp. (*n*= 156) was E, with a resistance rate of 32.7%.

The majority (73.7%) of the coliforms that was tested (*n*=14/19) was sensitive to CIP, E and AMO, while 36.8% was resistant to VA. Coliforms had the highest resistance to C (15.8%) in comparison to any of the other bacterium species that were tested. With regards to *E. coli* (*n*= 13), C and CIP were the most effective with a susceptibility rate of 84.6% for each of the antibiotics. AMO was the least effective with a resistance rate of 38.5% (Table 18).

Table 18: Antibacterial sensitivity patterns of five pre-specified bacteria isolated from 58 stethoscopes and sphygmomanometers used at the DUT CDC in relation to five commonly used antibiotic discs

		C 30 µg	CIP 5 µg	VA 30 µg	E 15 µg	AMO 25 µg
CoNS	**Resistant**	0.0%	4.5%	19.2%	35.9%	34.6%
***n*=156**	**Intermediate**	3.2%	3.2%	0.6%	16.7%	1.3%
	Susceptible	96.8%	92.3%	80.1%	47.4%	64.1%
S. aureus	**Resistant**	0.0%	4.0%	15.2%	22.2%	27.3%
***n*=99**	**Intermediate**	3.0%	2.0%	0.0%	22.2%	1.0%
	Susceptible	97.0%	94.0%	84.8%	55.6%	71.7%
Micrococcus	**Resistant**	1.9%	3.2%	17.9%	32.7%	9.6%
spp.	**Intermediate**	0.0%	0.0%	0.0%	0.6%	16.0%
***n*=156**	**Susceptible**	98.1%	96.8%	82.1%	66.7%	74.4%
Coliforms	**Resistant**	15.8%	15.8%	36.8%	26.3%	15.8%
***n*=19**	**Intermediate**	15.8%	10.5%	0.0%	0.0%	10.5%
	Susceptible	68.4%	73.7%	63.2%	73.7%	73.7%
E.coli	**Resistant**	0.0%	0.0%	30.8%	30.8%	38.5%
***n*=13**	**Intermediate**	15.4%	15.4%	0.0%	0.0%	7.7%
	Susceptible	84.6%	84.6%	69.2%	69.2%	53.8%
Grand total	**Resistant**	2.0%	4.3%	19.0%	31.2%	23.5%
***n*=443**	**Intermediate**	2.3%	2.5%	0.2%	11.1%	7.0%
	Susceptible	95.7%	93.2%	80.8%	57.7%	69.5%

C=Chloramphenicol, CIP=Ciprofloxacin, VA=Vancomycin, E=Erythromycin, AMO= Amoxicillin, µg= microgram, CoNS= coagulase-negative staphylococci, %= percentage, *n*= number of isolates tested

In a similar study, Shiferaw *et al.* (2013) identified much higher resistance levels of CoNS, *S. aureus* and coliforms to C, CIP, VA and E. Other studies also found higher resistance levels in various bacterial isolates, including CoNS, *S. aureus, E. coli, Bacillus* spp. and coliforms against different antibiotics, but in the same classes as used in the current study (Uneke *et al.* 2010; Leontsini, Papapetropoulos and Vantarakis 2013; Gupta *et al.* 2014; Pal *et al.* 2015). These differences could potentially be attributed to the following:

- The use of the same antibiotics than in the current study, but at lower concentrations.
- As the studies cited above were conducted in developing countries, an over prescription of specific antibiotics due to a lack of other antibiotics or other options could have led to the development of antibiotic resistant bacteria. Additionally, lack of adequate medical supplies could have resulted in patients not receiving full courses of antibiotics which could also have led to antibacterial resistance (ABR).
- Poor patient compliance could have produced these results, as they might have failed to complete the antibiotic course as instructed.

- The majority of patients in healthcare settings such as those in which the cited studies were conducted, suffer from diseases of an organic nature that require treatment in the form of antibiotics. The duration of stay in a hospital setting varies significantly depending on the seriousness of the condition. Some patients might only be exposed to the hospital environment for a few hours, while others might stay in the hospital for a few weeks. In contrast, the majority of patients presenting at the DUT CDC have conditions of mechanical origin, which are treated with various manual therapy techniques that do not include the prescription of any pharmaceutical medications. Some of these patients might also present with a cough at the same time as back pain; however, the scope of the chiropractic treatment will focus on the back pain only. Patients who do present with diseases of an organic nature do not receive treatment at the CDC, but are referred to either a general practitioner or a specialist. The duration of a consultation at the DUT CDC varies between 45 to 210 minutes, depending on whether it is an initial or follow-up consultation. Thus a patient is unlikely to be exposed to the CDC environment for more than 210 minutes. These facts could possibly explain why the ABR levels were lower in the DUT CDC in comparison to a hospital setting (McDonald 2014).

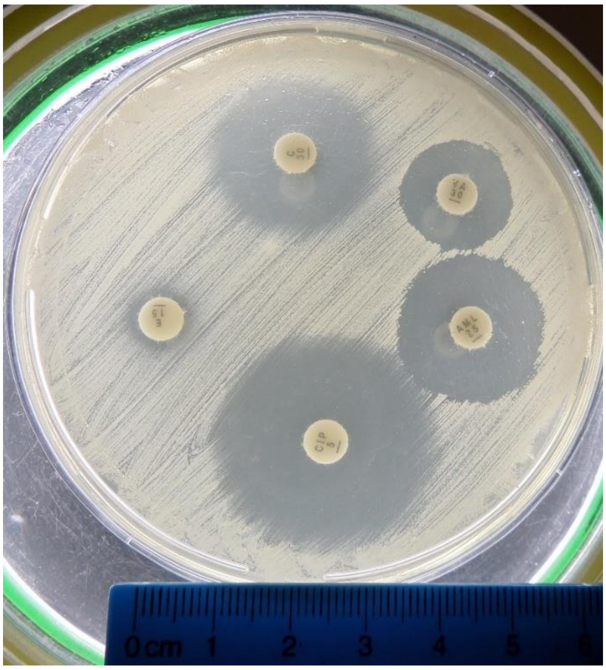


Figure 30: Visual illustration of the various sizes of the zones of inhibition of each of the five antibiotics when tested against a CoNS isolate on a MHA plate.

Micrococcus spp. are rarely the causative agent for infections in humans and are thus largely considered to be non-pathogenic (Albertson, Natsios and Gleckman 1978). The finding that *Micrococcus* spp. were resistant to the antibiotics that were tested was of concern, particularly because almost a quarter of the isolates were found to be resistant to E. The ABR developed by the *Micrococcus* spp. could be passed on via horizontal gene transfer or conjugation to various surrounding pathogenic bacteria such as *S. aureus* and *E. coli* which could result in serious HCAIs (United States Department of Health: Centers for Disease Control and Prevention 2013; World Health Organization 2014; Munita and Arias 2016).

The high resistance levels of CoNS and *S. aureus* to AMO suggest the presence of MRSA and methicillin-resistant coagulase negative staphylococci isolates. Resistance to the penicillin class and in particular to penicillin itself is very common, as penicillin was the first antibiotic that was made available for use by the public in 1943. The first case of penicillin resistance by staphylococci was reported in 1947. Similarly, methicillin was made available to the public in 1960, and the first finding of MRSA was reported within two years (United States Department of Health: Centers for Disease Control and Prevention 2013). The high overall resistance to VA is another matter of concern, as VA is considered the most important reserve drug against life-threatening infections caused by MRSA (Longmore *et al.* 2010). However, VA was found not be very effective against Gram-negative bacteria due to the big molecule being unable to penetrate their outer membranes (Todar 2012). Nonetheless, the resistance of the Gram-positive bacteria that were tested against it was still high at an average of 17.4%, thus indicating the presence of vancomycin-resistant *Staphylococcus aureus.*

One-way ANOVA and Tukey post hoc tests were used to determine whether there were any statistically significant differences in the degree of sensitivity to various antibiotics by the bacteria as a whole. The results of these statistical analyses are presented in Table 19 below.

Table 19: Overview of the results of the one-way ANOVA and Tukey post hoc test analyses with regards to significant differences in the degree of sensitivity to various antibiotics by the bacteria tested

Antibiotic	Groups with statistically significant differences between them	One-way ANOVA test	Tukey post hoc test
C	Resistant and Susceptible	$F(2.440) = 5.816$, $p < 0.05$*	$p < 0.01$*
CIP	Between no groups	$F(2.440) = 0.416$, $p < 0.66$	N/A
VA	Between no groups	$F(2.440) = 1.178$, $p < 0.31$	N/A
E	Resistant and Intermediate	$F(2.440) = 14.139$, $p < 0.01$*	$p < 0.01$*
	Intermediate and Susceptible		$p < 0.01$*
AMO	Resistant and Intermediate	$F(2.440) = 15.139$, $p < 0.01$*	$p < 0.01$*
	Resistant and Susceptible		$p < 0.01$*
	Intermediate and Susceptible		$p < 0.01$*

C=Chloramphenicol, CIP=Ciprofloxacin, VA= Vancomycin, E= Erythromycin, AMO= Amoxicillin, N/A= not applicable, <= lesser than, *= statistically significant, *F*= *F*-distributions, *p*= *p*-value

In general, the majority (55.1%) of the bacterial isolates that were tested (*n*= 244/443) was susceptible to all five antibiotic classes, as indicated in Table 20. The most sensitive specie overall was *Micrococcus* spp. (*n*= 95/156), as 60.9% of the isolates was susceptible to all five antibiotics. A close second was *S. aureus* (*n*= 59/99) at a 59.6% susceptibility rate, followed by *E. coli* (*n*= 7/13) at 53.8%, CoNS (*n*= 76/156) at 48.7%, and the least sensitive being coliforms (*n*= 7/19) at 36.9%.

Almost a quarter (23.1%) of the *E. coli* isolates (*n*= 3/13) was resistant to three antibiotic classes. The other species also had isolates which fell into this category, but were at a lower frequency: 16.0% of CoNS (*n*=25/156), 10.5% of coliforms (*n*= 2/19), 8.1% of *S. aureus* (*n*= 8/99) and 6.4% of *Micrococcus* spp. (*n*=10/156). Only one (0.6%) *Micrococcus* spp. isolate was resistant to all five of the antibiotic classes that were tested (Table 20).

Shiferaw *et al.* (2013) tested the susceptibility of bacteria isolated from the diaphragms of stethoscopes to 14 antibiotics representing ten different antibiotic classes. The findings were as follows: 8.7% of the CoNS isolates and 17.7% of the *S. aureus* isolates were also resistant to eight classes, while 50.0% of the *E. coli* isolates was resistant to three antibiotic classes. The CoNS and *S. aureus* isolates were in particular resistant to C, CIP and E. Due to Shiferaw *et al.* (2013) not providing the full multidrug resistance patterns of all the bacterial isolates tested, no direct comparison could be made to the current study.

It was once again an alarming finding that only 60.9% of the micrococci isolates (*n*= 95/156) was overall susceptible to the antibiotics that were tested, due to reasons already mentioned previously (Table 20).

Table 20: Multidrug resistance patterns of five pre-specified bacteria isolated from 58 stethoscopes and sphygmomanometers used at the DUT CDC to five commonly used antibiotic discs

	No resistance	**Resistance to one antibiotic class**	**Resistance to two antibiotic classes**	**Resistance to three antibiotic classes**	**Resistance to four antibiotic classes**	**Resistance to all five antibiotic classes**
CoNS *n*=156	48.7%	24.4%	10.9%	16.0%	0.0%	0.0%
***S. aureus* *n*=99**	59.6%	20.2%	12.1%	8.1%	0.0%	0.0%
***Micrococcus* spp. *n*=156**	60.9%	21.2%	10.9%	6.4%	0.0%	0,6%
Coliforms *n*=19	36.9%	26.3%	26.3%	10.5%	0.0%	0.0%
***E.coli* *n*=13**	53.8%	15.4%	7.7%	23.1%	0.0%	0.0%
Grand total *n*=443	55.1%	22.1%	11.8%	10.8%	0.0%	0.2%

CoNs= coagulase-negative staphylococci, %= percentage, *n*= number of isolates tested

4.2.5 Objective 5

This objective was to determine if CSs' hygienic practices correlated with the data obtained from the bacterial contamination of equipment that was tested in this study.

When looking at the mean CFU counts for each Likert-scale answer to Question 10 in Table 21 pertaining to the stethoscope, no gradual increase is seen. Conversely with regards to the sphygmomanometers, a steady rise in the mean CFU counts is observed as shown in Table 22. However, upon closer evaluation of the standard deviation and standard error for each answer to this question, it becomes clear that these values are high, thus indicating that the data are distributed over a wide range of values from the mean.

A one-way ANOVA test was unable to determine a statistically significant difference of the mean CFU counts when compared against the answers supplied to Question 10 (S- $F(5,23)= 2.203$, $p= 0.09$ and B- $F(5,23)= 0.406$, $p= 0.84$).

Table 21: Correlation between respondents' answers to Question 10 regarding average stethoscope disinfection practice and the mean CFU counts isolated from their stethoscopes before their consultation with two separate new patients

	Likert-scale options for Question 10 (stethoscopes) n= 29					
	1 After every patient	**2 Daily**	**3 Weekly**	**4 Monthly**	**5 Yearly**	**6 Never**
	N=4	*N*=3	*N*= 8	*N*= 6	*N*= 2	*N*= 6
Mean CFU counts	32.3	17.2	16.2	17.8	74.0	18.3
Standard deviation	35.28	3.62	16.94	18.69	64.35	17.54
Standard error	17.64	2.09	5.99	7.63	45.50	7.16

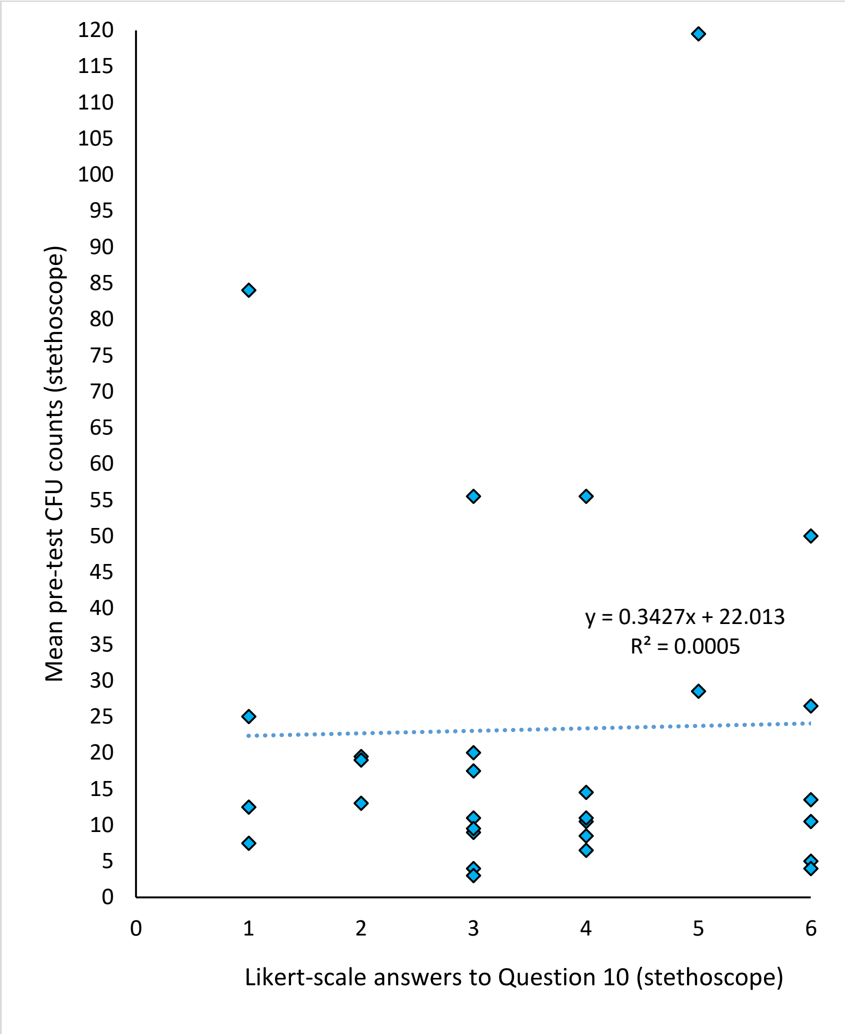
n= total sample size, *N*= number of respondents selecting that particular answer, CFU= colony-forming unit

Table 22: Correlation between respondents' answers to Question 10 regarding average sphygmomanometer disinfection practice and the mean CFU counts isolated from their sphygmomanometers before their consultation with two separate new patients

	Likert-scale options for Question 10 (sphygmomanometers) n= 29					
	1 After every patient	**2 Daily**	**3 Weekly**	**4 Monthly**	**5 Yearly**	**6 Never**
	N=4	*N*=3	*N*= 6	*N*= 6	*N*= 2	*N*= 8
Mean CFU count	15.8	18.8	22.9	32.0	30.5	53.6
Standard deviation	10.22	19.37	15.77	15.63	4.95	94.39
Standard error	5.11	11.18	6.44	6.38	3.50	33.37

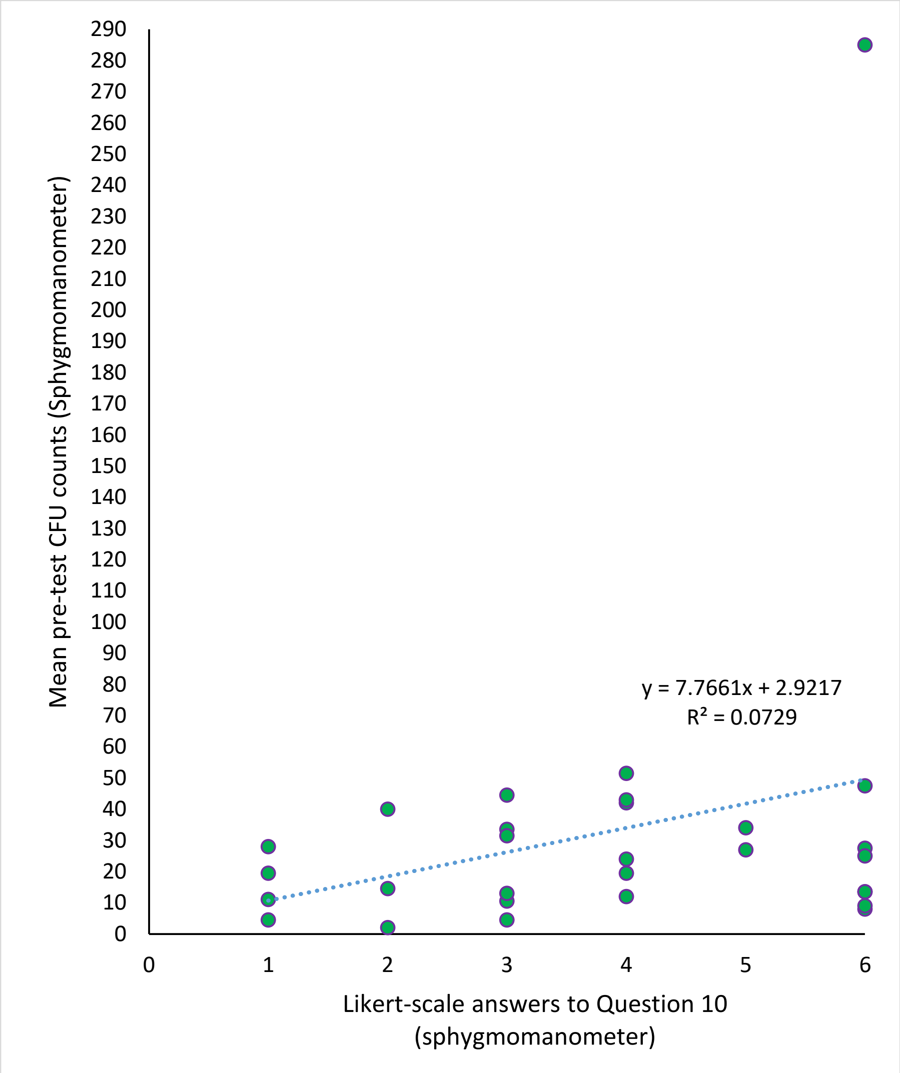
n= total sample size, *N*= number of respondents selecting that particular answer, CFU= colony-forming unit

When scatter diagrams were plotted, the distribution of the values over a wide range could easily be seen (see Figure 31- stethoscope and Figure 32- sphygmomanometer). In Figure 31 and Figure 32 the R^2 values were 0.0005 and 0.0729 respectively, thus indicating a very weak or poor correlation between the two variables.



R^2= coefficient of determination, CFU= colony-forming unit, Likert-scale answers= 1: After every patient; 2: Daily; 3: Weekly; 4: Monthly; 5: Yearly; and 6: Never

Figure 31: Respondents' answers to Question 10 regarding average stethoscope disinfection practice versus the mean CFU counts isolated from their stethoscopes before their consultation with two separate new patients.



R^2= coefficient of determination, CFU= colony-forming unit, Likert-scale answers= 1: After every patient; 2: Daily; 3: Weekly; 4: Monthly; 5: Yearly; and 6: Never

Figure 32: Respondents' answers to Question 10 regarding average sphygmomanometer disinfection practice versus the mean CFU counts isolated from their sphygmomanometers before their consultation with two separate new patients.

Similar the responses to Question 10 above, the mean CFU counts for each Likert-scale answer to Question 11 were tabulated for stethoscope and sphygmomanometer separately (see Table 23 and Table 24). The analyses that are presented in these two tables reveal similar findings to those pertaining to Question 10. This means that the mean CFU counts increased gradually but, once again, the standard deviation and standard error for each answer were high, indicating a wide distribution of the data.

The one-way ANOVA analysis did not indicate a statistically significant difference in the mean CFU counts when compared against the answers supplied to Question 11 (S- $F(4,24)= 0.316$, $p= 0.87$ and B- $F(4,24)= 0.626$, $p= 0.65$).

Table 23: Correlation between respondents' answers to Question 11 regarding last occurrence of stethoscope disinfection and the mean CFU counts isolated from their stethoscopes before their consultation with two separate new patients.

	Likert-scale options for Question 11 (stethoscope) n= 29				
	1 Today	**2 Within the last week**	**3 Within the last month**	**4 Within the last year**	**5 Never**
	N=2	*N*=11	N= 8	*N*= 1	*N*= 7
Mean CFUs	18.8	18.3	22.3	28.5	32.7
Standard deviation	8.84	22.57	20.81	-	41.48
Standard error	6.25	6.81	7.36	-	15.68

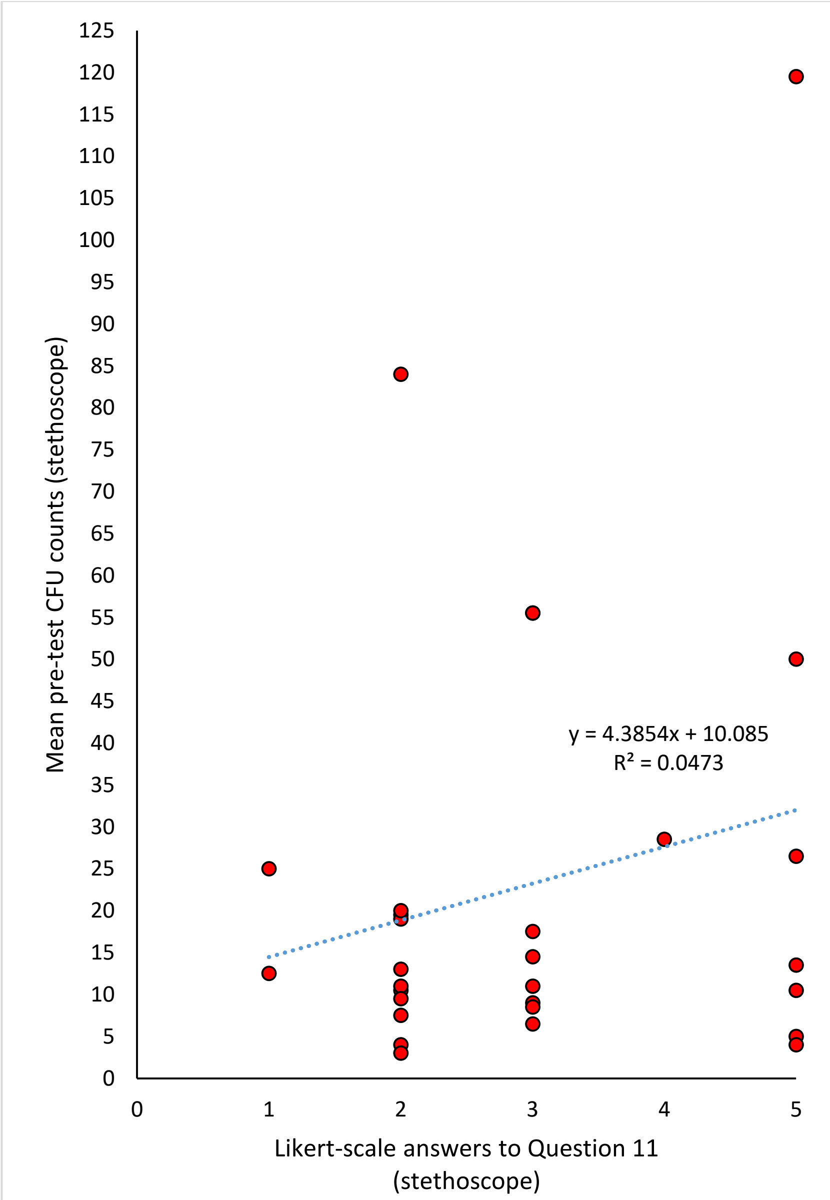
n= total sample size, *N*= number of respondents selecting that particular answer, CFUs= colony-forming units

Table 24: Correlation between respondents' answers to Question 11 regarding last occurrence of sphygmomanometer disinfection and the mean CFU counts isolated from their sphygmomanometers before their consultation with two separate new patients

	Likert-scale options for Question 11 (Sphygmomanometer) n= 29				
	1 Today	**2 Within the last week**	**3 Within the last month**	**4 Within the last year**	**5 Never**
	N=2	*N*=8	N= 8	*N*= 3	*N*= 8
Mean CFUs	12.0	15.3	32.8	33.7	53.6
Standard deviation	10.61	9.70	15.73	6.51	94.39
Standard error	7.50	3.43	5.56	3.76	33.37

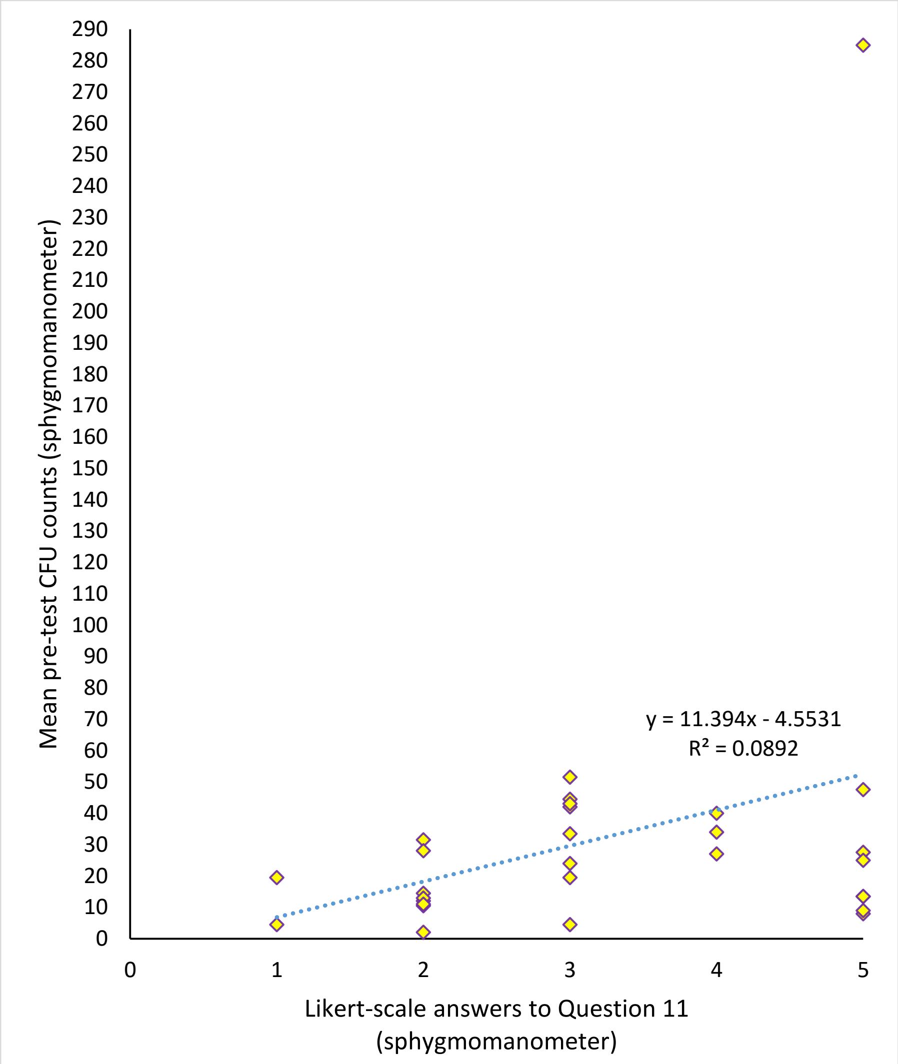
n= total sample size, *N*= number of respondents selecting that particular answer, CFUs= colony-forming units

The findings that are presented in Figure 33 (stethoscope group) and Figure 34 (sphygmomanometer group) on the next page, show that the data values pertaining to the corresponding tables were consistently distributed over a wide range. The R^2 values for Figure 33 and Figure 34 were 0.0473 and 0.0892 respectively, thus once again indicating very weak or poor correlations between the two variables.



CFU=Colony-forming unit, Likert-scale answers: 1: Today; 2: Within the last week; 3: Within the last month; 4: Within the last year; and 5: Never

Figure 33: Respondents' answers to Question 11 regarding last occurrence of stethoscope disinfection versus the mean CFU counts isolated from their stethoscopes before their consultation with two separate new patients.



CFU- Colony-forming unit, Likert-scale answers: 1: Today; 2: Within the last week; 3: Within the last month; 4: Within the last year; and 5:- Never

Figure 34: Respondents' answers to Question 11 regarding last occurrence of sphygmomanometer disinfection versus the mean CFU counts isolated from their sphygmomanometers before their consultation with two separate new patients.

Overall, no correlations were identified between the mean CFU counts isolated from the respondents' equipment and their reported average or last occurrence of equipment disinfection.

4.3 Synopsis of key findings

The overall findings suggested that the majority of the respondents was knowledgeable regarding the procedures of stethoscope and sphygmomanometer disinfection (see section 4.2.1.2), even though the majority reported not having received adequate education regarding both stethoscope (79.3%) and sphygmomanometer (86.2%) disinfection during their chiropractic training (Figure 4).

A minority (13.8%) of the respondents practised adequate stethoscope disinfection (Figure 11) as recommended by Shiferaw *et al.* (2013), Saloojee and Steenhoff (2001), Jain *et al.* (2013), Campos-Murguia *et al.* (2014) and Gupta *et al.* (2014). Conversely, 75.9% of the respondents practised adequate hand hygiene (Figure 13) as recommended by Evans *et al.* (2009g). This could possibly be linked to the fact that all of the respondents agreed that they had received adequate training pertaining to hand hygiene (Figure 5). This highlights the need for incorporating information regarding adequate equipment disinfection practices into the existing chiropractic curriculum. Other than inadequate education/training pertaining to hand and equipment hygiene, other obstacles hindering adequate equipment disinfection practices included forgetfulness, lack of time, and inadequate disinfectant availability (Figure 15).

The majority of the pre- and post-test stethoscope and sphygmomanometer samples (Figure 17) was found to be contaminated with bacteria at the time of sampling. However, these rates decreased to a large extent (Figure 18) when the presence of $\geq$ 20 CFUs per sample was considered to constitute significant bacterial contamination (Shiferaw *et al.* 2013; Pal *et al.* 2015; Jeyakumari *et al.* 2017). Nevertheless, both findings point to the fact that the new chiropractic patients who attended the DUT CDC for consultations were unnecessarily exposed to bacteria, because the CSs used contaminated equipment during the physical examinations they conducted on these patients. This reinforces the suggestion that education pertaining to adequate disinfection practices be incorporated in the chiropractic curriculum at all levels to improve disinfection practices of CSs. This will minimise bacterial cross-contamination between patients and examiners and will lower the risk of HCAIs being contracted.

The total CFU counts for the post-stethoscope (2984 CFUs) and post-sphygmomanometer (2631 CFUs) sample groups (table 13) were higher when compared to their respective pre-test samples. The higher total CFU counts for both the post-test stethoscope and post-test sphygmomanometer samples could possibly have occurred as a result of bacterial transfer from the patients' skins onto the surfaces of both pieces of equipment. The higher total CFU counts for the post-test stethoscope group in comparison with the post-test sphygmomanometer group could be related to the higher rate of exposure of stethoscopes' surfaces to the patients' skin during the physical examinations. A greater distribution of the total CFUs for both the pre- and post-test stethoscope samples was observed at the diaphragms' edges in comparison to the rest of the diaphragms' surfaces (Table 14).

The vast majority of bacteria isolated from the pre- and post-test samples from both pieces of equipment comprised of CoNS, *Micrococcus* spp. and *S. aureus*, while the minority consisted of *Bacillus* spp., *Corynebacterium* spp., coliforms and *E. coli* (Table 15 and Figure 27). When these bacteria were divided into potentially pathogenic and non-pathogenic bacterial groups, it was determined that the majority of the bacteria isolated from the various samples was potentially pathogenic, except in the case of the post-sphygmomanometer sample group (Table 16 and Figure 28).

The results of the efficacy testing of water, 70% isopropyl alcohol, 70% ethanol and hand sanitizer (62% ethanol) indicated that all four disinfectants failed to kill even one of the 443 bacterial isolates used during testing (Table 17). This was in direct contrast to the published literature (Davis 2009; Lecat *et al.* 2009; Paul *et al.* 2011; Gupta *et al.* 2014; Jeyakumari *et al.* 2017), and thus suggests that this particular finding of this study was anomalous. The anomaly was possibly due to a flawed methodology, as was discussed in section 4.2.3.

The most effective antibiotic in relation to inhibiting all species isolated was chloramphenicol with an overall bacterial susceptibility rate of 95.7%, followed by ciprofloxacin (93.2%), vancomycin (80.8%), amoxicillin (69.5%) and erythromycin (57.7%) (Table 18). The finding pertaining to the resistance of *Micrococcus* spp. to the antibiotics that were tested was of concern, as this resistance could theoretically be passed on via horizontal gene transfer or conjugation to various surrounding pathogenic bacteria and potentially result in the contraction of HCAIs (United States Department of Health: Centers for Disease Control and Prevention 2013; World Health Organization 2014; Munita and Arias 2016). The high resistance levels of CoNS and *S. aureus* to AMO suggests the presence of MRSA and methicillin-resistant coagulase negative staphylococci isolates (Table 18). Overall, the majority (55.1%) of the bacterial isolates (*n*= 244/443) was susceptible to all the antibiotic classes that were tested (table 20). The order from overall most sensitive to least sensitive specie was as follows: *Micrococcus* spp. 60.9% (*n*= 95/156), *S. aureus* 59.6% (*n*= 59/99), *E. coli* 53.8% (*n*= 7/13), CoNS 48.7% (*n*= 76/156) and coliforms 36.9% (*n*= 7/19).

No correlations were identified between the mean CFU count isolates from the respondents' equipment and their reported average or last occurrence of equipment disinfection (Tables 21-24 and Figures 31- 34).

CHAPTER 5: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

5.1 Conclusions

In order to draw significant conclusions, the results will be considered in relation to the aims and objectives of the study. Objective 4 was presented first in order to provide an understanding of the context of this study, and thus to better contextualise and explain the results that were obtained in relation to objectives 1 to 3. The following are the main conclusions:

- Objective 4 was to evaluate the hygiene practices of 29 CSs regarding the disinfection of their stethoscopes and sphygmomanometers. A Likert-scale structured questionnaire was used to elicit their responses.
    - The respondents were knowledgeable in terms of adequate stethoscope and sphygmomanometer disinfection procedures and they demonstrated a positive attitude towards these procedures.
    - While the respondents were of the view that adequate education pertaining to stethoscope and sphygmomanometer disinfection was lacking in their chiropractic course, the contrary was true with regards to hand hygiene.
    - The respondents generally failed to observe their fellow students disinfecting their stethoscopes and sphygmomanometers on a regular basis.
    - The respondents failed to adhere to adequate stethoscope and sphygmomanometer disinfection practices; however, their practices were satisfactory regarding hand hygiene.
    - The two most common disinfectants used by the respondents for equipment disinfecting purposes were alcohol based wipes, and surgical alcohol and cotton wool.
    - Forgetfulness, lack of time, insufficient education regarding the importance and application of equipment disinfection, the belief that disinfection is unimportant, and inadequate disinfectant availability hindered adequate equipment disinfection by the respondents.

- Objective 1 was to determine the presence, quantities and identification of bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC, before and after 58 new patient consultations using conventional bacterial plate count techniques and identification tests.

    - Bacterial contamination was present on both stethoscopes and sphygmomanometers before and after the physical examination of new patients at the DUT CDC. This means that these new patients were unnecessarily exposed to health-threatening bacteria and confirms that both pieces of equipment acted as reservoirs for bacterial growth.
    - Both stethoscopes and sphygmomanometers served as sources of bacterial transfer between the surfaces of the equipment and the patients' skin.
    - The quantity of bacterial transfer from the patients' skin to the equipment seemed proportional the number of exposures of the surface of the equipment to the skin.
    - The edges of the stethoscopes had higher levels of bacterial contamination in comparison to the rest of the diaphragms' surface.
    - The bacteria that were isolated from both pieces of equipment were *Bacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., coliforms, CoNS, *S. aureus*, and *E. coli.* The latter four species are considered potentially pathogenic as a result of their involvement in the development of HCAIs.

- Objective 2 was to assess the efficacy of the disinfectants that are generally used by CSs in the DUT CDC for cleaning stethoscopes and sphygmomanometers using a modified AOAC use dilution method.

  As the validity and reliability of the modified AOAC use dilution method methodology had been brought into question, it was decided not to draw any conclusions from the findings associated with this objective.

- Objective 3 was to determine the presence and quantities of antibiotic-resistant bacteria on stethoscopes and sphygmomanometers used by CSs in the DUT CDC, using therapeutic antibiotics with the disk diffusion assay to determine bacterial susceptibility to antibiotics.

    - Antibiotic-resistant bacteria, including MRSA and methicillin-resistant coagulase negative staphylococci, were detected on both pieces of equipment.
    - The most effective antibiotic tested was chloramphenicol (95.7%), while the least effective was erythromycin (57.7%).

- The findings pertaining to antibiotic-resistant *Micrococcus* spp. isolates engendered the notion that, even though the *Micrococcus* spp. is mainly considered non-pathogenic, it could contribute towards the spread of antibiotic resistance between different species.
- Bacteria that were resistant to multiple antibiotics were isolated from both the stethoscopes and sphygmomanometers.

- Objective 5 was to determine if CSs' hygiene practices correlated with the data that were obtained from the bacterial contamination testing of equipment that was conducted in this study.
  - No correlations were identified between the bacterial contamination of the CSs' stethoscopes and sphygmomanometers and their corresponding reported average or last occurrence of equipment disinfection.

5.2 Limitations of the research study

- Due to financial constraints the following limitations were experienced:
  - API® kits or the Biolog microstation could not be used during the identification process.
  - The antibiotic-sensitivity testing was done in duplicate and not in triplicate as is recommended.
  - The AOAC use dilution method had to be modified and the study was limited to bacterial cultures.
  - The diaphragm of the stethoscopes and the designated area of the cuff of the sphygmomanometers could not be assessed for the presence of fungi, protozoa and viruses.

- Other limitations pertained to the following:
  - It cannot be assumed that all the CSs interpreted the questions in the questionnaire in a similar manner, even though the questionnaire had been scrutinized and modified by a focus group and pilot study in order to limit any such misinterpretations or misunderstandings (Weber and He 2010).
  - Although the CSs had been requested to be truthful during the completion of the research questionnaire, there was no manner in which this could be confirmed.

- On the day of data collection, the CSs were encouraged to follow the same procedures and protocols as they would with any new patient; however, the Hawthorne effect should not be underestimated (McCambridge, Witton and Elbourne 2014; Hagel *et al.* 2015).
- The researcher had limited prior experience in the identification of bacteria and thus errors could potentially have been made and included into the results. To prevent this from occurring, the identification process consisted of multiple steps (i.e., macroscopic and light microscopic morphology, outcomes of the oxidase, catalase and coagulation tests as well as colony morphology on the MAC, MSA, EMB and Baird- Parker plates).
- This research project was conducted by the researcher in compliance with the requirements for obtaining a Master's Degree in Technology: Chiropractic. This could possibly have resulted in limitations to the study in terms of both budget and time constraints.

5.3 Recommendations

5.3.1 Recommendations to improve the study

- A larger sample size should be used in order to avoid type II errors and to allow the study population to be more accurately represented.
- To increase reliability with regards to the identification of the bacteria, API® kits, the BioLog or BioMerieux VITEK® 2 systems should be used.
- To increase the validity and reliability of the results of the antibiotic-sensitivity testing, triplicates should be made of each bacterium that is tested instead of duplicates.
- The implementation of a pre- and post-test design as proposed by Gupta *et al.* (2014) and Lecat *et al.* (2009) when assessing the efficacy of disinfectants is endorsed. The process regarding the implementation of such a method is fully explained in section 4.2.3.1.
- Regarding the questionnaire, a 'before and after every patient' option must be added to the options available to the respondents for Question 9: "How often do you think should your equipment be disinfected?" as well as for Question 10: "On average, how often do you disinfect your equipment?"

5.3.2 Recommendations for future research

- This research project was limited to CSs who consulted with patients at the DUT CDC. The study could be replicated using CSs at the University of Johannesburg's CDC locally, or at any other university internationally that offers chiropractic as a programme, for comparison with the findings of this study.
- This study was conducted within the quantitative paradigm. The questionnaire aspect could be done in the qualitative paradigm using a semi-structured questionnaire with open-ended questions or by conducting interviews, which could provide a deeper understanding of the hygiene practices of the CSs, as well as the reasons for these practices.
- A similar study could be conducted involving fourth year CSs who attend a hospital on a weekly basis where they perform physical examinations on the patients. These results could then be compared to the findings of this study to establish whether there were any differences in the type and quantity of bacteria identified between the hospital and the chiropractic clinic setting.
- A future study could be performed to determine the effect of an educational intervention on CSs' knowledge, attitudes and practices regarding equipment disinfection and on the bacterial contamination levels of these pieces of equipment. Samples and questionnaires should be taken and distributed before and after the intervention period.
- Further research is required to determine the presence and degree of bacterial contamination on the therapeutic equipment used at the DUT CDC. Therapeutic equipment includes Interferential Current, Transcutaneous Electrical Nerve Stimulation, ultrasound, and Electric Muscle Stimulator machines. If these electro-modalities are not adequately disinfected, bacteria could survive on the surfaces for a prolonged period of time as has been established in the case of other inanimate objects (Kramer, Schwebke and Kampf 2006).
- Future studies could determine the bacterial build-up on the diagnostic equipment over a set period while also tracking the occurrence of equipment disinfection over the same period.
- A study should be performed on the efficacy of the disinfectants used by CSs at various contact time periods, e.g., at 5 s, 10 s, 20 s, 30 s, 45 s, 60 s, etc. This would determine the optimal contact time needed for adequate equipment disinfection.
- Future studies could focus on the different materials used for the manufacturing of sphygmomanometer cuffs. These studies should determine whether these fabrics contribute towards the quantity of bacteria found on them and to what extent they influence the efficacy of the disinfectants used to disinfect them.
- A study with an observational component could be performed to determine whether there is a correlation between the quantity of bacteria that are isolated on the equipment and the number of exposures of the surfaces of the equipment to patients' skin.

5.3.3 Recommendations for the DUT Chiropractic department, DUT CDC and the CSs

- As part of the Chiropractic curriculum, CSs should be provided with adequate education pertaining to equipment disinfection, with specific emphasis on the importance and correct techniques for this procedure.
- CSs who have already completed their academic requirements for their Master's Degree and who thus no longer attend any scheduled lecture sessions, should be compelled to attend a workshop where information regarding adequate equipment disinfection is conveyed to them.
- CSs who recently graduated prior to the completion of this study should receive information regarding the importance of and procedures for equipment disinfection via e-mail.
- The placement of visual reminders and easily accessible disinfectants at strategic locations throughout the DUT CDC will assist in improving the practice of adequate equipment disinfection.
- The recommended protocols for chiropractic table and equipment disinfection as well as hand hygiene practices must be included in the chiropractic clinic manual.
- To prevent any bacterial cross-contamination between patients and the CSs themselves, the CSs should disinfect their diagnostic equipment before and after every patient consultation. This precaution will help minimise the risk of the development of HCAIs in the patients as well as in the CSs.
- The CSs should also disinfect their diagnostic kit bags at least weekly to prevent bacterial cross-contamination between the diagnostic equipment and the kit bag.

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APPENDIXES

Appendix A: Permission obtained from Clinic Director

MEMORANDUM

To : Prof Puckree
Chair : RHDC

Prof Adam
Chair : IREC

From : Dr Charmaine Korporaal
Clinic Director : FoHS Clinic

Date : 15.09.2016

Re : Request for permission to use the Chiropractic Day Clinic for research purposes

---

Permission is hereby granted to :

Ms Johmari Logtenberg (Student Number: 20818916)

Research title : "Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants."

It is requested that Ms Logtenberg a copy of her RHDC / IREC approved proposal to the Clinic Administrators, after her registration as a masters student, before she starts with her research in order that any special procedures with regards to her research can be implemented prior to the commencement of data capture.

Should her research approval require piloting of her procedures prior to any approvals being granted, this will be considered at that time and special arrangements made in line with the request from RHDC / IREC.

Thank you for your time.

Kind regards

[redacted]

Dr Charmaine Korporaal
Clinic Director : FoHS Clinic

Cc: Mrs Pat van den Berg : Chiropractic Day Clinic
Dr L O'Connor : Research co-ordinator
Dr F Swalaha : Research Supervisor
Dr C Kell : Research Co-supervisor

Appendix B: Letter to the Head of Biotechnology Department

DUT DURBAN UNIVERSITY OF TECHNOLOGY

FACULTY OF HEALTH SCIENCES

Department of Chiropractic and Somatology: Chiropractic Clinic
Faculty of Health Sciences
Ritson Campus
Durban University of Technology

11 Ritson Road, Berea, Durban 4001

P O Box 1334, Durban, 4000, South Africa

Tel: (031)373 2205

www.dut.ac.za

Professor Kugen Permaul
Head of the Biotechnology Department
Faculty of Applied Sciences

9 September 2016

Dear Prof Perumal,

My name is Johmari Logtenberg, I am a fifth year Chiropractic student at the Durban University of Technology. In order to complete my Master of Technology in Chiropractic, I must complete a dissertation.

Diagnostic equipment is often contaminated with microorganisms and can result in health care-associated infections (HCAIs), if proper protocol regarding disinfection is not followed. I will be conducting a quantitative investigation to determine if the stethoscopes and sphygmomanometers used by the chiropractic students in the Chiropractic Day Clinic, serve as reservoirs for microbial growth (including antibiotic-resistant microorganisms) and subsequently act as a source for microbial transfer.

The objectives of the study are:

- To determine the presence and quantities of microorganisms (including antibiotic- resistant microorganisms) on the students' stethoscopes and sphygmomanometers after each new patient consultation.
- To assess the efficacy of disinfectants used by the students to clean their stethoscopes and sphygmomanometers.
- To evaluate the student's hygienic practices regarding the cleaning of their stethoscopes and sphygmomanometers.
- To compare the microbiological findings with the data generated by the questionnaires.

In order to achieve this, I ask permission to use your Microbiology Laboratory situated on the Steve Biko Campus for the incubation and analysis of the samples collected from the students' stethoscopes and sphygmomanometers.

Your assistance with the above request will be greatly appreciated.

Yours sincerely,

[redacted] ______ 26/9/2016 ______
Ms. J. Logtenberg — Date

[redacted] ______ 26/9/2016 ______
Date

[redacted] ______ 26/09/2016 ______
Co- Supervisor: Dr C. M. Kell — Date

[redacted] ______ 24/10/2016 ______
Approved by: Prof Kugen Permual — Date
Head of Biotechnology Department

Appendix C: Chiropractic student - Letter of information and consent form

DUT DURBAN UNIVERSITY OF TECHNOLOGY

FACULTY OF HEALTH SCIENCES

DEPARTMENT OF CHIROPRACTIC AND SOMATOLOGY

Department of Chiropractic and Somatology: Chiropractic Clinic
Faculty of Health Sciences
Ritson Campus
Durban University of Technology

11 Ritson Road, Berea, Durban 4001

P O Box 1334, Durban, 4000, South Africa

Tel: (031)373 2205

www.dut.ac.za

Letter of information for chiropractic student

Dear Chiropractic student

Thank you for taking time to consider your participation in my study.

Study title:
Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants.

Introduction to the study:
Heath care-associated infections (HCAIs) are infections patients' contract whilst in a hospital or any healthcare facility, such as the Chiropractic Day Clinic, for reasons other than that for which they are receiving care. HCAIs are commonly caused by micro-organisms that can spread due to poor hand hygiene or usage of contaminated diagnostic equipment. The purpose of this study is to determine if diagnostic equipment used by the chiropractic students in the Chiropractic Day Clinic, serve as reservoirs for microbial growth, including antibiotic-resistant microorganisms and subsequently act as a source of microbial transfer.

Outline of the procedures:
You are requested not to alter any of your clinical procedure irrespective of the research procedure outlined below.

This is how the study will affect you. The study consists of two phases:

Phase one - The study will only be conducted on chiropractic students that consult with new patients. The researcher will provide your new patient with a letter of information and consent asking for their participation in the research study. This documentation will be completed together with the relevant patient information documentation supplied by the receptionist.

If both the patient and you give consent, the researcher will collect your personal diagnostic kit bag containing all your diagnostic equipment. This will be in order to collect the pre-test samples. When you leave the room to inform the clinician about your new patient, you will be given your bag back. After you have performed the vitals and full physical examination on the patient, you will be asked to bring your bag to the researcher when you leave your room to report your findings to the clinician. The researcher will then collect the post-test samples. The researcher will then return the bag to you. The sample collection will take 5 minutes each.

Phase two – After all the samples for all the required new patients have been collected with regards to the pre-tests and post-tests sampling of the diagnostic equipment, you will be given a questionnaire to complete with reference to this study. The questionnaire will take 5 minutes to fill out.

Risk or discomforts to the chiropractic student:
There is no risk involved in participating in this research.

Benefits:
The results of this research will be used to improve education and protocols regarding disinfection, which could be beneficial to future patients and chiropractic students.

Reason/s why the participant may be withdrawn from the study:
If it is noted by the researcher that should a participant purposely try to skew or manipulate the samples collected from their equipment, said participant and any associated data will be excluded from the study.

Confidentiality:
All data collected will be kept confidential. When the data is analysed and recorded, it will be coded so that no names are associated with the data.

Persons to contact in the event of any problems or queries:
Please contact the researcher (0824061182), my supervisor: Dr Swalaha (0313732689), my co-supervisor: Dr Kell (0313732393) or the Institutional Research Ethics administrator on 031 373 2900. Complaints can be reported to the DVC: Prof S. Moyo on 031 373 2576 or moyos@dut.ac.za

Thank you for reading this information. I hope you will decide to participate in my study.

Yours sincerely,

[redacted]

Johmari Logtenberg
M. Tech Chiropractic
Researcher

[redacted]

Dr. F.M. Swalaha
D. Tech. Biotechnology
Supervisor

[redacted]

Dr. C.M. Kell
M. Tech. Homeopathy
Co- supervisor

Chiropractic student consent

Statement of agreement to participate in the research study:

- I hereby confirm that I have been informed by the researcher, Johmari Logtenberg, about the nature, conduct, benefits and risks of this study – Research Ethics Clearance Number: REC 54/ 16
- I have also received, read and understood the above written information (Letter of information for chiropractic student) regarding the study.
- I am aware that the results of this study will be confidentially 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 Chiropractic Student **Date** **Time** **Signature**

I, , herewith confirm that the above chiropractic student has been fully informed about the nature, conduct and risks of the above study.

______________________ ______ _______ ______________

Full name of Researcher **Date** **Time** **Signature**

Appendix D: Chiropractic patient - Letter of information and consent form

DUT
DURBAN UNIVERSITY OF TECHNOLOGY

FACULTY OF HEALTH SCIENCES

DEPARTMENT OF CHIROPRACTIC AND SOMATOLOGY

Department of Chiropractic and Somatology: Chiropractic Clinic
Faculty of Health Sciences
Ritson Campus
Durban University of Technology

11 Ritson Road, Berea, Durban 4001

P O Box 1334, Durban, 4000, South Africa

Tel: (031)373 2205

www.dut.ac.za

Letter of information for chiropractic patient

Dear Chiropractic patient

Thank you for taking the time to consider your participation in my research study.

Study title:
Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants.

Introduction to the study:
Microbial flora is located on the surface of the skin covering the human body. This microbial flora consists of a range of commensal (not harmful to a person), mutualistic (beneficial to a person) and pathogenic (can cause disease and is therefore harmful to a person) bacteria. The majority of these bacteria contribute to human health, but pathogenic bacteria can cause diseases in especially immune-compromised people. Microbial flora can be transferred from the skin to any surface making contact with it, such as diagnostic equipment. The purpose of this study is to determine if diagnostic equipment used by the chiropractic students in the Chiropractic Day Clinic, serve as reservoirs for microbial growth and subsequently act as a source of microbial transfer.

Outline of the procedures:
Consent must be obtained from both the patient and the chiropractic student as microbial flora will be collected indirectly, by collecting microbial samples before and after the use of the chiropractic student's diagnostic equipment. This study will have a minimal influence on the total duration of the consultation, as samples are obtained at times when the diagnostic equipment is not needed by the chiropractic students. This will therefore mean that the consultation will not be any longer than would usually be the case. Sampling of the equipment will be done on two occasions and should not take more than 5 minutes each.

Risk or discomforts to the chiropractic patient:
There is no risk involved in participating in this research.

Benefits:
The results of the research will be used to improve education and disinfection protocols, which could be beneficial to future patients and chiropractic students.

Reason/s why the participant may be withdrawn from the study:
If it is noted by the researcher that you or the chiropractic student purposely tried to skew or manipulate the samples collected from their equipment, then you and any data from your participation in the study will be excluded.

Confidentiality:
All data collected from the chiropractic student will be kept confidential. When the data is analysed and recorded, it will be coded so that no name is associated with the data.

Persons to contact in the event of any problems or queries:
Please contact the researcher (0824061182), my supervisor: Dr Swalaha (0313732689), my co-supervisor: Dr Kell (0313732393) or the Institutional Research Ethics administrator on 031 373 2900. Complaints can be reported to the DVC: Prof S. Moyo on 031 373 2576 or moyos@dut.ac.za.

Thank you for reading this information. I hope you will decide to participate in my study.
Yours sincerely,

[redacted]

Johmari Logtenberg
M.Tech Chiropractic
Researcher

[redacted]

Dr. F.M. Swalaha
D. Tech. Biotechnology
Supervisor

[redacted]

Dr. C. M. Kell
M. Tech. Homeopathy
Co- supervisor

Chiropractic patient consent

Statement of agreement to participate in the research study:

- I hereby confirm that I have been informed by the researcher, Johmari Logtenberg, about the nature, conduct, benefits and risks of this study – Research Ethics Clearance Number: REC 54/ 16
- I have also received, read and understood the above written information (Letter of information for chiropractic patient) regarding the study.
- I am aware that the results of this study will be confidentially 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 Chiropractic patient	**Date**	**Time**	**Signature**

I, , 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**	**Time**	**Signature**

Appendix E: Disinfection Questionnaire (Pre-focus group)

Disinfection Questionnaire (Pre-focus group)

This is a questionnaire about your current thoughts and practices regarding the disinfection of your stethoscope and sphygmomanometers. Please place a cross over a single answer that best described your attitude or actions regarding disinfection at the Durban University of Technology Chiropractic Day Clinic. Your participation and answers are confidential, so please be as honest as possible. Please do not write any personal identifying information on this form.

IMPORTANT NOTICE – Disinfection of stethoscope refers to the diaphragm of the stethoscope. Disinfection of sphygmomanometers refers to the cuff part that is in direct contact with the patient's skin.

Are you	Male	Female

What year are you in	Fifth	Sixth	Seventh	Eight

Do you think that the following equipment can be possible sources of cross-contamination of pathogenic agents?

Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Do you think it is important to disinfect the surfaces of these equipment?

Stethoscope	Very important	Important	Neutral	Unimportant	Very unimportant
Sphygmomanometers	Very important	Important	Neutral	Unimportant	Very unimportant

On average, how often do you disinfect your equipment?

Stethoscope	After every patient	Daily	Twice daily	Weekly	Monthly	Yearly	Never
Sphygmomanometers	After every patient	Daily	Twice daily	Weekly	Monthly	Yearly	Never

When was the last time you disinfected your equipment?

Stethoscope	Today	Within the last week	Within the last month	Within the last year	Never	Other
Sphygmomanometers	Today	Within the last week	Within the last month	Within the last year	Never	Other

If other – please specify ……………………………………………………………………

What do you use to disinfect your equipment?

Stethoscope	Nothing	Water only	Soapy water	Alcohol based wipes	Surgical alcohol and cotton	Hand sanitizer	Other
Sphygmomanometers	Nothing	Water only	Soapy water	Alcohol based wipes	Surgical alcohol and cotton	Hand sanitizer	Other

If other – please specify ……………………………………………………………………

On average, do you have enough time to disinfect your equipment?

Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Are you confident in knowing how to properly disinfect your equipment?

Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Do you regularly see other students disinfect their equipment?

Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Do you think it is important for students to disinfect their equipment?					
Stethoscope	Very important	Important	Neutral	Unimportant	Very unimportant
Sphygmomanometers	Very important	Important	Neutral	Unimportant	Very unimportant

How often do you think should your equipment be disinfected?							
Stethoscope	After every patient	Daily	Twice daily	Weekly	Monthly	Yearly	Never
Sphygmomanometers	After every patient	Daily	Twice daily	Weekly	Monthly	Yearly	Never

If you don't disinfect your equipment, what is the reason?						
Stethoscope	Unimportant	No disinfectant equipment available	Lack of time	Forgetfulness	Uneducated about importance	Other
Sphygmomanometers	Unimportant	No disinfectant equipment available	Lack of time	Forgetfulness	Uneducated about importance	Other
If other – please specify ……………………………………………………………………						

Have you ever received any education regarding disinfection of your equipment during your chiropractic course?					
Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree

On average, how often do you disinfect your hands after seeing a patient?				
After each patient	After every second patient	Once daily	Twice daily	More than twice daily

Have you ever received any education on good hand hygiene practice during your chiropractic course?				
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Do you think education regarding hand hygiene and equipment disinfection is important?				
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

Thank you very much for taking the time to complete this questionnaire.

Adapted from (Evans Jr *et al.* 2009; Puhl *et al.* 2011; Saunders, Hryhorskyj and Skinner 2013)

Appendix F: Consent from authors to use their questionnaires

- **Email from Dr A. Puhl.**

Hi Johmari,
Thanks for your email and thank you for your interest in our bacterial load/disinfection study. I would be happy for you to use our survey Instrument as part of your analysis of student hygiene habits. Let me know if you have any questions or concerns regarding such a study that I can help you with; otherwise, best of luck with your research!
Best regards,
Aaron

Dr. Aaron Puhl, BSc, MSc, DC
Chiropractor, Able Body Health Clinic
1212-3 avenue South
Lethbridge, AB
T1J 0J9

- **Email from Dr C. Saunders**

Dear Johmari,

Thank you for your email; I am glad that you found our article of interest!

I am very happy for you to use the questionnaire. We asked the students in our study to complete the questionnaire online using a Google Forms questionnaire. This was very easy to set up, collated all the results into a useful format, and allowed students to remain anonymous. You will also notice that we had an optional prize draw for participants as an attempt to increase participant numbers. You can hopefully see the online questionnaire using the following URL: https://docs.google.com/forms/d/128D4Kthyx512MJ_34NmRViKG3AVUIutMRmj1tS9TX5k/viewform?usp=send_form

Please let me know if you run into any problems. Otherwise, I wish you all the best with your research and your studies and it will be interesting to hear what you find.

Kind regards,

Dr Chris Saunders.

- **Email from Dr W. Evans**

Hi Johmari- sure. Feel free to use it as long as you cite the paper. Technically, it is a copyright of the journal but I did design the survey so you can certainly use it. Also, we published several papers in this area. I will attach them here in case you don’t have all of them.

Will Evans, DC, PhD, MCHES, CWP

Executive Vice-president and Provost

University of Western States

Appendix G: Letter of information for focus group participants

Letter of information for focus group participants

Dear Participant

Thank you for showing interest in my study by taking time to participate in the focus group.

Study title:
Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants.

Introduction to the study:
Heath care-associated infections (HCAIs) are infections patients contract while being in a hospital or any healthcare facility, such as the Chiropractic Day Clinic, for reasons other than that for which they are receiving care. HCAIs are commonly caused by microorganisms that can spread due to poor hand hygiene or usage of contaminated diagnostic equipment.
An aspect of the research study will be evaluating the chiropractic students' hygienic practices regarding the cleaning of their stethoscopes and sphygmomanometers.

Purpose of focus group:
The purpose of this focus group is to validate the use of the Disinfection Questionnaire, which will be used to collect information from the chiropractic students. The focus group with discuss each question and make amendments were necessary to ensure the Questionnaire's efficiency and accuracy.

Your participation in this focus group is much appreciated and much valued. We want to encourage participants to speak freely and to participate in the discussion, as this will assist the research process. All suggestions, comments and contributions to the Questionnaire will remain confidential.

The results of this focus group will be used for research purposes only.
For more information regarding this research study, please to contact the researcher at johmarilogtenberg@yahoo.com

Thank you for your participation.
Yours sincerely,

[redacted]

Johmari Logtenberg
M.Tech Chiropractic
Researcher

[redacted]

Dr. F.M. Swalaha
D. Tech. Biotechnology
Supervisor

[redacted]

Dr. C.M. Kell
M. Tech. Homeopathy
Co-supervisor

Appendix H: Focus group informed consent form

Statement of Agreement to participate in the focus group:

- I hereby confirm that I have been informed by the researcher**,** Johmari Logtenberg, about the nature of this study – Research Ethics Clearance Number: REC 54/16
- I have received, read and understood the written information (Letter of information for focus group) regarding the study.
- I am aware that the results of this study will be confidentially 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.

________________________ ______ _______. ______________________.

Full name of Participant **Date** **Time** **Signature**

I, , herewith confirm that the above participant has been fully informed about the nature of the above study.

______________________. ______. _______. __________________.

Full name of Researcher **Date** **Time** **Signature**

Appendix I: Confidentiality statement for focus group

Declaration

- All information contained in the research documents as well as the information discussed during the focus group meeting, will be kept private and confidential. This is especially binding to any information that may identify any of the participants in the research process.
- All participants' files will be coded and kept anonymous in the research process.
- None of the information regarding this research study shall be communicated to any other individual or organization outside of this focus group.
- The information from this focus group will be made public in terms of a dissertation publication and a possible journal publication, which will in no way identify any participants of this research.

If you have read and agreed to the above, please complete the appropriate information below and sign to acknowledge agreement.

______________________.	______.	_______.	______________.
Full name of Participant	**Date**	**Time**	**Signature**
______________________.	______.	_______.	______________.
Full name of Researcher	**Date**	**Time**	**Signature**
______________________.	______.	_______.	______________.
Full name of Supervisor	**Date**	**Time**	**Signature**
__________________________.	______.	_______.	______________.
Full name of Co- Supervisor	**Date**	**Time**	**Signature**
______________________.	______.	_______.	______________.
Full name of Witness	**Date**	**Time**	**Signature**

Appendix J: Letter to Head of Homeopathy Department

DUT
DURBAN UNIVERSITY OF TECHNOLOGY

FACULTY OF HEALTH SCIENCES

Department of Chiropractic and Somatology: Chiropractic Clinic
Faculty of Health Sciences
Ritson Campus
Durban University of Technology

11 Ritson Road, Berea, Durban 4001

P O Box 1334, Durban, 4000, South Africa

Tel: (031)373 2205

www.dut.ac.za

Doctor Cornelia Hall
Head of the Homeopathy Department
Faculty of Health Sciences

9 September 2016

Dear Doctor Hall,

My name is Johmari Logtenberg, I am a fifth year Chiropractic student at the Durban University of Technology. In order to complete my Master of Technology in Chiropractic, I must complete a dissertation.

Diagnostic equipment is often contaminated with microorganisms and can result in health care-associated infections (HCAIs), if proper protocol regarding disinfection is not followed. I will be conducting a quantitative investigation to determine if the stethoscopes and sphygmomanometers used by the chiropractic students in the Chiropractic Day Clinic, serve as reservoirs for microbial growth (including antibiotic-resistant microorganisms) and subsequently act as a source for microbial transfer.

The objectives of the study are:

- To determine the presence and quantities of microorganisms (including antibiotic- resistant microorganisms) on the students' stethoscopes and sphygmomanometers after each new patient consultation.
- To assess the efficacy of disinfectants used by the students to clean their stethoscopes and sphygmomanometers.
- To evaluate the student's hygienic practices regarding the cleaning of their stethoscopes and sphygmomanometers.
- To compare the microbiological findings with the data generated by the questionnaires.

In order to achieve this, I ask permission to approach a number of your senior students for their participation in my pilot study. The purpose of this pilot study is to test the adequacy of the Disinfection Questionnaire which will be used to collect information from the chiropractic students, as well as the procedure of administration of this questionnaire.

Your assistance with the above request will be greatly appreciated.

Yours sincerely,

[redacted] ______________   26/9/2016
Ms. J. Logtenberg   Date
Student No: 20818916

[redacted] ______________   26/9/2016
Supervisor: Dr F.M. Swalaha   Date

[redacted] ______________   26/09/2016
Co-Supervisor: Dr C. M. Kell   Date

[redacted] ______________   11/10/2016.
Approved by: Doctor Cornelia Hall   Date
Head of Homeopathy Department

Appendix K: Letter of information for pilot study participants

Letter of information for pilot study participants

Dear Participant

Thank you for showing interest in my study by taking time to participate in the pilot study.

Study title:
Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants.

Introduction to the study:
Heath care-associated infections (HCAIs) are infections patients contract while being in a hospital or any healthcare facility, such as the Chiropractic Day Clinic, for reasons other than that for which they are receiving care. HCAIs are commonly caused by microorganisms that can spread due to poor hand hygiene or usage of contaminated diagnostic equipment.
An aspect of this research study will be evaluating the chiropractic students' hygienic practices regarding the cleaning of their stethoscopes and sphygmomanometers.

Purpose of pilot study:
The purpose of this pilot study is to test the adequacy of the Disinfection Questionnaire which will be used to collect information from the chiropractic students, as well as the procedure of administration of this questionnaire.

Participation in this pilot study is much appreciated and much valued. We want to encourage you to speak freely when providing feedback on the questionnaire when given the opportunity, as this will assist the research process. Participation as well as contributions to the Questionnaire will remain confidential.

The results of this pilot study will be used for research purposes only.
For more information regarding this research study, please contact the researcher at johmarilogtenberg@yahoo.com

Thank you for your participation.
Yours sincerely,

[redacted]
Johmari Logtenberg
M.Tech Chiropractic
Researcher

[redacted]
Dr. F.M. Swalaha
D. Tech. Biotechnology
Supervisor

[redacted]
Dr. C.M. Kell
M. Tech. Homeopathy
Co-supervisor

Appendix L: Pilot study informed consent form

Statement of Agreement to participate in the pilot study:

- I hereby confirm that I have been informed by the researcher**,** Johmari Logtenberg, about the nature of this study – Research Ethics Clearance Number: REC 54/16
- I have received, read and understood the written information (Letter of information for pilot study) regarding the study.
- I am aware that the results of this study will be confidentially 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.

______________________. ______. _______. ______________.

Full name of Participant **Date** **Time** **Signature**

I, , herewith confirm that the above participant has been fully informed about the nature of the above study.

______________________. ______. _______. ______________.

Full name of Researcher **Date** **Time** **Signature**

Appendix M: Confidentiality statement for pilot study

Declaration

- All information contained in the research documents as well as the information discussed during the pilot study, will be kept private and confidential. This is especially binding to any information that may identify any of the participants in the research process.
- All participants' files will be coded and kept anonymous in the research process.
- None of the information regarding this research study shall be communicated to any other individual or organization outside of this pilot study.
- The information from this pilot study will be made public in terms of a dissertation publication and a possible journal publication, which will in no way identify any participants of this research.

If you have read and agreed to the above, please complete the appropriate information below and sign to acknowledge agreement.

____________________.	______.	_______.	______________.
Full name of Participant	**Date**	**Time**	**Signature**
____________________.	______.	_______.	______________.
Full name of Researcher	**Date**	**Time**	**Signature**
____________________.	______.	_______.	______________.
Full name of Witness	**Date**	**Time**	**Signature**

Appendix N: Data collection sheet one - Enumeration of bacteria

Date:	**Time:**	**Chiropractic student code:**

Pre-test sample of stethoscope	Bacteria				Fungi			
Quantity of colony forming units								
Location of colony forming units	Edge		Diaphragm		Edge		Diaphragm	

Post-test sample of stethoscope	Bacteria				Fungi			
Quantity of colony forming units								
Location of colony forming units (Stethoscope only)	Edge		Diaphragm		Edge		Diaphragm	

Pre-test sample of BP cuff	Bacteria	Fungi
Quantity of colony forming units		

Post-test sample of BP cuff	Bacteria	Fungi
Quantity of colony forming units		

***-** Presence of fungal growth indicates contamination of sample

Appendix O: Data collection sheet two - Identification of bacteria to genus level

Date:	**Time:**	**Equipment:**	**Chiropractic student code:**

New patient	First	Second

Specimen	Pre-test sample	Post- test sample

Quantity	

Macroscopic colony characteristics		
	Size:	Margin:
	Colour of colony:	Surface appearance:
	Colour change of agar:	Odour:
	Shape:	Elevation:

Bacterial cellular morphology		
	Shape:	Arrangement:
	Spores:	

Gram staining characteristics		
	Gram-positive:	Gram-negative:

Oxidase test characteristics		
	Positive:	Negative:

Catalase test characteristics		
	Positive:	Negative:

Coagulase test characteristics			
	Positive:	Negative:	Not applicable:

Bacterium identified as:

Adapted from (Logtenberg 2009)

Appendix P: Data collection sheet three - Identification of bacteria to specie level

Date:	**Time:**	**Equipment:**	**Chiropractic student code:**

New patient	First	Second

Specimen	Pre-test sample	Post- test sample

Quantity

At genus level bacterium identified as:

Specialised media used:

Macroscopic colony characteristics		
	Size:	Margin:
	Colour of colony:	Surface appearance:
	Colour change of agar:	Odour:
	Shape:	Elevation:

Appearance on Baird-Parker if applicable:

At specie level bacterium identified as:

Adapted from (Logtenberg 2009)

Appendix Q: IREC- Full approval

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

9 December 2016

IREC Reference Number: **REC 54/16**

Ms J Logtenberg
66 Lewis Drive
Amanzimtoti
4126

Dear Ms Logtenberg

Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants

The Institutional Research Ethics Committee acknowledges receipt of your final data collection tool for review.

We are pleased to inform you that the questionnaire has been approved. Kindly ensure that participants used for the pilot study are not part of the main study.

In addition, the IREC acknowledges receipt of your gatekeeper permission letters.

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

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2016 -12- 09
INSITUTUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA

Appendix R: Data collection sheet four - Efficacy of disinfectants

Date:	Time:	Equipment:	Chiropractic student code:

New patient	First	Second

Specimen	Pre-test sample	Post- test sample

Bacterium

Disinfectant	Test tube 1	Test tube 2	Test tube 3	Negative control	Positive control
Water					
70% Isopropyl alcohol					
70% Ethanol					
Hand sanitizer (62% ethanol)					

Conclusion	

Appendix S: Data collection sheet five - Antibiotic-resistant bacteria

Date:	**Time:**	**Equipment:**	**Chiropractic student code:**

New patient	First	Second

Specimen	Pre-test sample	Post- test sample

At specie level bacterium identified as:

Antibiotic	**Zone of inhibition size Plate one**	**Zone of inhibition size Plate two**	**Average zone of inhibition size**	**Resistant/ Intermediate/ Sensitive**
Chloramphenicol 30 µg				
Ciprofloxacin 5 µg				
Vancomycin 30 µg				
Erythromycin 15 µg				
Amoxicillin 25 µg				

Appendix T: Gatekeeper permission obtained from Prof. S. Moyo

DURBAN UNIVERSITY OF TECHNOLOGY — DIRECTORATE FOR RESEARCH AND POSTGRADUATE SUPPORT

Directorate for Research and Postgraduate Support
Durban University of Technology
Tromso Annexe, Steve Biko Campus
P.O. Box 1334, Durban 4000
Tel.: 031-3732576/7
Fax: 031-3732946
E-mail: moyos@dut.ac.za

5th December 2016

Ms Johmari Logtenberg
c/o Department of Chiropractic
Faculty of Health Sciences
Durban University of Technology

Dear Ms Logtenberg

PERMISSION TO CONDUCT RESEARCH AT THE DUT

Your email correspondence in respect of the above refers. I am pleased to inform you that the Institutional Research Committee (IRC) has granted full permission for you to conduct your research "Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants" at the Durban University of Technology.

We would be grateful if a summary of your key research findings can be submitted to the IRC on completion of your studies.

Kindest regards.
Yours sincerely

[redacted]

PROF. S. MOYO
DIRECTOR: RESEARCH AND POSTGRADUATE SUPPORT

Appendix U: Amendments proposed by focus group to questionnaire

Changes made to sequence of questions in order to improve the flow. Please see pre-focus questionnaire and post- focus group questionnaire to see change in sequence of questions.

The following alterations refer to changes made to the pre-focus group questionnaire:

- Question 1 – Change from “are you” to “gender”.
- Question 2 – Change from “what year are you in” to “year of study”.
- Question 5 – Removed the option “twice daily”.
- Question 6 – Removed the option “other”.
- Question 7- Indicate next to question that participant may select more than one option.
- Question 8- Question was deleted
- Question 9 – Wording of question changed to “Are you knowledgeable in the disinfection procedures?”
- Question 11 – Beginning of question changed to start with “In your opinion”.
- Question 12- Deleted option “twice daily”.
- Question 13 - Indicate next to question that participant may select more than one option. Added options not applicable and perception of patient hygiene.
- Question 14 – Changed wording to “In your opinion, have you received adequate education regarding disinfection of the following equipment during your chiropractic course? (You may select more than one option)”. Also changed options to stethoscope, thermometer, otoscope, speculums, ophthalmoscope and sphygmomanometer.
- Question 15 – Deleted option “after every second patient”.
- Question 16- Rephrased the wording.

Additions to the questionnaire:

- Questions are numbered accordingly.
- From what material is your sphygmomanometer cuff made up of? Options: Plastic, fabric and other.
- Do you think your perception of disinfection protocols would alter between DUT Chiropractic Day Clinic versus private practice? Option: yes or no. if yes. Please specify why?
- Do you have any comments?

Appendix V: Research questionnaire (Post-focus group questionnaire)

Department of Chiropractic and Somatology: Chiropractic Clinic
Faculty of Health Sciences
Ritson Campus
Durban University of Technology

11 Ritson Road, Berea, Durban 4001

P O Box 1334, Durban, 4000, South Africa

Tel: (031)373 2205

www.dut.ac.za

Disinfection Questionnaire

This is a questionnaire about your current thoughts and practices regarding the disinfection of your stethoscope and sphygmomanometers. Please place a cross over a single answer that best describes your attitude or actions regarding disinfection at the Durban University of Technology Chiropractic Day Clinic. Your participation and answers are confidential, so please be as honest as possible. Please do not write any personal identifying information on this form. Please note that at some questions, more than one option can be selected.

IMPORTANT NOTICE – Disinfection of stethoscope refers to the diaphragm of the stethoscope. Disinfection of sphygmomanometers refers to the cuff part that is in direct contact with the patient's skin.

1. Gender	Male			Female	
2. Year of study	Fifth	Sixth	Seventh	Other	
If other – please specify					
3. From what material is your sphygmomanometer cuff made up of?					
Fabric		Plastic		Other	
If other – please specify					
4. Do you think that the following equipment can be possible sources of cross-contamination of pathogenic agents?					
Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
5. Do you think it is important to disinfect the surfaces of these equipment?					
Stethoscope	Very important	Important	Neutral	Unimportant	Very unimportant
Sphygmomanometers	Very important	Important	Neutral	Unimportant	Very unimportant
6. Are you knowledgeable in the disinfection procedures?					
Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
7. Do you regularly see other students disinfect their equipment?					
Stethoscope	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Sphygmomanometers	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
8. In your opinion, is it important for students to disinfect their equipment?					
Stethoscope	Very important	Important	Neutral	Unimportant	Very unimportant
Sphygmomanometers	Very important	Important	Neutral	Unimportant	Very unimportant

9. How often do you think should your equipment be disinfected?						
Stethoscope	After every patient	Daily	Weekly	Monthly	Yearly	Never
Sphygmomanometers	After every patient	Daily	Weekly	Monthly	Yearly	Never

10. On average, how often do you disinfect your equipment?						
Stethoscope	After every patient	Daily	Weekly	Monthly	Yearly	Never
Sphygmomanometers	After every patient	Daily	Weekly	Monthly	Yearly	Never

11. When was the last time you disinfected your equipment?					
Stethoscope	Today	Within the last week	Within the last month	Within the last year	Never
Sphygmomanometers	Today	Within the last week	Within the last month	Within the last year	Never

12. What do you use to disinfect your equipment? (You may select more than one option)							
Stethoscope	Water only	Soapy water	Alcohol based wipes	Surgical alcohol and cotton	Hand sanitizer	Other	Nothing
Sphygmomanometers	Water only	Soapy water	Alcohol based wipes	Surgical alcohol and cotton	Hand sanitizer	Other	Nothing

If other – please specify ....

13. If you don't disinfect your equipment, what is the reason? (You may select more than one option)					
Stethoscope	Not applicable	Unimportant	Forgetfulness	Lack of time	Other
	Uneducated about importance	Perception of patient hygiene	Lack disinfectant equipment available		
Sphygmomanometers	Not applicable	Unimportant	Forgetfulness	Lack of time	Other
	Uneducated about importance	Perception of patient hygiene	Lack disinfectant equipment available		

If other – please specify ....

14. In your opinion, have you received adequate education regarding disinfection of the following equipment during your chiropractic course? (You may select more than one option)					
Stethoscope	Thermometer	Otoscope	Speculums	Ophthalmoscope	Sphygmomanometers

15. On average, how often do you disinfect your hands while in clinic?				
After each patient	Daily	Twice daily	More than twice daily	Never

16. Have you received adequate education on good hand hygiene practice during your chiropractic course?				
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

17. Do you think education regarding hand hygiene and equipment disinfection is important?				
Strongly agree	Agree	Neutral	Disagree	Strongly disagree

18. Do you think your perception of disinfection protocols would alter between DUT Chiropractic Day Clinic versus private practice?		
Yes	No	Neutral

If yes- please specify why - ....

19. Do you have any comments?

Comment- ....

Thank you very much for taking the time to complete this questionnaire.

Adapted from (Evans Jr *et al.* 2009; Puhl *et al.* 2011; Saunders, Hryhorskyj and Skinner 2013)

Appendix W: Explanation of techniques / methods

- **Streak plate technique (Swalaha 2013)**

The laminar flow (Labtec Bioflow II, South Africa) was swiched on and disinfected with 95% ethanol. The bunsen burner was lit and used to sterilize the inoculation loop. After cooling down, the inoculation loop was used to make contact with a representative CFU. The inoculation loop was then drawn in a zig- zag pattern, with no streaks overlapping, across a quarter of the surface of a new TSA plate (Merck). The TSA plate was returned to its inverted position and rotated 90˚anticlockwise. Once cooled after re-sterilization, the inoculation loop was used to draw paralell streaks from the first quarter extending into a second quarter of the plate. This procedure of resterilization and streaking was followed until all four quarters of the TSA plate was inoculated. Once finished, the inoculation loop was flamed once more.

- **Gram staining procedure (Tille 2014; Bruckner 2016)**

After the inoculation loop was sterilized, a loopful of de-ionised water was placed on the labelled microscope slide. Working within the sterile area created by the bunsen burner, a cool sterile loop was used to transfer a small sample of the pure culture to the water and spread across the slide. The smear was then left to air dry, whereafter it was heat fixed and placed on the staining tray to cool.

Once cooled, crystal violet was dropped onto the smear for a minute, whereafter excess stain was rinsed off with water. The smear was then flooded with iodine for another minute before being rinsed off again. The slide was tilted while ethanol was dropped onto the smear for roughly five to 15 s, after which the leftover ethanol was rinsed off. The smear was then covered with the secondary stain, safranin for 30 s before being rinsed off with water. Once the slide had air-dryed, a microscope was used to evaluate the cellular morphology of the bacterium.

- **Oxidase test (Tille 2014)**

Oxidase tests strips (Oxoid) were used to perform the test. These strips were already impregnanted with the substrate 1% tetramethyl-p-phenylenediamine dihydrochloride. Working within the laminar flow and close to the bunsen burner, a steile wooden stick was used to collect a CFU from the pure culture and smeared it onto a test trip. The inoculated area on the strip was then observed for 10 s for any colour changes. Positive result: Inoculated area turn purple/ blue within 10 s. Negative result: No colour change within 10 s. This process was repeated for every pure culture with a new test strip and a sterile wooden stick.

- **Catalase test (Tille 2014)**

Within the confine of the laminar flow and near the bunsen burner's flame, a small sample was collected from the pure culture with the use of a steile wooden stick and smeared onto a clean microscope slide. A sterile pipette was used to drop 1 mL of 30% hydrogen peroxide (Merck) onto the culture smear. The culture on the slide was observed for the formation of oxygen bubbles. Positive result: Formation of copius bubbles. Negative result: No or few bubbles are formed. This process was repeated for every pure culture with a microscope slide and sterile wooden stick.

- **Coagulase test (Sigma-Aldrich 2013)**

The coagulase test was performed with the usage of the Staphylo Monotec test kit plus (Sigma-Aldrich) which consisted of the test reagent, the control reagent and analysis cards. Both of the reagents were taken out of the refrigator beforehand in order to reach room temperature. A vortex (Vortex Genie 2, Scientific Industries) was then used to agitate the reagents for 10 s. A drop of the test reagents was placed onto the allocated area of the analysis card. Working within the confines of the laminar flow and close to the bunsen burner, a steile wooden stick was then used to mix a CFU from the pure culture with the reagent droplet for 20 s. After 20 s, the analysis card was tilted to observe for agglutination. The result was then repeated with the control reagent. Positive result: The isolate is *S. aureus* if agglutination occurs in the test reagent, but not in the control reagent. Negative result: The isolate is not *S. aureus* if the test reagent does not agglutinate or if both the test and negative control reagent agglutinate

- **Procedure for modified AOAC use dilution method (US Environmental Protection Agency Office of Pesticide Programs 2009; Microchem Laboratory 2015)**

A test tube containing nutrient broth (NB)(Merck) was inoculated with the selected bacterium and incubated at 37℃ for 24 to 48 hours. A stock solution of the inoculated broth was prepared to a 0, 5 McFarland standard with the use of a Biochrom Libra S21 spectrophotometer. Four sterile carriers were placed individually into the test tube containing the stock solution for 30 s each. The carriers were then removed and placed into a sterile Pertri dish to air dry. Once dry, three carriers were transferred to a test tube filled with 10 mL of the disinfectant being tested for 30 s each. After 30 s, the three disinfected carriers were moved each to a test tube containing NB. The fourth contaminated carrier were placed into a test tube containing NB, without being exposed to the disinfectant. This test tube served as our negative control. A fifth sterile carrier was placed in the test tube containing 10 mL of the disinfectant being tested for 30 s. After 30 s, the disinfected carriers were moved to a test tube containing NB. This test tube served as our positive control. The test tubes were then incubated at 37℃ for 24 to 48 hours. After incubation, the test tubes were assessed for growth and the results recorded on Data collection sheet four (Appendix R). For a pass, all three test tubes (excluding the controls) must have shown no growth. The presence of growth in one or more test tubes indicated that the disinfectant was ineffective against the tested bacterium.

Note: For one bacterium, this procedure were repeated four times as to test the efficacy of each of the four disinfectants. Each one of the 232 bacteriological samples collected from the chiropractic students' equipment, which exhibited the growth of one or more of the selected bacteria, were included in this testing.

- **Spread-plate technique (Logtenberg 2009)**

A stock solution of the inoculated broth was prepared to a 0.5 McFarland standard to ensure a standardized bacterial cell concentration of each bacterium being tested across the board. Inside the laminar flow, a pipette was used to transfer 100 microliters of the stock solution onto a MHA plate (Merck). With the use of a sterile swab, the inoculum was spread across the entire surface of the MHA plate by moving the swab from side to side starting at the top and moving down to the bottom. The plate was then rotated 90˚ and the above process was then repeated to ensure that the entire surface was covered completely.

Appendix X: DUT Chiropractic programme breakdown

Qualification	Year of study	Subjects	Hours per week
National diploma: Chiropractic	1st year	Anatomy I (Gross Anatomy)	5
		Anatomy I (Topographic and Radiographic)	2.5
		Anatomy I (Histology)	5
		Biology	8
		Physiology I	6
		Chemistry I	7
		Physics I	5
		Philosophy, History and Principles I - Module 1	3
		Philosophy, History and Principles I - Module 2	3
	2nd year	Anatomy II (Gross Anatomy)	5
		Anatomy II (Clinical Anatomy)	5
		Anatomy II (Topographic and Radiographic)	2.5
		Biochemistry II	6
		Epidemiology II	6
		General Pathology II	4
		Medical microbiology II	7
		Physiology II	6
		Social studies I	2
	3rd year	Auxillary therapeutics III	4
		Diagnostics III	9
		Psychopathology II	2
		Chiropractic principles & practice III - Module 1	10
		Chiropractic principles & practice III - Module	6
		Systemic Pathology III (Pathology)	8
		Systemic Pathology III (Pharmacology)	2
Bachelors degree in Technology: Chiropractic	4th year	Diagnostics IV	8
		Clinical biomechanics and kinesiology IV	4
		Clinical Chiropractic IV	9
		Chiropractic Principles & Practice IV	7
		Radiology IV	6
		Research methods and techniques I	2
Masters degrees in Technology: Chiropractic	5th year	Clinical biomechanics and kinesiology V	6
		Clinical chiropractic V	20
		Chiropractic principles & practice V	4
		Research project and dissertation	13
		Practice management and jurisprudence V	2

Source: (Faculty of Health 2017)

Appendix Y: Raw data

Table 1: Correlation between Equipment possible sources: Stethoscope and opinion: Disinfect surfaces of equipment: Stethoscope

Correlations				
			Equipment possible sources: Stethoscope	Opinion: Disinfect surfaces of equipment: Stethoscope
Spearman's rho	Equipment possible sources: Stethoscope	Correlation Coefficient	1.000	.515**
		Sig. (2-tailed)	.	.004
		N	29	29
	Opinion: Disinfect surfaces of equipment:Stethoscope	Correlation Coefficient	.515**	1.000
		Sig. (2-tailed)	.004	.
		N	29	29
**. Correlation is significant at the 0.01 level (2-tailed).				

Equipment possible sources: Stethoscope * Opinion: Disinfect surfaces of equipment:Stethoscope Crosstabulation						
			Opinion: Disinfect surfaces of equipment: Stethoscope			
			SA	A	Neutral	Total
Equipment possible sources: Stethoscope	SA	Count	6	0	0	6
		% within Equipment possible sources: Stethoscope	100.0%	0.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	40.0%	0.0%	0.0%	20.7%
		% of Total	20.7%	0.0%	0.0%	20.7%
	A	Count	8	9	1	18
		% within Equipment possible sources: Stethoscope	44.4%	50.0%	5.6%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	53.3%	75.0%	50.0%	62.1%
		% of Total	27.6%	31.0%	3.4%	62.1%
	N	Count	1	2	1	4

		% within Equipment possible sources: Stethoscope	25.0%	50.0%	25.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	6.7%	16.7%	50.0%	13.8%
		% of Total	3.4%	6.9%	3.4%	13.8%
	Disagree	Count	0	1	0	1
		% within Equipment possible sources: Stethoscope	0.0%	100.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	0.0%	8.3%	0.0%	3.4%
		% of Total	0.0%	3.4%	0.0%	3.4%
Total		Count	15	12	2	29
		% within Equipment possible sources: Stethoscope	51.7%	41.4%	6.9%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	100.0%	100.0%	100.0%	100.0%
		% of Total	51.7%	41.4%	6.9%	100.0%

Correlation between Equipment possible sources: Sphygmomananameter and Opinion: Disinfect surfaces of equipment:Sphygmomananameter

Correlations				
			Equipment possible sources: BP	Opinion: Disinfect surfaces of equipment:BP
Spearman's rho	Equipment possible sources: Sphygmomananameter	Correlation Coefficient	1.000	.445*
		Sig. (2-tailed)	.	.015
		N	29	29
	Opinion: Disinfect surfaces of equipment:Sphygmomananameter	Correlation Coefficient	.445*	1.000
		Sig. (2-tailed)	.015	.
		N	29	29

Equipment possible sources: Sphygmomananameter * Opinion: Disinfect surfaces of equipment:Sphygmomananameter Crosstabulation						
			Opinion: Disinfect surfaces of equipment:BP			Total
			SA	Agree	Neutral	
Equipment possible sources: Sphygmomananameter	SA	Count	4	0	0	4
		% within Equipment possible sources: Sphygmomananameter	100.0%	0.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	26.7%	0.0%	0.0%	13.8%
		% of Total	13.8%	0.0%	0.0%	13.8%
	A	Count	9	7	1	17
		% within Equipment possible sources: Sphygmomananameter	52.9%	41.2%	5.9%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	60.0%	58.3%	50.0%	58.6%
		% of Total	31.0%	24.1%	3.4%	58.6%
	Neutral	Count	2	3	1	6
		% within Equipment possible sources: Sphygmomananameter	33.3%	50.0%	16.7%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	13.3%	25.0%	50.0%	20.7%
		% of Total	6.9%	10.3%	3.4%	20.7%
	Disagree	Count	0	2	0	2
		% within Equipment possible sources: Sphygmomananameter	0.0%	100.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	0.0%	16.7%	0.0%	6.9%
		% of Total	0.0%	6.9%	0.0%	6.9%

Total	Count	15	12	2	29
	% within Equipment possible sources: Sphygmomananameter	51.7%	41.4%	6.9%	100.0%
	% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	100.0%	100.0%	100.0%	100.0%
	% of Total	51.7%	41.4%	6.9%	100.0%

Correlations

			Equipment possible sources: Stethoscope	How often do you disinfected:Stethoscope
Spearman's rho	Equipment possible sources: Stethoscope	Correlation Coefficient	1.000	.108
		Sig. (2-tailed)	.	.575
		N	29	29
	How often do you disinfected:Stethoscope	Correlation Coefficient	.108	1.000
		Sig. (2-tailed)	.575	.
		N	29	58

Relationship between Equipment possible sources: Sphygmomananameter and How often do you disinfected:Sphygmomananameter

Correlations

			Equipment possible sources: BP	How often do you disinfected:BP
Spearman's rho	Equipment possible sources: BP	Correlation Coefficient	1.000	.145
		Sig. (2-tailed)	.	.453
		N	29	29
	How often do you disinfected:BP	Correlation Coefficient	.145	1.000
		Sig. (2-tailed)	.453	.
		N	29	58

Relationship between Equipment possible sources: Stethoscope and when was the last time you disinfected:Stethoscope

Correlations				
			Equipment possible sources: Stethoscope	When was last time you disinfected:Stethoscope
Spearman's rho	Equipment possible sources: Stethoscope	Correlation Coefficient	1.000	.164
		Sig. (2-tailed)	.	.395
		N	29	29
	When was the last time you disinfected:Stethoscope	Correlation Coefficient	.164	1.000
		Sig. (2-tailed)	.395	.
		N	29	29

Correlation between Equipment possible sources: Sphygmomananameter and When was the last time you disinfected:Sphygmomananameter

Correlations				
			Equipment possible sources: BP	When was the last time you disinfected:BP
Spearman's rho	Equipment possible sources: Sphygmomananameter	Correlation Coefficient	1.000	.215
		Sig. (2-tailed)	.	.262
		N	29	29
	When was the last time you disinfected:Sphygmomananameter	Correlation Coefficient	.215	1.000
		Sig. (2-tailed)	.262	.
		N	29	29

Correlation between Think : Disinfect surfaces of equipment:Stethoscope and Knowledge in the procedures:Stethoscope

Correlations				
			Think : Disinfect surfaces of equipment:Stethoscope	Knowledge in the procedures:Stethoscope
Spearman's rho		Correlation Coefficient	1.000	.366
		Sig. (2-tailed)	.	.051

	Think : Disinfect surfaces of equipment:Stethoscope	N	29	29
	Knowledge in the procedures:Stethoscope	Correlation Coefficient	.366	1.000
		Sig. (2-tailed)	.051	.
		N	29	29

Correlation between Think: Disinfect surfaces of equipment: Sphygmomananameter and Knowledge in the procedures:Sphygmomananameter

Correlations				
			Think : Disinfect surfaces of equipment: Sphygmomananameter	Knowledge in the procedures:Sphygmomananameter
Spearman's rho	Think : Disinfect surfaces of equipment: Sphygmomananameter	Correlation Coefficient	1.000	.425*
		Sig. (2-tailed)	.	.022
		N	29	29
	Knowledge in the procedures:Sphygmomananameter	Correlation Coefficient	.425*	1.000
		Sig. (2-tailed)	.022	.
		N	29	29
*. Correlation is significant at the 0.05 level (2-tailed).				

Think: Disinfect surfaces of equipment: Sphygmomananameter * Knowledge in the procedures:Sphygmomananameter Crosstabulation						
			Knowledge in the procedures:BP			Total
			SA	A	Neutral	
Think : Disinfect surfaces of equipment: Sphygmomananameter	VA	Count	3	5	2	10
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	30.0%	50.0%	20.0%	100.0%
		% within Knowledge in the procedures:BP	75.0%	45.5%	14.3%	34.5%
		% of Total	10.3%	17.2%	6.9%	34.5%
	Important	Count	1	5	10	16
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	6.3%	31.3%	62.5%	100.0%

		% within Knowledge in the procedures:BP	25.0%	45.5%	71.4%	55.2%
		% of Total	3.4%	17.2%	34.5%	55.2%
	Neutral	Count	0	0	2	2
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	0.0%	0.0%	100.0%	100.0%
		% within Knowledge in the procedures:BP	0.0%	0.0%	14.3%	6.9%
		% of Total	0.0%	0.0%	6.9%	6.9%
	Unimportant	Count	0	1	0	1
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	0.0%	100.0%	0.0%	100.0%
		% within Knowledge in the procedures:Sphygmomananameter	0.0%	9.1%	0.0%	3.4%
		% of Total	0.0%	3.4%	0.0%	3.4%
Total		Count	4	11	14	29
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	13.8%	37.9%	48.3%	100.0%
		% within Knowledge in the procedures:BP	100.0%	100.0%	100.0%	100.0%
		% of Total	13.8%	37.9%	48.3%	100.0%

Correlation between Think: Disinfect surfaces of equipment:Stethoscope and Opinion: Disinfect surfaces of equipment:Stethoscope

Correlations				
			Think : Disinfect surfaces of equipment:Stethoscope	Opinion: Disinfect surfaces of equipment:Stethoscope
Spearman's rho	Think : Disinfect surfaces of equipment:Stethoscope	Correlation Coefficient	1.000	.770**
		Sig. (2-tailed)	.	.000
		N	29	29
		Correlation Coefficient	.770**	1.000
		Sig. (2-tailed)	.000	.

	Opinion: Disinfect surfaces of equipment:Stethoscope	N	29	29

Think : Disinfect surfaces of equipment:Stethoscope * Opinion: Disinfect surfaces of equipment:Stethoscope Crosstabulation

			Opinion: Disinfect surfaces of equipment:Stethoscope			Total
			SA	A	Neutral	
Think : Disinfect surfaces of equipment:Stethoscope	Very important	Count	11	0	0	11
		% within Think : Disinfect surfaces of equipment:Stethoscope	100.0%	0.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	73.3%	0.0%	0.0%	37.9%
		% of Total	37.9%	0.0%	0.0%	37.9%
	Important	Count	4	12	1	17
		% within Think : Disinfect surfaces of equipment:Stethoscope	23.5%	70.6%	5.9%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	26.7%	100.0%	50.0%	58.6%
		% of Total	13.8%	41.4%	3.4%	58.6%
	Unimportant	Count	0	0	1	1
		% within Think : Disinfect surfaces of equipment:Stethoscope	0.0%	0.0%	100.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	0.0%	0.0%	50.0%	3.4%
		% of Total	0.0%	0.0%	3.4%	3.4%
Total		Count	15	12	2	29
		% within Think : Disinfect surfaces of equipment:Stethoscope	51.7%	41.4%	6.9%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Stethoscope	100.0%	100.0%	100.0%	100.0%

	% of Total	51.7%	41.4%	6.9%	100.0%

Correlation between Think: Disinfect surfaces of equipment: Sphygmomananameter and Opinion: Disinfect surfaces of equipment:Sphygmomananameter

Correlations				
			Think : Disinfect surfaces of equipment: Sphygmomananameter	Opinion: Disinfect surfaces of equipment:Sphygmomananameter
Spearman's rho	Think : Disinfect surfaces of equipment: Sphygmomananameter	Correlation Coefficient	1.000	.652**
		Sig. (2-tailed)	.	.000
		N	29	29
	Opinion: Disinfect surfaces of equipment:Sphygmomananameter	Correlation Coefficient	.652**	1.000
		Sig. (2-tailed)	.000	.
		N	29	29

Think : Disinfect surfaces of equipment: Sphygmomananameter * Opinion: Disinfect surfaces of equipment:Sphygmomananameter Crosstabulation						
			Opinion: Disinfect surfaces of equipment:Sphygmomananameter			Total
			SA	Agree	Neutral	
Think : Disinfect surfaces of equipment: Sphygmomananameter	VI	Count	10	0	0	10
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	100.0%	0.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	66.7%	0.0%	0.0%	34.5%
		% of Total	34.5%	0.0%	0.0%	34.5%
	I	Count	4	11	1	16
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	25.0%	68.8%	6.3%	100.0%

		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	26.7%	91.7%	50.0%	55.2%
		% of Total	13.8%	37.9%	3.4%	55.2%
	Neutral	Count	1	1	0	2
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	50.0%	50.0%	0.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	6.7%	8.3%	0.0%	6.9%
		% of Total	3.4%	3.4%	0.0%	6.9%
	Unimportant	Count	0	0	1	1
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	0.0%	0.0%	100.0%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	0.0%	0.0%	50.0%	3.4%
		% of Total	0.0%	0.0%	3.4%	3.4%
Total		Count	15	12	2	29
		% within Think : Disinfect surfaces of equipment: Sphygmomananameter	51.7%	41.4%	6.9%	100.0%
		% within Opinion: Disinfect surfaces of equipment:Sphygmomananameter	100.0%	100.0%	100.0%	100.0%
		% of Total	51.7%	41.4%	6.9%	100.0%

Correlation between Knowledge in the procedures: Stethoscope and Opinion: Disinfect surfaces of equipment: Stethoscope

Correlations				
			Knowledge in the procedures: Stethoscope	Opinion: Disinfect surfaces of equipment: Stethoscope
Spearman's rho	Knowledge in the procedures: Stethoscope	Correlation Coefficient	1.000	.256
		Sig. (2-tailed)	.	.180
		N	29	29
	Opinion: Disinfect surfaces of equipment: Stethoscope	Correlation Coefficient	.256	1.000
		Sig. (2-tailed)	.180	.
		N	29	29

Correlation between Knowledge in the procedures:Sphygmomananameter and Opinion: Disinfect surfaces of equipment:Sphygmomananameter

Correlations				
			Knowledge in the procedures:BP	Opinion: Disinfect surfaces of equipment:BP
Spearman's rho	Knowledge in the procedures:Sphygmomananameter	Correlation Coefficient	1.000	.244
		Sig. (2-tailed)	.	.201
		N	29	29
	Opinion: Disinfect surfaces of equipment:Sphygmomananameter	Correlation Coefficient	.244	1.000
		Sig. (2-tailed)	.201	.
		N	29	29

Correlation between Knowledge in the procedures:Stethoscope and Received adequate education: Stethoscope

Correlations				
			Knowledge in the procedures:Stethoscope	Received adequate education: Stethoscope
Spearman's rho	Knowledge in the procedures:Stethoscope	Correlation Coefficient	1.000	-.056
		Sig. (2-tailed)	.	.775
		N	29	29

	Received adequate education: Stethoscope	Correlation Coefficient	-.056	1.000
		Sig. (2-tailed)	.775	.
		N	29	29

Opinion: Disinfect surfaces of equipment:Stethoscope was not significantly related with What do you use to disinfect:Sphygmomananameter: Alcohol based wipes, What do you use to disinfect:Sphygmomananameter:Surgical alcohol and cotton, and If you do not disinfect: Sphygmomananameter: Not applicable (p>0.05).

			Opinion: Disinfect surfaces of equipment:Stethoscope
Spearman's rho	Opinion: Disinfect surfaces of equipment:Stethoscope	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	29
	What do you use to disinfect:Sphygmomananameter: Alcohol based wipes	Correlation Coefficient	-.283
		Sig. (2-tailed)	.137
		N	29
	What do you use to disinfect:Sphygmomananameter: Surgical alcohol and cotton	Correlation Coefficient	.172
		Sig. (2-tailed)	.374
		N	29
	If you do not disinfect: Sphygmomananameter: Not applicable	Correlation Coefficient	-.067
		Sig. (2-tailed)	.728
		N	29

How often think disinfected:Stethoscope was significantly positively correlated with How often do you disinfected:Stethoscope (r=0.542, p=0.02), and When was the last time you disinfected:Stethoscope (r=0.642, p<0.01).

			How often think disinfected:Stethoscope
Spearman's rho	How often think disinfected:Stethoscope	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	29
	How often do you disinfected:Stethoscope	Correlation Coefficient	.542**
		Sig. (2-tailed)	.002
		N	29

	When was the last time you disinfected:Stethoscope	Correlation Coefficient	.642**
		Sig. (2-tailed)	.000
		N	29

Correlations

			How often think disinfected:Sphygmomananameter	How often do you disinfected:Sphygmomananameter	When was the last time you disinfected:Sphygmomananameter
Spearman's rho	How often think disinfected:Sphygmomananameter	Correlation Coefficient	1.000	.415*	.404*
		Sig. (2-tailed)	.	.025	.030
		N	29	29	29
	How often do you disinfected:Sphygmomananameter	Correlation Coefficient	.415*	1.000	.884**
		Sig. (2-tailed)	.025	.	.000
		N	29	58	29
	When was the last time you disinfected:Sphygmomananameter	Correlation Coefficient	.404*	.884**	1.000
		Sig. (2-tailed)	.030	.000	.
		N	29	29	29

			How often do you disinfected:Stethoscope
Spearman's rho	How often do you disinfected:Stethoscope	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	58
	Think : Disinfect surfaces of equipment:Stethoscope	Correlation Coefficient	.064
		Sig. (2-tailed)	.742
		N	29
	Knowledge in the procedures:Stethoscope	Correlation Coefficient	.188
		Sig. (2-tailed)	.328
		N	29
	Regularly see others disinfect:Stethoscope	Correlation Coefficient	.489**
		Sig. (2-tailed)	.007
		N	29
	Opinion: Disinfect surfaces of equipment:Stethoscope	Correlation Coefficient	.273
		Sig. (2-tailed)	.152
		N	29
		Correlation Coefficient	.902**

	When was the last time you disinfected:Stethoscope	Sig. (2-tailed)	.000
		N	29
	If you do not disinfect: Stethoscope: Not applicable	Correlation Coefficient	.353
		Sig. (2-tailed)	.060
		N	29
	Received adequate education: Stethoscope	Correlation Coefficient	.223
		Sig. (2-tailed)	.244
		N	29
	How often disinfect hands	Correlation Coefficient	.010
		Sig. (2-tailed)	.958
		N	29
			How often do you disinfected:Sphygmomananameter
Spearman's rho	How often do you disinfected:Sphygmomananameter	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	58
	Think : Disinfect surfaces of equipment: Sphygmomananameter	Correlation Coefficient	.233
		Sig. (2-tailed)	.223
		N	29
	Knowledge in the procedures:Sphygmomananameter	Correlation Coefficient	.419*
		Sig. (2-tailed)	.024
		N	29
	Regularly see others disinfect:Sphygmomananameter	Correlation Coefficient	.415*
		Sig. (2-tailed)	.025
		N	29
	Opinion: Disinfect surfaces of equipment:Sphygmomananameter	Correlation Coefficient	.165
		Sig. (2-tailed)	.392
		N	29
	When was the last time you disinfected:Sphygmomananameter	Correlation Coefficient	.884**
		Sig. (2-tailed)	.000
		N	29
	If you do not disinfect: Stethoscope: Not applicable	Correlation Coefficient	.193
		Sig. (2-tailed)	.316
		N	29
	How often disinfect hands	Correlation Coefficient	-.014
		Sig. (2-tailed)	.944
		N	29

Correlations

			When was the last time you disinfected:Stethoscope	Received adequate education: Stethoscope
Spearman's rho	When was the last time you disinfected:Stethoscope	Correlation Coefficient	1.000	.213
		Sig. (2-tailed)	.	.267
		N	29	29
	Received adequate education: Stethoscope	Correlation Coefficient	.213	1.000
		Sig. (2-tailed)	.267	.
		N	29	29

			Received adequate education: Stethoscope
Spearman's rho	Received adequate education: Stethoscope	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	29
	Adequate education on good hand hygiene	Correlation Coefficient	-.005
		Sig. (2-tailed)	.978
		N	29
	Think education regarding hand hygiene	Correlation Coefficient	-.393*
		Sig. (2-tailed)	.035
		N	29

Correlations

			How often disinfect hands	Adequate education on good hand hygiene
Spearman's rho	How often disinfect hands	Correlation Coefficient	1.000	.201
		Sig. (2-tailed)	.	.296
		N	29	29
	Adequate education on good hand hygiene	Correlation Coefficient	.201	1.000
		Sig. (2-tailed)	.296	.
		N	29	29

Correlations				
			Adequate education on good hand hygiene	Think education regarding hand hygiene
Spearman's rho	Adequate education on good hand hygiene	Correlation Coefficient	1.000	.141
		Sig. (2-tailed)	.	.466
		N	29	29
	Think education regarding hand hygiene	Correlation Coefficient	.141	1.000
		Sig. (2-tailed)	.466	.
		N	29	29

Frequency Table

Prestetho_GA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	56	96.6	96.6	96.6
	No growth	2	3.4	3.4	100.0
	Total	58	100.0	100.0	

Poststetho_GA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	58	100.0	100.0	100.0

Prestetho_GA20

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	21	36.2	36.2	36.2
	No growth	37	63.8	63.8	100.0
	Total	58	100.0	100.0	

Poststetho_GA20

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	43	74.1	74.1	74.1
	No growth	15	25.9	25.9	100.0
	Total	58	100.0	100.0	

PreBP_GA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	55	94.8	94.8	94.8
	No growth	3	5.2	5.2	100.0
	Total	58	100.0	100.0	

postBP_GA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	58	100.0	100.0	100.0

preBP_GA20

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	23	39.7	39.7	39.7
	No growth	35	60.3	60.3	100.0
	Total	58	100.0	100.0	

PostBP_GA20

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Growth	30	51.7	51.7	51.7
	No growth	28	48.3	48.3	100.0
	Total	58	100.0	100.0	

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Prestetho_GA	1.03	58	.184	.024
	Poststetho_GA	1.00	58	.000	.000
Pair 2	Prestetho_GA20	1.64	58	.485	.064
	Poststetho_GA20	1.26	58	.442	.058
Pair 3	PreBP_GA	1.05	58	.223	.029
	postBP_GA	1.00	58	.000	.000
Pair 4	preBP_GA20	1.60	58	.493	.065

PostBP_GA20	1.48	58	.504	.066

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Prestetho_GA & Poststetho_GA	58	.	.
Pair 2	Prestetho_GA20 & Poststetho_GA20	58	.035	.792
Pair 3	PreBP_GA & postBP_GA	58	.	.
Pair 4	preBP_GA20 & PostBP_GA20	58	.007	.957

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Prestetho_GA - Poststetho_GA	.034	.184	.024	-.014	.083	1.427	57	.159
Pair 2	Prestetho_GA20 - Poststetho_GA20	.379	.644	.085	.210	.549	4.484	57	.000
Pair 3	PreBP_GA - postBP_GA	.052	.223	.029	-.007	.110	1.763	57	.083
Pair 4	preBP_GA20 - PostBP_GA20	.121	.703	.092	-.064	.305	1.308	57	.196

Oneway

Descriptives

Mean CFUs-stetho

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
After every patient	4	32.250	35.2763	17.6381	-23.882	88.382	7.5	84.0
Daily	3	17.167	3.6171	2.0883	8.181	26.152	13.0	19.5
Weekly	8	16.188	16.9346	5.9873	2.030	30.345	3.0	55.5
Monthly	6	17.750	18.6862	7.6286	-1.860	37.360	6.5	55.5
Yearly	2	74.000	64.3467	45.5000	-504.132	652.132	28.5	119.5
Never	6	18.250	17.5350	7.1586	-.152	36.652	4.0	50.0
Total	29	23.241	26.3949	4.9014	13.201	33.281	3.0	119.5

ANOVA

Mean CFUs-stetho

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6316.675	5	1263.335	2.203	.089
Within Groups	13190.635	23	573.506		
Total	19507.310	28			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Mean CFUs-stetho

Tukey HSD

(I) Likert Answer-Stethoscope Question 10	(J) Likert Answer-Stethoscope Question 10	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
After every patient	Daily	15.0833	18.2906	.960	-41.672	71.839
	Weekly	16.0625	14.6651	.878	-29.443	61.568
	Monthly	14.5000	15.4584	.932	-33.467	62.467
	Yearly	-41.7500	20.7396	.366	-106.105	22.605
	Never	14.0000	15.4584	.941	-33.967	61.967
Daily	After every patient	-15.0833	18.2906	.960	-71.839	41.672
	Weekly	.9792	16.2129	1.000	-49.329	51.288
	Monthly	-.5833	16.9338	1.000	-53.129	51.962
	Yearly	-56.8333	21.8614	.138	-124.669	11.003
	Never	-1.0833	16.9338	1.000	-53.629	51.462
Weekly	After every patient	-16.0625	14.6651	.878	-61.568	29.443
	Daily	-.9792	16.2129	1.000	-51.288	49.329
	Monthly	-1.5625	12.9334	1.000	-41.695	38.570
	Yearly	-57.8125	18.9325	.056	-116.560	.935
	Never	-2.0625	12.9334	1.000	-42.195	38.070
Monthly	After every patient	-14.5000	15.4584	.932	-62.467	33.467
	Daily	.5833	16.9338	1.000	-51.962	53.129
	Weekly	1.5625	12.9334	1.000	-38.570	41.695
	Yearly	-56.2500	19.5534	.080	-116.924	4.424
	Never	-.5000	13.8264	1.000	-43.403	42.403
Yearly	After every patient	41.7500	20.7396	.366	-22.605	106.105
	Daily	56.8333	21.8614	.138	-11.003	124.669
	Weekly	57.8125	18.9325	.056	-.935	116.560
	Monthly	56.2500	19.5534	.080	-4.424	116.924
	Never	55.7500	19.5534	.084	-4.924	116.424

Never	After every patient	-14.0000	15.4584	.941	-61.967	33.967
	Daily	1.0833	16.9338	1.000	-51.462	53.629
	Weekly	2.0625	12.9334	1.000	-38.070	42.195
	Monthly	.5000	13.8264	1.000	-42.403	43.403
	Yearly	-55.7500	19.5534	.084	-116.424	4.924

Homogeneous Subsets

Mean CFUs-stetho

Tukey HSD[a,b]

Likert Answer- Stethoscope Question 10	N	Subset for alpha = 0.05 1	2
Weekly	8	16.188	
Daily	3	17.167	
Monthly	6	17.750	
Never	6	18.250	
After every patient	4	32.250	32.250
Yearly	2		74.000
Sig.		.933	.187

Oneway

Descriptives

Mean CFUs-stetho

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Today	2	18.750	8.8388	6.2500	-60.664	98.164	12.5	25.0
Within the last week	11	18.273	22.5747	6.8065	3.107	33.439	3.0	84.0
Within the last month	8	22.250	20.8155	7.3594	4.848	39.652	6.5	55.5
Within the last year	1	28.500	.	.	.	.	28.5	28.5
Never	7	32.714	41.4818	15.6786	-5.650	71.079	4.0	119.5
Total	29	23.241	26.3949	4.9014	13.201	33.281	3.0	119.5

ANOVA

Mean CFUs-stetho

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	975.575	4	243.894	.316	.865
Within Groups	18531.735	24	772.156		
Total	19507.310	28			

Oneway

Descriptives

Mean CFUs-BP

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
After every patient	4	15.750	10.2184	5.1092	-.510	32.010	4.5	28.0
Daily	3	18.833	19.3671	11.1816	-29.277	66.944	2.0	40.0
Weekly	6	22.917	15.7684	6.4374	6.369	39.465	4.5	44.5
Monthly	6	32.000	15.6301	6.3810	15.597	48.403	12.0	51.5
Yearly	2	30.500	4.9497	3.5000	-13.972	74.972	27.0	34.0
Never	8	53.625	94.3904	33.3721	-25.287	132.537	8.0	285.0
Total	29	32.379	50.6149	9.3990	13.126	51.632	2.0	285.0

ANOVA

Mean CFUs-BP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5812.828	5	1162.566	.406	.840
Within Groups	65919.500	23	2866.065		
Total	71732.328	28			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Mean CFUs-BP

Tukey HSD

(I) Likert Answer-Sphygmomanometer Question 10	(J) Likert Answer-Sphygmomanometer Question 10	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
After every patient	Daily	-3.0833	40.8885	1.000	-129.961	123.794
	Weekly	-7.1667	34.5571	1.000	-114.398	100.064
	Monthly	-16.2500	34.5571	.997	-123.481	90.981
	Yearly	-14.7500	46.3632	1.000	-158.615	129.115
	Never	-37.8750	32.7838	.853	-139.603	63.853
Daily	After every patient	3.0833	40.8885	1.000	-123.794	129.961
	Weekly	-4.0833	37.8554	1.000	-121.549	113.382
	Monthly	-13.1667	37.8554	.999	-130.632	104.299

	Yearly	-11.6667	48.8711	1.000	-163.314	139.981
	Never	-34.7917	36.2438	.926	-147.256	77.673
Weekly	After every patient	7.1667	34.5571	1.000	-100.064	114.398
	Daily	4.0833	37.8554	1.000	-113.382	121.549
	Monthly	-9.0833	30.9088	1.000	-104.994	86.827
	Yearly	-7.5833	43.7117	1.000	-143.221	128.054
	Never	-30.7083	28.9126	.891	-120.424	59.007
Monthly	After every patient	16.2500	34.5571	.997	-90.981	123.481
	Daily	13.1667	37.8554	.999	-104.299	130.632
	Weekly	9.0833	30.9088	1.000	-86.827	104.994
	Yearly	1.5000	43.7117	1.000	-134.137	137.137
	Never	-21.6250	28.9126	.973	-111.341	68.091
Yearly	After every patient	14.7500	46.3632	1.000	-129.115	158.615
	Daily	11.6667	48.8711	1.000	-139.981	163.314
	Weekly	7.5833	43.7117	1.000	-128.054	143.221
	Monthly	-1.5000	43.7117	1.000	-137.137	134.137
	Never	-23.1250	42.3236	.993	-154.455	108.205
Never	After every patient	37.8750	32.7838	.853	-63.853	139.603
	Daily	34.7917	36.2438	.926	-77.673	147.256
	Weekly	30.7083	28.9126	.891	-59.007	120.424
	Monthly	21.6250	28.9126	.973	-68.091	111.341
	Yearly	23.1250	42.3236	.993	-108.205	154.455

Homogeneous Subsets

Mean CFUs-BP

Tukey HSD[a,b]

Likert Sphygmomanometer Answer-Question 10	N	Subset for alpha = 0.05 1
After every patient	4	15.750
Daily	3	18.833
Weekly	6	22.917
Yearly	2	30.500
Monthly	6	32.000
Never	8	53.625
Sig.		.917

Oneway

Descriptives

Mean CFUs-BP

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Today	2	12.000	10.6066	7.5000	-83.297	107.297	4.5	19.5
Within the last week	8	15.313	9.7025	3.4303	7.201	23.424	2.0	31.5
Within the last month	8	32.813	15.7252	5.5597	19.666	45.959	4.5	51.5
Within the last year	3	33.667	6.5064	3.7565	17.504	49.829	27.0	40.0
Never	8	53.625	94.3904	33.3721	-25.287	132.537	8.0	285.0
Total	29	32.379	50.6149	9.3990	13.126	51.632	2.0	285.0

ANOVA

Mean CFUs-BP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6778.348	4	1694.587	.626	.648
Within Groups	64953.979	24	2706.416		
Total	71732.328	28			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Mean CFUs-BP

Tukey HSD

(I) Likert Answer-Sphygmomanometer Question 11	(J) Likert Answer-Sphygmomanometer Question 11	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Today	Within the last week	-3.3125	41.1280	1.000	-124.477	117.852
	Within the last month	-20.8125	41.1280	.986	-141.977	100.352
	Within the last year	-21.6667	47.4905	.990	-161.575	118.242
	Never	-41.6250	41.1280	.847	-162.789	79.539
Within the last week	Today	3.3125	41.1280	1.000	-117.852	124.477
	Within the last month	-17.5000	26.0116	.960	-94.131	59.131
	Within the last year	-18.3542	35.2199	.984	-122.113	85.405
	Never	-38.3125	26.0116	.589	-114.943	38.318
Within the last month	Today	20.8125	41.1280	.986	-100.352	141.977
	Within the last week	17.5000	26.0116	.960	-59.131	94.131
	Within the last year	-.8542	35.2199	1.000	-104.613	102.905

	Never	-20.8125	26.0116	.928	-97.443	55.818
Within the last year	Today	21.6667	47.4905	.990	-118.242	161.575
	Within the last week	18.3542	35.2199	.984	-85.405	122.113
	Within the last month	.8542	35.2199	1.000	-102.905	104.613
	Never	-19.9583	35.2199	.979	-123.717	83.800
Never	Today	41.6250	41.1280	.847	-79.539	162.789
	Within the last week	38.3125	26.0116	.589	-38.318	114.943
	Within the last month	20.8125	26.0116	.928	-55.818	97.443
	Within the last year	19.9583	35.2199	.979	-83.800	123.717

Homogeneous Subsets

Mean CFUs-BP

Tukey HSD[a,b]

Likert Sphygmomanometer 11 Answer-Question	N	Subset for alpha = 0.05 1
Today	2	12.000
Within the last week	8	15.313
Within the last month	8	32.813
Within the last year	3	33.667
Never	8	53.625
Sig.		.778

Oneway

Warnings

Post hoc tests are not performed for Mean CFUs-stetho because at least one group has fewer than two cases.

Descriptives

Mean CFUs-stetho

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Today	2	18.750	8.8388	6.2500	-60.664	98.164	12.5	25.0
Within the last week	11	18.273	22.5747	6.8065	3.107	33.439	3.0	84.0
Within the last month	8	22.250	20.8155	7.3594	4.848	39.652	6.5	55.5
Within the last year	1	28.500	.	.	.	.	28.5	28.5
Never	7	32.714	41.4818	15.6786	-5.650	71.079	4.0	119.5
Total	29	23.241	26.3949	4.9014	13.201	33.281	3.0	119.5

ANOVA

Mean CFUs-stetho

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	975.575	4	243.894	.316	.865
Within Groups	18531.735	24	772.156		
Total	19507.310	28			

Descriptives

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Prestetho-patho	58	0	139	14.52	25.282
Pre-stetho- non-patho	58	0	58	8.78	12.225
Post-stetho-patho	58	0	167	35.24	37.326
Post-stetho-nonpatho	58	0	91	16.21	19.166
prebp-patho	58	0	520	20.29	68.728
prebp-nonpatho	58	0	52	12.07	13.480
postBP-patho	58	0	335	22.22	46.571
postbp-nonpatho	58	0	278	23.14	42.390
Valid N (listwise)	58				

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Prestetho-patho	14.52	58	25.282	3.320
	Post-stetho-patho	35.24	58	37.326	4.901
Pair 2	Pre-stetho- non-patho	8.78	58	12.225	1.605
	Post-stetho-nonpatho	16.21	58	19.166	2.517
Pair 3	prebp-patho	20.29	58	68.728	9.024
	postBP-patho	22.22	58	46.571	6.115
Pair 4	prebp-nonpatho	12.07	58	13.480	1.770
	postbp-nonpatho	23.14	58	42.390	5.566

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Prestetho-patho & Post-stetho-patho	58	.071	.594
Pair 2	Pre-stetho- non-patho & Post-stetho-nonpatho	58	.045	.737
Pair 3	prebp-patho & postBP-patho	58	.228	.085
Pair 4	prebp-nonpatho & postbp-nonpatho	58	.031	.817

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Prestetho-patho - Post-stetho-patho	-20.724	43.562	5.720	-32.178	-9.270	-3.623	57	.001
Pair 2	Pre-stetho- non-patho - Post-stetho-nonpatho	-7.431	22.263	2.923	-13.285	-1.577	-2.542	57	.014
Pair 3	prebp-patho - postBP-patho	-1.931	73.691	9.676	-21.307	17.445	-.200	57	.843
Pair 4	prebp-nonpatho - postbp-nonpatho	-11.069	44.082	5.788	-22.660	.522	-1.912	57	.061

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Total_quantity-Prestet	23.29	58	31.483	4.134
	Total_quantity-poststet	51.45	58	41.398	5.436
Pair 2	Edge - prestet	20.07	58	25.788	3.386
	Edge-poststet	42.62	58	36.429	4.783
Pair 3	Diaphragm-prestet	3.22	58	8.848	1.162
	Diaphragm-poststet	8.83	58	10.337	1.357
Pair 4	CoNS-prestet	10.91	58	22.993	3.019
	CoNS-poststet	28.22	58	35.786	4.699
Pair 5	S.aureus-Prestet	3.41	58	6.421	.843
	S.aureus-poststet	5.91	58	9.561	1.255
Pair 6	Micrococcus-prestet	7.00	58	12.563	1.650
	Micrococcus-poststet	13.81	58	19.194	2.520
Pair 7	Bacillus-prestet	1.33	58	2.591	.340
	Bacillus-poststet	1.67	58	3.005	.395
Pair 8	E.coli-prestet	.03	58	.184	.024
	E.coli-prebp	.14	58	.605	.080
Pair 9	Coliforms_(exc E.coli)-prestet	.16	58	.586	.077
	Coliforms_(exc E.coli)-poststet	.64	58	1.764	.232
Pair 10	Corynebacterium- Presteth	.45	58	1.558	.205
	Corynebacterium-poststet	.72	58	3.077	.404

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Total_quantity-Prestet & Total_quantity-poststet	58	.079	.558
Pair 2	Edge - prestet & Edge-poststet	58	.075	.574
Pair 3	Diaphragm-prestet & Diaphragm-poststet	58	.047	.725
Pair 4	CoNS-prestet & CoNS-poststet	58	.127	.342
Pair 5	S.aureus-Prestet & S.aureus-poststet	58	-.168	.207
Pair 6	Micrococcus-prestet & Micrococcus-poststet	58	.107	.424
Pair 7	Bacillus-prestet & Bacillus-poststet	58	-.162	.225
Pair 8	E.coli-prestet & E.coli-prebp	58	.271	.039
Pair 9	Coliforms_(exc E.coli)-prestet & Coliforms_(exc E.coli)-poststet	58	-.046	.729
Pair 10	Corynebacterium- Presteth & Corynebacterium-poststet	58	.037	.781

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Total_quantity-Prestet - Total_quantity-poststet	-28.155	50.002	6.566	-41.303	-15.008	-4.288	57	.000
Pair 2	Edge - prestet - Edge-poststet	-22.552	43.017	5.648	-33.862	-11.241	-3.993	57	.000
Pair 3	Diaphragm-prestet - Diaphragm-poststet	-5.603	13.285	1.744	-9.097	-2.110	-3.212	57	.002
Pair 4	CoNS-prestet - CoNS-poststet	-17.310	40.004	5.253	-27.829	-6.792	-3.295	57	.002
Pair 5	S.aureus-Prestet - S.aureus-poststet	-2.500	12.380	1.626	-5.755	.755	-1.538	57	.130

Pair 6	Micrococcus-prestet - Micrococcus-poststet	-6.810	21.786	2.861	-12.539	-1.082	-2.381	57	.021
Pair 7	Bacillus-prestet - Bacillus-poststet	-.345	4.274	.561	-1.469	.779	-.614	57	.541
Pair 8	E.coli-prestet - E.coli-prebp	-.103	.583	.077	-.257	.050	-1.351	57	.182
Pair 9	Coliforms_(exc E.coli)-prestet - Coliforms_(exc E.coli)-poststet	-.483	1.885	.247	-.978	.013	-1.951	57	.056
Pair 10	Corynebacterium-Presteth - Corynebacterium-poststet	-.276	3.397	.446	-1.169	.617	-.619	57	.539

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Total_quantity-prebp	32.36	58	72.389	9.505
	Total_quantity-postbp	45.36	58	66.139	8.684
Pair 2	CoNS- presbp	16.53	58	68.635	9.012
	CoNS-postbp	18.97	58	44.728	5.873
Pair 3	S.aureus-prebp	3.43	58	6.580	.864
	S.aureus-postbp	2.72	58	5.699	.748
Pair 4	Micrococcus-prebp	10.28	58	12.597	1.654
	Micrococcus-postbp	20.40	58	41.942	5.507
Pair 5	Bacillus-prebp	1.45	58	6.816	.895
	Bacillus-postbp	2.14	58	5.714	.750
Pair 6	Corynebacterium-prebp	.34	58	.664	.087
	Corynebacterium-postbp	.60	58	1.589	.209
Pair 7	Coliforms_(exc E.coli)-prebp	.19	58	.847	.111
	Coliforms_(exc E.coli)-postbp	.41	58	1.929	.253
Pair 8	E.coli-prebp	.14	58	.605	.080
	E.coli-postbp	.12	58	.703	.092

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Total_quantity-prebp & Total_quantity-postbp	58	.113	.397
Pair 2	CoNS- presbp & CoNS-postbp	58	.245	.064

Pair 3	S.aureus-prebp & S.aureus-postbp	58	.257	.051
Pair 4	Micrococcus-prebp & Micrococcus-postbp	58	.001	.996
Pair 5	Bacillus-prebp & Bacillus-postbp	58	.736	.000
Pair 6	Corynebacterium-prebp & Corynebacterium-postbp	58	.199	.135
Pair 7	Coliforms_(exc E.coli)-prebp & Coliforms_(exc E.coli)-postbp	58	-.049	.716
Pair 8	E.coli-prebp & E.coli-postbp	58	-.040	.767

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Total_quantity-prebp - Total_quantity-postbp	-13.000	92.360	12.128	-37.285	11.285	-1.072	57	.288
Pair 2	CoNS- presbp - CoNS-postbp	-2.431	72.177	9.477	-21.409	16.547	-.257	57	.798
Pair 3	S.aureus-prebp - S.aureus-postbp	.707	7.516	.987	-1.269	2.683	.716	57	.477
Pair 4	Micrococcus-prebp - Micrococcus-postbp	-10.121	43.785	5.749	-21.633	1.392	-1.760	57	.084
Pair 5	Bacillus-prebp - Bacillus-postbp	-.690	4.665	.613	-1.916	.537	-1.126	57	.265
Pair 6	Corynebacterium-prebp - Corynebacterium-postbp	-.259	1.596	.210	-.678	.161	-1.234	57	.222
Pair 7	Coliforms_(exc E.coli)-prebp - Coliforms_(exc E.coli)-postbp	-.224	2.144	.282	-.788	.340	-.796	57	.429
Pair 8	E.coli-prebp - E.coli-postbp	.017	.946	.124	-.231	.266	.139	57	.890

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Stethoscope:Reason:Unimportant	1.97	29	.186	.034
	Sphygmomanometer:Reason:Unimportant	1.90	29	.310	.058
Pair 2	Stethoscope:Reason:Not_applicable	1.72	29	.455	.084
	Sphygmomanometers:Reason:Not_applicable	1.83	29	.384	.071

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Stethoscope:Reason:Unimportant & Sphygmomanometer:Reason:Unimportant	29	.556	.002
Pair 2	Stethoscope:Reason:Not_applicable & Sphygmomanometers:Reason:Not_applicable	29	.740	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Stethoscope:Reason:Unimportant - Sphygmomanometer:Reason:Unimportant	.069	.258	.048	-.029	.167	1.440	28	.161
Pair 2	Stethoscope:Reason:Not_applicable - Sphygmomanometers:Reason:Not_applicable	-.103	.310	.058	-.221	.014	-1.797	28	.083

Correlations

Correlations

		Stethoscope:Knowledgeable_in_disinfection_procedures	Stethoscope:How_often_think_should_disinfect
Stethoscope:Knowledgeable_in_disinfection_procedures	Pearson Correlation	1	-.036
	Sig. (2-tailed)		.855
	N	29	29
Stethoscope:How_often_think_should_disinfect	Pearson Correlation	-.036	1
	Sig. (2-tailed)	.855	
	N	29	29

Nonparametric Correlations

Correlations

			Sphygmomanometer:How_often_think_should_disinfect	Sphygmomanometer:Knowledgeable_in_disinfection_procedures
Spearman's rho	Sphygmomanometer:How_often_think_should_disinfect	Correlation Coefficient	1.000	-.022
		Sig. (2-tailed)	.	.911
		N	29	29
	Sphygmomanometer:Knowledgeable_in_disinfection_procedures	Correlation Coefficient	-.022	1.000
		Sig. (2-tailed)	.911	.
		N	29	29

Nonparametric Correlations

Correlations

			Stethoscope:How_often_think_should_disinfect	Stethoscope:Knowledgeable_in_disinfection_procedures
Spearman's rho	Stethoscope:How_often_think_should_disinfect	Correlation Coefficient	1.000	-.059
		Sig. (2-tailed)	.	.761
		N	29	29
	Stethoscope:Knowledgeable_in_disinfection_procedures	Correlation Coefficient	-.059	1.000
		Sig. (2-tailed)	.761	.
		N	29	29

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Opinion-Have_received_education_of_disinfection-stethoscope	1.79	29	.412	.077
	Opinion_have_received_education_of_disinfection-thermometer	1.14	29	.351	.065
Pair 2	Opinion-Have_received_education_of_disinfection-stethoscope	1.79	29	.412	.077
	Opinion-Have_received_education_of_disinfection-otoscope	1.41	29	.501	.093
Pair 3	Opinion-Have_received_education_of_disinfection-stethoscope	1.79	29	.412	.077
	opinion:Have_received_education_of_disinfection-speculums	1.41	29	.501	.093
Pair 4	Opinion-Have_received_education_of_disinfection-stethoscope	1.79	29	.412	.077
	opinion:Have_received_education_of_disinfection:Ophthalmoscope	1.55	29	.506	.094
Pair 5	Opinion:Have_received_education_of_disinfection:Sphygmomanometers	1.86	29	.351	.065
	Opinion-Have_received_education_of_disinfection-stethoscope	1.79	29	.412	.077
Pair 6	Opinion:Have_received_education_of_disinfection:Sphygmomanometers	1.86	29	.351	.065
	opinion:Have_received_education_of_disinfection:Ophthalmoscope	1.55	29	.506	.094
Pair 7	Opinion:Have_received_education_of_disinfection:Sphygmomanometers	1.86	29	.351	.065
	opinion:Have_received_education_of_disinfection-speculums	1.41	29	.501	.093

Pair 8	Opinion:Have_received_education_of_disinfection:Sphygmomanometers	1.86	29	.351	.065
	Opinion-Have_received_education_of_disinfection-otoscope	1.41	29	.501	.093
Pair 9	Opinion:Have_received_education_of_disinfection:Sphygmomanometers	1.86	29	.351	.065
	Opinion_have_received_education_of_disinfection-thermometer	1.14	29	.351	.065

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Opinion-Have_received_education_of_disinfection-stethoscope & Opinion_have_received_education_of_disinfection-thermometer	29	.204	.288
Pair 2	Opinion-Have_received_education_of_disinfection-stethoscope & Opinion-Have_received_education_of_disinfection-otoscope	29	.083	.667
Pair 3	Opinion-Have_received_education_of_disinfection-stethoscope & opinion:Have_received_education_of_disinfection-speculums	29	-.089	.645

Pair 4	Opinion-Have_received_education_of_disinfection-stethoscope & opinion:Have_received_education_of_disinfection:Ophthalmoscope	29	.395	.034
Pair 5	Opinion:Have_received_education_of_disinfection:Sphygmomanometers & Opinion-Have_received_education_of_disinfection-stethoscope	29	.536	.003
Pair 6	Opinion:Have_received_education_of_disinfection:Sphygmomanometers & opinion:Have_received_education_of_disinfection:Ophthalmoscope	29	.243	.205
Pair 7	Opinion:Have_received_education_of_disinfection:Sphygmomanometers & opinion:Have_received_education_of_disinfection-speculums	29	-.070	.718
Pair 8	Opinion:Have_received_education_of_disinfection:Sphygmomanometers & Opinion-Have_received_education_of_disinfection-otoscope	29	-.070	.718

Pair 9	Opinion:Have_received_education_of_disinfection:Sphygmomanometers & Opinion_have_received_education_of_disinfection-thermometer	29	-.130	.501

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Opinion-Have_received_education_of_disinfection-stethoscope - Opinion_have_received_education_of_disinfection-thermometer	.655	.484	.090	.471	.839	7.294	28	.000
Pair 2	Opinion-Have_received_education_of_disinfection-stethoscope - Opinion-Have_received_education_of_disinfection-otoscope	.379	.622	.115	.143	.616	3.285	28	.003
Pair 3	Opinion-Have_received_education_of_disinfection-stethoscope - opinion:Have_received_education_of_disinfection-speculums	.379	.677	.126	.122	.637	3.018	28	.005
Pair 4	Opinion-Have_received_education_of_disinfection-stethoscope - opinion:Have_received_education_of_disinfection:Ophthalmoscope	.241	.511	.095	.047	.436	2.544	28	.017

Pair 5	Opinion:Have_received_education_of_disinfection:Sphygmomanometers - Opinion-Have_received_education_of_disinfection-stethoscope	.069	.371	.069	-.072	.210	1.000	28	.326
Pair 6	Opinion:Have_received_education_of_disinfection:Sphygmomanometers - opinion:Have_received_education_of_disinfection:Ophthalmoscope	.310	.541	.101	.104	.516	3.087	28	.005
Pair 7	Opinion:Have_received_education_of_disinfection:Sphygmomanometers - opinion:Have_received_education_of_disinfection-speculums	.448	.632	.117	.208	.689	3.822	28	.001
Pair 8	Opinion:Have_received_education_of_disinfection:Sphygmomanometers - Opinion-Have_received_education_of_disinfection-otoscope	.448	.632	.117	.208	.689	3.822	28	.001
Pair 9	Opinion:Have_received_education_of_disinfection:Sphygmomanometers - Opinion_have_received_education_of_disinfection-thermometer	.724	.528	.098	.523	.925	7.392	28	.000

Nonparametric Correlations

Correlations

			Stethoscope-think_source_of_cross-contamination	Stethoscope-Think:important_to_disinfect_surface
Spearman's rho	Stethoscope-think_source_of_cross-contamination	Correlation Coefficient	1.000	.506**
		Sig. (2-tailed)	.	.005
		N	29	29
	Stethoscope-Think:important_to_disinfect_surface	Correlation Coefficient	.506**	1.000
		Sig. (2-tailed)	.005	.
		N	29	29

Nonparametric Correlations

Correlations

			Sphygmomanometer-Think:Important_to_disinfect_surface	Sphygmomanometer-think_source_of_cross-contamination
Spearman's rho	Sphygmomanometer-Think:Important_to_disinfect_surface	Correlation Coefficient	1.000	.596**
		Sig. (2-tailed)	.	.001
		N	29	29
	Sphygmomanometer-think_source_of_cross-contamination	Correlation Coefficient	.596**	1.000
		Sig. (2-tailed)	.001	.
		N	29	29

Nonparametric Correlations

Correlations

			Stethoscope:See_other_students_disinfect	Stethoscope:On_average-do_you_disinfect
Spearman's rho	Stethoscope:See_other_students_disinfect	Correlation Coefficient	1.000	.489**
		Sig. (2-tailed)	.	.007
		N	29	29
		Correlation Coefficient	.489**	1.000

	Stethoscope:On_average-do_you_disinfect	Sig. (2-tailed)	.007	.
		N	29	29

Nonparametric Correlations

Correlations

			Sphygmomanometer:See_other_students_disinfect	Sphygmomanometer:On_average-Do_you_disinfect
Spearman's rho	Sphygmomanometer:See_other_students_disinfect	Correlation Coefficient	1.000	.415*
		Sig. (2-tailed)	.	.025
		N	29	29
	Sphygmomanometer:On_average-Do_you_disinfect	Correlation Coefficient	.415*	1.000
		Sig. (2-tailed)	.025	.
		N	29	29

*. Correlation is significant at the 0.05 level (2-tailed).

Nonparametric Correlations

Correlations

			Stethoscope-think_source_of_cross-contamination	Stethoscope:On_average-do_you_disinfect
Spearman's rho	Stethoscope-think_source_of_cross-contamination	Correlation Coefficient	1.000	.108
		Sig. (2-tailed)	.	.575
		N	29	29
	Stethoscope:On_average-do_you_disinfect	Correlation Coefficient	.108	1.000
		Sig. (2-tailed)	.575	.
		N	29	29

Nonparametric Correlations

Correlations

			Sphygmomanometer-think_source_of_cross-contamination	Sphygmomanometer:On_average-Do_you_disinfect
Spearman's rho	Sphygmomanometer-think_source_of_cross-contamination	Correlation Coefficient	1.000	.145
		Sig. (2-tailed)	.	.453
		N	29	29
		Correlation Coefficient	.145	1.000

	Sphygmomanometer:On_average-Do_you_disinfect	Sig. (2-tailed)	.453	.
		N	29	29

Nonparametric Correlations

Correlations

			Sphygmomanometer:On_average-Do_you_disinfect	Sphygmomanometer:Knowledgeable_in_disinfection_procedures
Spearman's rho	Sphygmomanometer:On_average-Do_you_disinfect	Correlation Coefficient	1.000	.419*
		Sig. (2-tailed)	.	.024
		N	29	29
	Sphygmomanometer:Knowledgeable_in_disinfection_procedures	Correlation Coefficient	.419*	1.000
		Sig. (2-tailed)	.024	.
		N	29	29

*. Correlation is significant at the 0.05 level (2-tailed).

Nonparametric Correlations

Correlations

			Stethoscope:On_average-do_you_disinfect	Stethoscope:Knowledgeable_in_disinfection_procedures
Spearman's rho	Stethoscope:On_average-do_you_disinfect	Correlation Coefficient	1.000	.188
		Sig. (2-tailed)	.	.328
		N	29	29
	Stethoscope:Knowledgeable_in_disinfection_procedures	Correlation Coefficient	.188	1.000
		Sig. (2-tailed)	.328	.
		N	29	29

Nonparametric Correlations

Correlations

			Average-disinfect_hands_in_clinic	Think:Education_regarding_hand_hygiene_and_equipment_disinfectio_important
Spearman's rho	Average-disinfect_hands_in_clinic	Correlation Coefficient	1.000	-.065
		Sig. (2-tailed)	.	.736
		N	29	29
		Correlation Coefficient	-.065	1.000

	Think:Education_regarding_hand_hygiene_and_equipment_disinfectio_important	Sig. (2-tailed)	.736	.
		N	29	29

Frequency Table

Bacterium

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	CoNS	156	35.2	35.2	35.2
	S.aureus	99	22.3	22.3	57.6
	Micrococcus spp	156	35.2	35.2	92.8
	Coliforms	19	4.3	4.3	97.1
	E.coli	13	2.9	2.9	100.0
	Total	443	100.0	100.0	

Chloramphenicol

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Resistant	6	1.4	1.4	1.4
	Intermediate	13	2.9	2.9	4.3
	Susceptible	424	95.7	95.7	100.0
	Total	443	100.0	100.0	

Ciprofloxacin

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Resistant	19	4.3	4.3	4.3
	Intermediate	11	2.5	2.5	6.8
	Susceptible	413	93.2	93.2	100.0
	Total	443	100.0	100.0	

Vancomycin

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Resistant	84	19.0	19.0	19.0
	Intermediate	1	.2	.2	19.2
	Susceptible	358	80.8	80.8	100.0
	Total	443	100.0	100.0	

Erythromycin

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Resistant	138	31.2	31.2	31.2
	Intermediate	49	11.1	11.1	42.2
	Susceptible	256	57.8	57.8	100.0
	Total	443	100.0	100.0	

Amoxicillin

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Resistant	104	23.5	23.5	23.5
	Intermediate	31	7.0	7.0	30.5
	Susceptible	308	69.5	69.5	100.0
	Total	443	100.0	100.0	

Resistance_class/s

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No resistance	244	55.1	55.1	55.1
	Resistance to 1 class	98	22.1	22.1	77.2
	Resistance to 2 classes	53	12.0	12.0	89.2
	Resistance to 3 classes	47	10.6	10.6	99.8
	Resistance to 5 classes	1	.2	.2	100.0
	Total	443	100.0	100.0	

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Bacterium * Chloramphenicol	443	100.0%	0	0.0%	443	100.0%
Bacterium * Ciprofloxacin	443	100.0%	0	0.0%	443	100.0%
Bacterium * Vancomycin	443	100.0%	0	0.0%	443	100.0%
Bacterium * Erythromycin	443	100.0%	0	0.0%	443	100.0%
Bacterium * Amoxicillin	443	100.0%	0	0.0%	443	100.0%
Bacterium * Resistance_class/s	443	100.0%	0	0.0%	443	100.0%

Bacterium * Chloramphenicol Crosstabulation

Count

		Chloramphenicol			
		Resistant	Intermediate	Susceptible	Total
Bacterium	CoNS	0	5	151	156
	S.aureus	0	3	96	99
	Micrococcus spp	3	0	153	156
	Coliforms	3	3	13	19
	E.coli	0	2	11	13
Total		6	13	424	443

Bacterium * Ciprofloxacin Crosstabulation

Count

		Ciprofloxacin			
		Resistant	Intermediate	Susceptible	Total
Bacterium	CoNS	7	5	144	156
	S.aureus	4	2	93	99
	Micrococcus spp	5	0	151	156
	Coliforms	3	2	14	19
	E.coli	0	2	11	13
Total		19	11	413	443

Bacterium * Vancomycin Crosstabulation

Count

		Vancomycin			
		Resistant	Intermediate	Susceptible	Total
Bacterium	CoNS	30	1	125	156
	S.aureus	15	0	84	99
	Micrococcus spp	28	0	128	156
	Coliforms	7	0	12	19
	E.coli	4	0	9	13
Total		84	1	358	443

Bacterium * Erythromycin Crosstabulation

Count

		Erythromycin			
		Resistant	Intermediate	Susceptible	Total
Bacterium	CoNS	56	26	74	156
	S.aureus	22	22	55	99
	Micrococcus spp	51	1	104	156
	Coliforms	5	0	14	19
	E.coli	4	0	9	13
Total		138	49	256	443

Bacterium * Resistance_class/s Crosstabulation

Count

		Resistance_class/s					
		No resistance	Resistance to 1 class	Resistance to 2 classes	Resistance to 3 classes	Resistance to 5 classes	Total
Bacterium	CoNS	76	38	17	25	0	156
	S.aureus	59	20	12	8	0	99
	Micrococcus spp	95	33	17	10	1	156
	Coliforms	7	5	5	2	0	19
	E.coli	7	2	1	3	0	13
Total		244	98	52	48	1	443

Oneway

Descriptives

Bacterium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Resistant	6	3.50	.548	.224	2.93	4.07	3	4
Intermediate	13	2.54	1.613	.447	1.56	3.51	1	5
Susceptible	424	2.14	1.027	.050	2.05	2.24	1	5
Total	443	2.17	1.055	.050	2.08	2.27	1	5

ANOVA

Bacterium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.661	2	6.331	5.816	.003
Within Groups	478.955	440	1.089		
Total	491.616	442			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bacterium

Tukey HSD

(I) Chloramphenicol	(J) Chloramphenicol	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Resistant	Intermediate	.962	.515	.149	-.25	2.17
	Susceptible	1.356*	.429	.005	.35	2.36
Intermediate	Resistant	-.962	.515	.149	-2.17	.25
	Susceptible	.395	.294	.372	-.30	1.09
Susceptible	Resistant	-1.356*	.429	.005	-2.36	-.35
	Intermediate	-.395	.294	.372	-1.09	.30

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Bacterium

Tukey HSD[a,b]

Chloramphenicol	N	Subset for alpha = 0.05: 1	2
Susceptible	424	2.14	
Intermediate	13	2.54	2.54
Resistant	6		3.50
Sig.		.619	.060

Oneway

Descriptives

Bacterium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean: Lower Bound	Upper Bound	Minimum	Maximum
Resistant	19	2.21	1.134	.260	1.66	2.76	1	4

Intermediate	11	2.45	1.695	.511	1.32	3.59	1	5
Susceptible	413	2.16	1.032	.051	2.06	2.26	1	5
Total	443	2.17	1.055	.050	2.08	2.27	1	5

ANOVA

Bacterium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.927	2	.464	.416	.660
Within Groups	490.689	440	1.115		
Total	491.616	442			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bacterium

Tukey HSD

(I) Ciprofloxacin	(J) Ciprofloxacin	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Resistant	Intermediate	-.244	.400	.815	-1.18	.70
	Susceptible	.046	.248	.981	-.54	.63
Intermediate	Resistant	.244	.400	.815	-.70	1.18
	Susceptible	.290	.323	.641	-.47	1.05
Susceptible	Resistant	-.046	.248	.981	-.63	.54
	Intermediate	-.290	.323	.641	-1.05	.47

Bacterium

Tukey HSD[a,b]

Ciprofloxacin	N	Subset for alpha = 0.05 1
Susceptible	413	2.16
Resistant	19	2.21
Intermediate	11	2.45
Sig.		.653

Oneway

Descriptives

Bacterium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Resistant	84	2.29	1.178	.129	2.03	2.54	1	5
Intermediate	1	1.00	.	.	.	.	1	1
Susceptible	358	2.15	1.023	.054	2.04	2.26	1	5
Total	443	2.17	1.055	.050	2.08	2.27	1	5

ANOVA

Bacterium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.619	2	1.309	1.178	.309
Within Groups	488.998	440	1.111		
Total	491.616	442			

Oneway

Descriptives

Bacterium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Resistant	138	2.12	1.084	.092	1.94	2.31	1	5
Intermediate	49	1.49	.545	.078	1.33	1.65	1	3
Susceptible	256	2.33	1.060	.066	2.20	2.46	1	5
Total	443	2.17	1.055	.050	2.08	2.27	1	5

ANOVA

Bacterium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.688	2	14.844	14.139	.000
Within Groups	461.928	440	1.050		
Total	491.616	442			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bacterium

Tukey HSD

(I) Erythromycin	(J) Erythromycin	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Resistant	Intermediate	.633*	.170	.001	.23	1.03
	Susceptible	-.209	.108	.131	-.46	.05
Intermediate	Resistant	-.633*	.170	.001	-1.03	-.23
	Susceptible	-.842*	.160	.000	-1.22	-.47
Susceptible	Resistant	.209	.108	.131	-.05	.46
	Intermediate	.842*	.160	.000	.47	1.22

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Bacterium

Tukey HSD[a,b]

Erythromycin	N	Subset for alpha = 0.05: 1	2
Intermediate	49	1.49	
Resistant	138		2.12
Susceptible	256		2.33
Sig.		1.000	.339

Means for groups in homogeneous subsets are displayed.

Oneway

Descriptives

Bacterium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Resistant	104	1.83	1.092	.107	1.61	2.04	1	5
Intermediate	31	2.97	.706	.127	2.71	3.23	1	5
Susceptible	308	2.21	1.023	.058	2.10	2.33	1	5
Total	443	2.17	1.055	.050	2.08	2.27	1	5

ANOVA

Bacterium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.481	2	16.241	15.564	.000
Within Groups	459.135	440	1.043		
Total	491.616	442			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Bacterium

Tukey HSD

(I) Amoxicillin	(J) Amoxicillin	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Resistant	Intermediate	-1.141*	.209	.000	-1.63	-.65
	Susceptible	-.384*	.116	.003	-.66	-.11
Intermediate	Resistant	1.141*	.209	.000	.65	1.63
	Susceptible	.757*	.192	.000	.30	1.21
Susceptible	Resistant	.384*	.116	.003	.11	.66
	Intermediate	-.757*	.192	.000	-1.21	-.30

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Bacterium

Tukey HSD[a,b]

Amoxicillin	N	Subset for alpha = 0.05	
		1	2
Resistant	104	1.83	
Susceptible	308	2.21	
Intermediate	31		2.97
Sig.		.078	1.000

Appendix Z: Amendment to methodology

DUT DURBAN UNIVERSITY OF TECHNOLOGY

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Research and Postgraduate Support Directorate
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28 July 2017

Ms J Logtenberg
66 Lewis Drive
Amanzimtoti
4126

Dear Ms Logtenberg

Application for Amendment of Approved Research Proposal

Investigation of diagnostic equipment as reservoirs for microbial growth and sources of microbial transfer, hygiene practices of students and efficacy of disinfectants

I am pleased to inform you that your application for amendment to change from the modified Kirby Bauer technique to a modified AOAC use dilution method for assessing the efficacy of the disinfectants has been Approved. In addition changes to Appendix R has been approved

Yours Sincerely

[redacted]

Professor J K Adam
Chairperson: IREC

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2017 -07- 28
INSITUTUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA

Appendix AA: Table used for interpretation of microbial susceptibility to antibiotics

		Zone of inhibition in mm		
Antibiotic	**Bacterium**	**Resistant**	**Intermediate**	**Susceptible**
Chloramphenicol 30mg	Staphylococcus	< 12	13 -17	>18
	Enterobacteria	< 12	13 -17	>18
	Micrococci	**No values available Used readings for Staphylococcus**		
Ciprofloxacin 15 mg	Staphylococcus	< 15	16 -20	>21
	Enterobacteria	< 20	21- 30	>31
	Micrococci	**No values available Used readings for Staphylococcus**		
Erythromycin 15 mg	Staphylococcus	< 13	14-22	>23
	Enterobacteria	**No values available Used readings for Staphylococcus**		
	Micrococci			
Vancomycin 30 mg	Staphylococcus	**No values available Used readings for Enterococcus**		
	Enterobacteria			
	Micrococci			
	Enterococcus	< 14	15- 16	>17
Amoxicillin 25 mg	Other	< 13	14 -17	>18
	Staphylococcus	< 19		>20

Source: (Clinical and Laboratory standards Institute 2014)