Microbial Quality of Communal Hand Washing Water at African Funerals in the Mangaung Region

by

Ntsokolo Alliestar Moabi

Submitted in fulfilment of the requirements for the Degree

Magister Technologiae: Environmental Health

Department of Life Sciences

Faculty of Health and Environmental Sciences

Central University of Technology, Free State

BLOEMFONTEIN

2016

Declaration

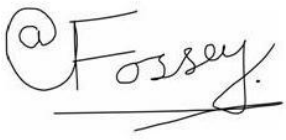
I, Ntsokolo Alliestar Moabi, identity number [redacted] and student number [redacted], do hereby declare that this research project submitted to the Central University of Technology, Free State, for the Degree Magister Technologiae in Environmental Health, is my own, independent work. This work complies with the code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person for the attainment of a qualification.

……

Ntsokolo Alliestar Moabi

2016

I certify that the above statement is correct.



……..

Professor Annabel Fossey (Supervisor)

Acknowledgements

To God, I give all the glory. I managed to complete this project through all the challenges I encountered over the years. Thank you for giving me strength, patience, wisdom and perseverance to complete this project.

To the following people I wish to offer my most heartfelt gratitude:

1. My research supervisor, Professor Annabel Fossey, thank you for your guidance and teachings. Through your constructive criticism and efforts, you helped to ensure that the final product became good quality research work. Dr Elsa Potgieter, co-supervisor, thank you for your guidance and valuable advice. Your contributions have assisted me immensely.

2. BT Funerals, particularly to Mr Petrus Baukudi and Ms Boitumelo Baukudi, I will never be able to thank you enough for your contribution to the success of this project. Through your efforts, I managed to successfully visit 42 funerals I needed for my project. Although this was unprecedented within our community and African culture, you managed to persuade the reluctant families to grant me their permission. To the families that participated in the project, I thank you.

3. To my father, Mr Thami James Moabi, thank you for all your support and my mother, Mrs Matiisetso Maria Moabi, your constant prayers and encouragement have been a great motivation to me. To my siblings; Nikiwe Mokitimi, Memmeng Lekhafola, Ngaka Mokitimi and Mphithizeli Moabi thank you for your support, love and encouragement. And to my beloved nephews; Lwethuthando and Lungelo, thank you for your love.

4. To the following members of my extended family, your prayers and advice did not go unnoticed:

   My brother and sister, Mr Paul Moeketsane and Mrs Mpho Moeketsane, your ongoing encouragement contributed to the success of this project. Abuti Tshepo Kingsley Phakisi, you have been a great pillar of strength and a voice of reason throughout the years of my study. Most of the time I used you as a punching bag to release all the frustrations. You brought so much comfort to me throughout this period. Bhut' Thabo James Boutu, your prayers, love and support played a pivotal role in this project. Abuti Thabang Molatou, thank you for your support and encouragement.

   To Bhut' Bongani Gqwetha, Mme Gontse Mosola, Mme Portia Mofokeng, Mme Nikiwe Lerotholi, Mme Nozi Moroka, Mme Elisa Sibeko, Mme Lineo Modisaesi and Mrs Lulu du Bruyn (Mommy) I thank you for your constant support, your prayers, love and advices. Indeed, I can say it is finished, thank you GOD!

I love you all very much, and to all of you I say these words: "the Lord blesses you and keeps you; the Lord makes his face shine upon you and be gracious to you; the Lord turns his face toward you and give you peace."

Table of Contents

List of Tables

List of Figures

Abstract

African people's lives are defined by various ritual practices. At funerals a cleansing ritual is performed to wash away bad omens attracted at the cemetery. This ritual entails the washing of hands in a communal hand washing bowl of standing water outside the gate of the bereaved family's house. Many mourners wash their hands in the same bowl without replenishment of the water. Depending on mourners' hygiene practices, they introduce microorganisms into the water during the ritual. These microorganisms may be pathogenic or of faecal origin. Because mourners allow their hands to air dry and traditionally eat their funeral meal with their bare hands, they are at risk of ingesting pathogens. Therefore, the aim of this study was to investigate the microbial quality of communal hand washing ritual water at 42 African funerals in the Mangaung region of Central South Africa.

Microbial water quality was determined for water samples collected from the yard tap (the control), and from a communal hand washing bowl before and after the ritual. Total aerobic bacteria were enumerated using the heterotrophic plate count (HPC) method, while coliform bacteria and *Escherichia coli* were enumerated using the Colilert-18 method. Residual free chlorine was also determined as an indicator of efficacy of the disinfection process. A health potential index (HPI) that describes the potential health threat of a water sample was also calculated for each sample.

Residual free chlorine levels, HPC values, number of coliforms and number of *E. coli* were all within the prescribed limits for all yard tap samples. Free chlorine levels were mostly less than the prescribed limit for more than 50% of the samples taken before the ritual, while all samples taken after the ritual had less free chlorine than prescribed. Percentages of funerals with compliant water samples taken from the bowls before the ritual were for HPC, 95.2%, for coliforms, 78.6% and for *E. coli*, 88.0%. This demonstrates that some bowls were contaminated before the commencement of the ritual. A substantial decline in water quality was evident for the samples collected after the ritual. The

percentage of compliant funerals for the water samples taken from the bowls after the ritual were for HPC, 45.2%, for coliforms, 2.4% and for *E. coli*, 47.6%.

The calculated HPI also showed that all the yard tap water samples were safe. The water samples collected before and after the ritual showed an increase in the number of samples that posed an intermediate or high health threat to mourners. For the samples collected before the ritual, 7.1% funerals posed an intermediate threat and 7.1% funerals posed a high threat to mourners. For the samples collected after the ritual, 26.2% funerals posed an intermediate threat and 59.5% funerals posed a high threat to mourners.

This study supports the notion that the water in the bowls used for the hand washing ritual were contaminated before the ritual, which can only be attributed to prior contamination of the bowls before or during the preparation of the ritual water. Usually the bowls are also used for other domestic purposes, which could have contributed to the contamination of the bowls. Furthermore, the high levels of faecally derived microorganisms in this ritual water may also be indicative of a lack of proper hand hygiene practices by the people preparing the water. The high levels of faecally derived microorganisms in the water after the performance of the ritual are indicative of the introduction of microorganisms by the hands of the mourners during the ritual. This evidence supports the idea that proper hand hygiene practices were not observed by a large number of mourners. Mourners are thus at risk of being exposed to harmful pathogens, when eating the funeral meal with their bare hands.

This study strongly suggests that hand washing ritual practices at African funerals have the potential for disease transmission convergence. This study has shown that disease transmission could be linked to poor hand sanitation and poor personal hygiene practices, which puts mourners in a vulnerable position, particularly children, the elderly and the immune-compromised.

Chapter 1

Introduction

1.1 Introduction

African people have great respect and honour for their traditional rituals and symbols. African people's lives are defined by various ritual practices. Rituals are considered to be a way of expressing their beliefs. They perform rituals to strengthen their relations and interactions with family members who have died and believe that they have passed over to the afterlife (Kastenbaum 2003). Dead family members become the respected ancestors in African cultures and it is believed by the living that ancestors are watching over the living and protecting them (Kastenbaum 2003).

In African culture it is also believed that people visiting the cemetery will invite bad omens and become unclean. Therefore, people that visit the cemetery will perform a cleansing ritual of self-purification once they have returned from the cemetery to remove bad omens. This cleansing ritual involves the washing of hands in a communal hand washing bowl that is placed outside the gate of the bereaved family's house. Mourners rinse their hands in a communal hand washing bowl of standing (stagnant) water that may contain pieces of cut aloe leaf (Kastenbaum 2003). Many African people believe that aloe will remove bad omens during self-purification.

The communal hand washing bowl will remain outside the gate of the bereaved family's house for the duration of the funeral without replenishment of the water. Hand washing is conducted in groups of three to six people at a time before mourners enter the bereaved family's house to enjoy the funeral meal, before their departure and return to their homes (Kastenbaum 2003). Once the mourners had completed the cleansing ritual; the shovels used at the cemetery will also be cleansed by sprinkling them with the water from the communal hand washing bowl. Africans believe that the water in the

communal hand washing bowl contains all the washed off dirt and bad omens gathered by the mourners at the cemetery.

Once mourners returned from the cemetery, they are invited to the bereaved family's house for the funeral meal. The meal is offered as a token of appreciation from the bereaved family to the mourners. According to African culture, people are expected to consume the food with their bare hands. When mourners wash hands in the communal hand washing bowl of standing water, microorganisms are introduced into the water. The load of microorganisms increases as more mourners continue to wash their hands. The concentration of microorganisms will depend on the personal hygiene maintained by the mourners (Abd-Elaleem et al. 2014). Because mourners eat with their bare hands, they may now be exposed to harmful microorganisms that have been transferred to their hands during the hand washing ritual (O'Toole et al. 2009). Washing of hands in the communal hand washing bowl could potentially become a source for the transmission of harmful microorganisms, which include faecal and coliform bacteria as well as a wide spectrum of other pathogenic microorganisms.

Water-borne diseases are typically caused by enteric pathogens which are transmitted via the faecal–oral route (Barna & Kádár 2012). Faecally derived microorganisms are excreted with faeces and indirectly consumed by unsuspecting individuals in a form of faecally contaminated water or food. Other pathogens, which are not necessarily faecally excreted, can also be transmitted by water. These are opportunistic pathogens which may be part of the normal flora on the human body (Moosavi & Lofti 2009). These types of pathogens can then be released into the water from wounds, lesions or ulcers found on a person's hands. Furthermore, the dirt that accumulates on hands and underneath nails of people may also introduce harmful microorganisms into the communal hand washing bowls used for the hand washing rituals at African funerals.

1.2 Aim and objectives

From personal experience and newspaper reports (Maletsky 2007; Hlungwani et al. 2010; Lamond & Kinyanjui 2012) it is known that many people attending African funerals become ill after the funerals. These people typically demonstrate symptoms of diarrhoeal related diseases. No empirical study of the potential transfer of harmful microorganisms via the communal hand washing bowls at African funerals could be found after an extensive search of the literature. Therefore, the aim of this study was to investigate microbial quality of communal hand washing ritual water at African funerals in the Mangaung region of Central South Africa.

In order to address this aim, the following objectives were devised:

- To liaise prior to water sample collection with a funeral undertaker, whom communicated with the bereaved family and obtained written permission for the collection of water samples;
- To collect water samples at funerals from the municipal water supply yard tap on the premises and communal hand washing bowls before and after the hand washing ritual;
- To determine the microbiological quality of the water samples, together with the measurement of free residual chlorine;
- To attempt to count the number of mourners that had washed their hands in the communal hand washing bowls; and
- To statistically analyse the data and draw conclusions.

1.3 Layout of the dissertation

The dissertation is partitioned into six chapters, namely:

Chapter 1: *Introduction*

In this chapter, the research project is introduced, the problem, aim and the objectives stated.

Chapter 2: *Literature Review*

In this chapter, a comprehensive review of the literature pertaining to use of water in African rituals, particularly at African funerals, is presented. The importance of water quality and potential dangers of contaminated water used in rituals are highlighted.

Chapter 3: *Materials and Methods*

In this chapter, all materials and methods used in this research project is presented.

Chapter 4: *Quality of Ritual Water Used at African Funerals*

In this chapter, the microbial quality of the hand washing water used at 42 African funerals in the Mangaung Metropolitan Municipality is presented.

Chapter 5: *Health Potential Index of Ritual Water Used at African Funerals*

In this chapter, the formulation of a Health Potential Index (HPI) is presented. The results of the application of the HPI on the microbial quality of the water samples, collected at 42 African funerals, is presented.

Chapter 6: *Discussion and Conclusion*

In this chapter, an overall discussion of the microbial quality of the hand washing ritual water used at 42 African funerals in the Mangaung Metropolitan Municipality is presented together with concluding remarks.

References: The references of this dissertation were generated with the reference manager Mendeley.

Annexure: Letter of permission in presented.

Chapter 2

Literature Review

2.1 Introduction to African rituals

In the lives of the African people, rituals and symbols play an important role (Mankga 2013). Through rituals, tribes of African people express their beliefs and culture, thereby keeping their social structure intact. Although there are many definitions and descriptions of rituals, generally, a ritual can be described as a sequence of activities performed by any tribe or individual, involving gestures, words and objects at predestined places, depending upon the particular ritual (Castle & Phillips 2003).

African rituals differ across cultures and tribes. Rituals give a symbolic expression of certain feelings and thoughts of individuals or groups of individuals (Castle & Phillips 2003). In African culture, rituals are perceived as a form of identity for African people and the manner in which they live their lives. Even though some ritual practices may be common across different African tribes, there are a number of rituals which can separate one tribe from another in terms of how they are performed.

Generally rituals are performed at different stages of life for particular purposes. Some of these rituals include the introduction of a new baby in a family, the celebration of the rite of passage of females and males from childhood entering into adulthood, the performance of the rite of marriage, and the journey to the afterlife (Bruce & Yearley 2006). African people will also perform rituals when they ask for blessings and for protection in life, or when they perform thanksgiving ceremonies (Cocks & Dold 2008).

In most African cultures, when a baby is born the family must perform a ritual to introduce the new member to the ancestors and also to ask for protection from any harm, especially harm that can be brought by witchcraft and evil spirits (Cocks & Dold 2008). At these rituals the families slaughter

animals; according to the rules of a particular tribe (Cocks & Dold 2008). The shedding of the animal's blood is perceived as a symbol of communication with the family's ancestors. In some cultures when a baby is born, rituals to safeguard the new-born are also performed. These rituals protect the growing child from fearful circumstances, intimidation and ridicule (Abid 2014). If the ritual is performed soon after childbirth, it is believed that the growing child will be able to confront any adversary. In tribes of the Xhosa nation, smoke of burning leaves of a 'sifudu' tree is used in this safeguarding ritual. This ritual is performed by passing a baby through the smoke and strong aroma caused by the burning leaves (Abid 2014). The baby's head is held downwards into the smoke and the baby is turned around several times. After completing this act, the baby is handed back to the mother who is seated close by. She then swiftly passes the baby over and under her legs (Abid 2014). Thereafter, the baby is washed and then coated with a white substance called 'ingceke', which is collected from a river bank and mixed with grounded 'mtomboti' wood (Abid 2014). The tribe believes that the strong pleasant odour of the 'mtomboti' wood will cling to a baby's body repelling any evil spirits that may attack the baby (Abid 2014).

In the culture of Basotho people, when a woman gives birth, the baby's gender is announced to the father in a unique way. Traditionally, the relatives and friends of the family will pour water over the baby's father if it is a girl, but if it is a boy he will be beaten with sticks (Ntšihlele 2003). The different acts of the ritual symbolises the future life of the new-born. The water pouring act symbolises the domestic duties of a female, while the beating act symbolises the warrior and protection duties of a male in the family (Ntšihlele 2003). Thereafter, the mother and baby remain in the house for at least three months after birth. The seclusion is ended when the baby is brought outside and introduced to his or her first rainfall.

The rite of passage from childhood to adulthood is a ritual that has been practiced by most African tribes for many years and differs from tribe to tribe (Bruce & Yearley 2006). This ritual prepares young

adolescent females and males for adulthood. The rituals are performed separately for females and males in a secluded location, usually away from the public. During the period of seclusion, young females and males are taught skills for adulthood. After the period of seclusion, the adult females and males are reintroduced into the community with a graduation ceremony. The ceremony is accompanied by the slaughtering of an animal, enjoyment of food and traditional dancing. The shedding of the slaughtered animal's blood symbolises the invitation and attendance of the ancestors.

Many traditions surround marriage in African cultures. Usually when a man has identified a potential bride, delegates from his family will undertake 'lobola' negotiations with delegates of her family (Zibani 2002; Nkosi 2011; Semenya 2014). Once a price agreement has been reached for the 'lobola', it is paid either in the form of live head of cattle or money representing the value of the cattle (Zibani 2002; Nkosi 2011; Semenya 2014). After payment of the 'lobola' has been made, the preparations for the wedding ceremony commences. On the wedding day, animals are slaughtered and gifts are exchanged. After welcoming the new bride into her husband's family, she is renamed and shares her first meal with his family (Semenya 2014). After the celebrations have concluded the bride will accompany her husband to his house.

African people are firm believers in the existence of their ancestors and the possibility of new life after death. When a family member dies, a funeral is accompanied by several rituals which are performed to send the deceased person's spirit to the world of the afterlife (Baloyi & Makobe-Rabothata 2013). These rituals are performed to ensure that the deceased's spirit is properly reincarnated with the ancestors in the afterlife (Baloyi & Makobe-Rabothata 2013). The deceased members of a family are regarded as the family's ancestors who offer guidance and protection to the living (Nyawose 2000).

2.2 African death rituals

Various death rituals are performed according to traditions of different African tribes. After the announcement of a death in a family; extended family members, friends and community members visit the deceased's house to offer their support to the family, particularly to the chief mourner (Kastenbaum 2003; Mankga 2013). In the more rural areas, the windows of the bereaved family's house may be coated with an ash and water mixture to announce a death in the family (Kastenbaum 2003). The chief mourner is usually a close female relative of the deceased, for example, a wife, a mother or an important female elder (Tshapa 2016). All female mourners are expected to cover their heads and shoulders as a symbol of mourning and respect for the dead. The chief mourner sits on the floor on a mattress in the bedroom of the deceased with other mourning woman (Kastenbaum 2003). The clothing of the deceased is kept together with these mourners in the room. A candle is lit and kept burning until after the funeral service (Setsiba 2012; Tshapa 2016). The burning candle symbolises life and provides light for the deceased person's journey to the afterlife.

Prayer services are held at the bereaved family's house until the day of the funeral to provide spiritual support and comfort to the grieving family and friends (Kastenbaum 2003). A few days before the funeral service, some family members will visit the funeral undertaker to prepare the deceased by washing and clothing the body. On the day before the funeral service, some members of the family will fetch the coffin containing the deceased's body and place it in the room where the mourning women are seated (Kastenbaum 2003; Mapaya & Mugovhani 2014). This is followed by the slaughtering of an animal and the preparation of food to be served at the funeral (Mankga 2013; Kgatla 2014). It is believed that the shedding of an animal's blood plays an important role in reincarnating the deceased with the ancestors (Kgatla 2014).

On the night before the funeral service, a vigil or prayer service will be held at the bereaved family's house (Nyawose 2000; Kastenbaum 2003). On the morning of the funeral the coffin will be opened for

the family and friends to view the deceased so that they can pay their last respects and say farewell. After viewing, the coffin is closed and taken to the place where the funeral service will be held. Services are usually performed at the house of the deceased, at the church, or at a community hall (Nyawose 2000). Because African funerals are community affairs, many mourners will attend a funeral to pay their last respects to the deceased and to show support to the grieving family (Kastenbaum 2003; Mapaya & Mugovhani 2014).

After the funeral service, the coffin is taken to the cemetery. Depending on particular cultural practices of the family, a cattle hide or sheep skin of the slaughtered animal may be used to cover the coffin (Kastenbaum 2003; Kgatla 2014). In some instances a new blanket is used to cover the coffin after it has been lowered into the grave (Nyawose 2000; Mapaya & Mugovhani 2014). The covering of the coffin represents a blanket that will provide warmth to the deceased. After the lowering of the coffin, men take turns to cover the coffin with soil (Nyawose 2000; Mankga 2013).

While mourners are at the cemetery, women continue with the preparation of the funeral meal and the preparation of water for the cleansing ritual. A cleansing ritual is practiced in most African tribes by mourners when they return from the cemetery. The women who remained at the bereaved family's house prepare bowls of water for the cleansing ritual. Usually two bowls are filled with water and often cut aloe pieces are added to the water (Kastenbaum 2003). Amongst African people, aloe is perceived to play an important role during any form of cleansing ritual. In some Zulu tribes, a small portion of the chime and bile from the slaughtered animal will be poured into water for the hand washing ritual (Nyawose 2000). These water bowls are then placed outside the gate of the bereaved family's house until the cortege returns to the house from the cemetery (Kastenbaum 2003). When the mourners arrive at the house, before entering the yard, mourners will in turn wash their hands in one of the communal hand washing bowls placed outside the gate (Maloka 1998; Yawa 2010; Mankga 2013). Usually, washing of hands takes place in groups of three to six people (Countries and their culture,

n.d.). Hand washing takes place without any form of replenishment of the water in the communal hand washing bowls. This ritual is practiced because African people believe that any visit to a cemetery will attract bad omens linked to death, which will surround them and bring about bad luck (Maloka 1998; Yawa 2010). If the bad omens and dust of the cemetery are not washed off by performing a ritual, the bad omens will always stay at the bereaved family's house and follow mourners back to their homes (Figure 2.1). The hand washing ritual must, therefore, be performed before the mourners enter the yard and accept the funeral meal, which is traditionally consumed with bare hands. The spades used to cover the grave with soil are placed outside the yard and once mourners have completed the cleansing ritual, the spades will be cleansed by sprinkling the water from the communal hand washing bowl (Nyawose 2000). Because it is believed that the hand washing ritual water contains bad omens and dirt, this water is disposed of outside the gate of the bereaved family's house.

(a) (b) (c)

Figure 2.1 African burial rituals; (a) covering of the grave with soil, (b) hand washing ritual at communal hand washing bowl and (c) mourners enjoying the funeral meal

2.3 Hand contamination

2.3.1 Introduction

In the world, various religious and cultural groups perform some or other form of cleansing. These practices are performed for various reasons, which may be for hygienic purposes, ritualistic reasons or for symbolic reasons (Allegranzi & Pittet 2009). Hand cleansing practices are prominent amongst

most religious and cultural groups (Allegranzi & Pittet 2009; Mishra et al. 2013). In various religions, believers perform cleansing practices before praying (Allegranzi & Pittet 2009). For example, in Christianity, these cleaning practices symbolises the washing away of sin and attaining the level of purity (Zhong & Liljenquist 2006).

At African funerals, many attending mourners participate in a hand washing ritual. The large number of mourners that attend an African funeral can be viewed as a planned mass gathering (WHO 2008; Abubakar et al. 2012). At such events the potential occurrence of communicable disease increases, because of the gathering of so many mourners from diverse areas (Memish et al. 2014). These mourners washing their hands in a communal hand washing bowl are people with a wide range of hygiene complexities, age, health and susceptibility to illness. (Al-Tawfiq et al. 2016). Of particular concern is the hand washing ritual performed at the bereaved family's house prior to the enjoyment of the funeral meal.

People are exposed to a great variety of microorganisms in their homes and community. Some of these microorganisms may be pathogenic and could cause infectious diseases (Bloomfield et al. 2007). Substantial evidence exists that suggests a causal link between hand washing and infection (Bryn et al. 1995). Hands can gather dirt and contaminants such as microorganisms directly or indirectly; through contact with various contaminated sources; for example through touching surfaces, other humans and by using contaminated water (Jumaa 2005; Lingaas & Fagernes 2009; Dodrill et al. 2011). Thus, it has been recognised that hands play a major role in the transmission of infectious diseases (Jumaa 2005). Hand washing has been shown to be an important means to reduce the transmission of infectious diseases (Aiello et al. 2008; Istenes et al. 2013). It has been estimated that using soap when washing hands could save a million lives a year (Jumaa 2005). However, there is a lack of understanding of the role of hand hygiene and the spreading of infectious diseases, particularly in developing countries (Anargh et al. 2013; Ataei et al. 2013; FitzGerald et al. 2013).

2.3.2 Hands and microbial populations

The microbial population of human skin is diverse. Although evidence has shown that skin microbial populations differ from person to person, they remain relatively uniform for an individual (Jumaa 2005). The number of microorganisms on intact areas of skin of the same person can vary from 100 to $10^6/cm^2$ (Jumaa 2005). Skin microbial populations can be divided into two categories; the resident group and the transient group. The resident group of microorganisms are generally associated with the deeper layers of the skin, such as the sebaceous glands. Hand hygiene treatments are not effective on these microorganisms (Jumaa 2005). Transient skin microorganisms colonise the superficial layers of the skin and may be removed through hand hygiene preparations.

The resident microbial population group consists mainly of coagulase-negative staphylococci, *Corynebacterium* spp. and anaerobes, such as *Propioni bacterium* spp. (Jumaa 2005). These microorganisms rarely cause infections. The transient group of microorganisms, on the other hand, can be transferred by direct contact between humans (WHO 2009) and contact with inanimate objects and surfaces (Lopez et al. 2013; Nwankwo et al. 2014). Transient microbial populations include microorganisms that are often associated with nosocomial infections. Because viruses are not considered as part of a normal microbial population, they are included as transient or contaminating microorganisms (Jumaa 2005).

2.3.3 Faecal hand contamination

Hands harbour many microorganisms, most often of faecal origin. Faecally derived microorganisms are one of the major causes of diarrhoea, particularly in developing countries (Bryn et al. 1995). Diarrhoea remains the second leading cause of death among children under five year of age globally (Veneman & Chan 2009).

Most microorganisms that cause diarrhoea in an infected person has a similar mode of transmission. The route of transmission of microorganisms of faecal origin is faecal-oral (Veneman & Chan 2009). Faecal-oral transmission of disease occurs when faecal microorganisms are passed from one host and introduced into the oral cavity of another host. The process whereby transmission is accomplished may be simple or relatively complex. For example, faecal microorganisms may be transmitted via faecally contaminated water, food, surfaces, soiled nappies or vectors such as houseflies, to the hands of a host. Through oral contamination, this could lead to disease. One of the main causes of faecal-oral disease, such as diarrhoea, in developing countries is a lack of sanitation, particularly hand sanitation (Bryn et al. 1995).

Normal bacterial flora of the gastrointestinal tract consists of a wide range of microorganisms. The gastrointestinal tract is a complex ecosystem containing over 400 bacterial species (Gorbach 1996). Gastrointestinal microorganisms include anaerobic organisms, Gram-negative species of the enterobacteriaceae family, as well as Gram-positive enterococci. Common species of the enterobacteriaceae family include *Escherichia coli*, *Klebsiella*, *Enterobacter* and *Proteus* (Dodrill et al. 2011). The gastrointestinal flora also includes low populations of potentially pathogenic organisms such as *Clostridium difficile*. The spread of enteropathogens is evident by the recovery of faecally derived microorganisms from people's hands (Laborde et al. 1993). Viruses, such as rotavirus and calicivirus, are also known for their diarrhoeal causing properties in humans (Gorbach 1996). In a study that was conducted in five cities in the United Kingdom, faecal bacteria were detected on the hands of 28% of 404 commuters using public transport (Judah et al. 2010). Particularly, more men had faecally contaminated hands than woman in this study. In another study that took place in 20 schools in Leeds, United Kingdom, faecal bacteria were isolated from the hands of school children and from environmental surfaces. It was found that children from schools, with a history of gastrointestinal disease, demonstrated higher hand counts of the faecal indicator organisms than children from schools where such outbreaks had not been reported (Kaltenthaler et al. 1995).

2.3.4 Human interaction in hand contamination

Hands play a major role in human interaction and the transfer of microorganisms from one person to another. Hands harbour different amounts and types of microorganisms depending on a person's occupation, location and hand hygiene habits (Mela & Whitworth 2014). People tend to touch their own bodies, transferring microorganisms from various parts of their bodies to their hands. In most cultures various forms of greeting involves touching, for example, ritualistic touching of another person's body, hugging and handshaking (Mela & Whitworth 2014). Greeting in this manner may act as a potential vector for pathogenic microorganisms and cross infection between individuals (Ghareeb et al. 2013; Sklansky et al. 2014). The extended duration and the grip strength applied by people during handshaking seemingly increases the intimacy of association between hands, which in turn increases the potential of transmission of microorganisms between hands (Mela & Whitworth 2014). It has been demonstrated that the spores of *Clostridium difficile*, a common cause of diarrhoea, can be transferred between individuals during handshaking (Sklansky et al. 2014). These types of human contacts have been implicated in community-acquired pneumonia causing more than 60 000 deaths annually in the United States of America (File & Marrie 2010).

A further important human interaction that is implicated in hand contamination involves the care of patients. Besides caregivers, family members and friends are all at risk of being exposed to pathogenic microorganisms, particularly through touching and greeting. Thus, with poor hand hygiene practices these people could further transmit pathogenic microorganisms to other people (WHO 2009). Hand hygiene is considered as a leading measure for preventing the spread of pathogenic microorganisms including healthcare associated infections (Allegranzi & Pittet 2009).

2.3.5 Food and hand contamination

Foodborne illnesses, often referred to as food poisoning, results from the spoilage of food through contamination by microorganisms. Food contamination is caused through microorganisms which are

commonly present in kitchens and in the household environment (Macias-Rodriguez et al. 2013). Contamination of food occurs through handling, particularly raw food, and through food being exposed to contaminated surfaces and equipment in kitchens. The survival and multiplication of microorganisms in kitchens and household environments are favoured by the moist conditions, the availability of nutrients and the storage of food at room temperatures (Gorman et al. 2002; Macias-Rodriguez et al. 2013). Hands of food handlers have also been implicated as vectors in the spread of foodborne illnesses, mainly because of inadequate personal hygiene practices, and account for approximately 97% of foodborne illnesses resulting from kitchen and household environments (Lambrechts et al. 2014; Faour-Klingbeil et al. 2015).

Amongst microorganisms found in kitchen and household environments, pathogenic bacteria form a major source of foodborne illnesses (Newell et al. 2010). Therefore, improper handling, preparation or storage of food encourages the growth of pathogenic bacteria in food. Such microorganisms include *Listeria* spp., *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni* and *Bacillus cereus* (Josephson et al. 1997; Alwakeel 2007; Chavatte et al. 2014; Yu et al. 2016). The implementation of good hygiene practices before, during and after the preparation of food can significantly reduce the potential of food been contaminated by pathogenic bacteria and resultant foodborne illnesses. Routine hand washing is considered to be the most effective control measure against the spread of foodborne illnesses (Chavatte et al. 2014).

The occurrence of foodborne outbreaks has had a significant impact on public health systems worldwide. In 2011 it was estimated for the USA that domestically acquired foodborne outbreaks were responsible for approximately 47.8 million illnesses, 127 839 hospitalisations and 3 037 deaths (CDC 2011). The top five pathogens that caused illnesses, hospitalisations and death, included Norovirus, *Salmonella*, *Clostridium perfringens, Campylobacter spp*. and *S. aureus* (CDC 2011). In the developing world, foodborne illness causes an estimated 2.2 million deaths each year, of which 1.9

million are children (FAO/WHO 2005; Farahat et al. 2015). Symptoms related to the foodborne outbreaks can vary from mild gastroenteritis to life threatening neurologic, hepatic and renal syndromes (Mead et al. 1999). *Listeria monocytogenes* is one of the most virulent foodborne pathogens, with a mortality rate of 20 – 40% in humans, despite early antibiotic treatment (Stylianou et al. 2008). When ingesting raw and undercooked food or water containing *Toxoplasma gondii* oocysts, people may become infected with *Toxoplasma gondii* (Choi et al. 1997). In Korea, two separate foodborne outbreaks involving *Toxoplasma gondii* occurred (Choi et al. 1997). The first outbreak resulted because of adults eating a meal consisting of raw spleen and liver of wild pig. Three people developed unilateral chorioretinitis within three months of consuming the meat with *Toxoplasma gondii* oocysts (Choi et al. 1997). Similarly, in the second outbreak, five soldiers developed lymphadenopathy after consuming infected raw liver of the domestic pig (Choi et al. 1997).

Food handling and handlers play a prominent role in the spread of pathogenic microorganisms. Improper handling and processing food products have been linked to several foodborne outbreaks (Tajkarimi et al. 2013). In a study conducted in the Republic of Ireland, it was shown that raw food, particularly raw meat, was a major source for the introduction of pathogenic bacteria in the kitchens and the household environment. Evidence of cross-contamination of *Salmonella*, *Campylobacter* and *E. coli* was found on dishcloths (12%), food handler's hands (24%), refrigerator handles (4%), oven door handles (20%), kitchen counter-surfaces (24%) and draining boards (32%) (Gorman et al. 2002). Food handlers can spread foodborne diseases by hand due to poor personal hygiene and unhygienic living conditions (Tajkarimi et al. 2013). In a study on 250 nose and 250 hand samples of food handlers in 50 restaurants in Kuwait, a total of 174 isolates of staphylococcal species consisting of 174 *S. aureus* and coagulase-negative staphylococci were isolated (Udo et al. 1999).

2.3.6 Surfaces, inanimate objects and fabrics in hand contamination

Both porous and nonporous surfaces and inanimate objects can become contaminated with microorganisms that may be pathogenic and thus could serve as vehicles in the transmission of infectious diseases (Boone & Gerba 2007; Lopez et al. 2013). There is also strong evidence that contaminated surfaces and inanimate objects could play a role in the transmission of viral infections (Boone & Gerba 2007). Some of the diseases that are commonly spread by means of surfaces and objects include the common cold, cold sores, conjunctivitis, hand-foot-mouth disease, croup, *E. coli* and Staphylococcal infections, *Giardia* infection, impetigo, influenza, meningitis and rotavirus diarrhoea (Nwankiti et al. 2012) .

Surfaces serve as a potential reservoir of many different pathogens. Transmission of these pathogens can be directly, by surface-to-mouth contact, or indirectly, by contamination of fingers and subsequent hand-to-mouth, hand-to-eye, or hand-to-nose contact (Lopez et al. 2013). Bodily fluids such as saliva, mucus, nasal secretions, blood, urine, and faeces, may also act as potential routes for the transmission of pathogens from surfaces. Several studies have shown that surfaces found in day care centres (Barker et al. 2001), schools (Bright et al. 2010), office buildings (Boone & Gerba 2010), homes (Fekety et al. 1981), public areas (Reynolds et al. 2005), and hospitals (Huslage et al. 2010) can act as reservoirs of pathogens. In a study of nine different surfaces, Lopez et al. (2013) showed that *E. coli, S. aureus*, *Bacillus thuringiensis*, MS2 coliphage, and poliovirus 1 were transferred through finger contact with the surfaces. Rusin et al. (2002) and Lopez et al. (2013) showed that the efficiency of transfer of microorganisms from a contaminated surface to the hand increased by about 40% with repeated contact.

Viruses are probably the most common cause of infectious disease that can be acquired in closed areas (Barker et al. 2001). Viruses spread rapidly in crowded indoor areas, such as day care centres, schools, office buildings and hospitals (Boone & Gerba 2007). Particularly, viruses are shed in large

numbers after illness and could contaminate surfaces through direct contact with body secretions or fluids, sneezing, coughing, or vomiting, as well through the shaking of a contaminated blanket (Boone & Gerba 2007). If viruses remain viable on surfaces for long enough, only a small number is needed to infect a host through direct contact (Boone & Gerba 2007).

Inanimate objects such as phones, public as well as mobile phones, play a major role in the lives of most people. These phones may also serve as a means of transmission for infectious diseases (Jerković-Mujkić et al. 2013). Microorganisms have been isolated from the mouthpiece, earpiece, phone handle, mobile phone with buttons and keyboards, including smart phones (Annand et al., 2009; Lee et al., 2013). Microorganisms isolated from phones include Methicillin-sensitive *S. aureus*, *S. aureus*, *Staphylococcus epidermidis, Bacillus* spp., *Proteus* spp., *E. coli*, *Streptococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*., *Enterococcus faecalis*, coliforms, *Micrococcus* spp., and *Citrobacter* spp. (Annand et al. 2009; Girma 2015; Jerković-Mujkić et al. 2013). A study conducted on the mobile phones of 200 health care workers working in operating rooms and intensive care units, revealed that 94.5% of the phones showed evidence of microbiological contamination. *S. aureus* was isolated from 52% of the mobile phones (Ulger et al. 2009). Some of the mobile phones analysed also contained microorganisms of nosocomial origin. In a study in Nigeria, a total of 150 samples were collected from mobile phones of volunteers from the university, commercial centres and hospitals. Of the 150 samples analysed 124 showed evidence of microbiological contamination, which included *E. coli* (28.2%), *Pseudomonas aeruginosa* (22.6%), *Klebsiella* sp. (14.5%), *Serratia* sp. (13.7%), *S. aureus* (12.9%) and *Proteus vulgaris* (8.1%) (Famurewa & David 2009).

Other inanimate objects that have shown to harbour microorganisms are kitchen utensils and various items of jewellery. Poor hygiene and improper food preparation practices in the home could lead to cross-contamination of kitchen utensils. In a study where inoculated vegetables were cut and grated,

microorganisms were transferred to the knife and grater (Erickson et al. 2015). These utensils have the potential of contaminating the hands of any user thereafter.

Wearers of jewellery often demonstrate greater numbers of microorganisms on the skin underneath the jewellery (Jumaa 2005). For example, the number of microorganisms increases with the number of rings worn. Wearers of rings are at risk of carrying Gram-negative bacilli and *S. aureus* on their hands. Evidence exists that microorganisms found under rings may be carried for many months (Field et al. 1996; Trick et al. 2003; Al-Allak et al. 2008). Similarly, the skin underneath wristwatches also carries large numbers of microorganisms (Jumaa 2005).

Kitchen dishcloths and sponges may also act as reservoirs of microorganisms. Dishcloths are commonly used to clean surfaces, kitchen equipment and utensils, crockery and cutlery, enhancing the potential for cross-contamination between food-related environments (Kusumaningrum et al. 2003; Mattick et al. 2003; Scott et al. 2008; Macias-Rodriguez et al. 2013). Dishcloths and sponges have been reported to contain populations of bacteria of up to 10^8 bacteria, which were mostly members of *Enterobacteriaceae* (Gorman et al. 2002). Growth of microorganisms on dishcloths and sponges are also stimulated by the humid conditions of these materials (Scott et al. 1982; Erdogrul & Erbilir 2000). In a study that was conducted in ten household kitchens in the USA, the prevalence of staphylococci, *Pseudomonas*, coliforms and faecal coliforms were monitored (Josephson et al. 1997; Rusin et al. 1998). Large bacterial concentrations were detected in basins and kitchen sponges, particularly high incidences of faecal coliforms (Josephson et al. 1997). Evidence has been presented demonstrating that microorganisms can be transferred from dishcloths and sponges to hands, to other surfaces, as well as to food (Scott & Bloomfield 1990).

Similarly to dishcloths that harbour microorganisms, other fabrics such as clothing are also prone to harbouring microorganisms. Studies investigating microorganisms on clothing have focused on

hospital staff. Munoz-Price et al. (2012) conducted a study to determine the association between bacterial contamination of hands and the uniforms of 119 health care workers working in five different intensive care units. The study revealed a strong association between the bacteria present on the hands of the health care workers and their clothing.

Bathrooms, in particular, have plenty of areas that may harbour a wide range of microorganisms. Contact with these areas in the bathroom can expose people to infectious diseases. In a study conducted in public female bathrooms in Taif in the Kingdom of Saudi Arabia, 260 samples were collected from bathroom doors, bathroom handles, hand wash basins, toilet handles and toilet cubicle doors. Of the 260 samples, 187 samples were positive for bacterial contamination (Sabra 2013). The highest bacterial counts were found on the toilet handles (91.3%) followed by toilet cubicle doors (73.8%), hand wash basins (63.3%), bathroom handles (50%) and bathroom doors (35%) (Sabra, 2013). Of the microorganisms isolated, *S. aureus* (40.6%) was the most abundant, followed by *E. coli* (22.5%), *Bacillus* spp. and *Klebsiella pneumoniae* (21.4%), *Enterococcus faecalis* (13.4%), *Citrobacter* spp. (9.6%), *Pseudomonas aeruginosa* (8.6%) and *Proteus mirablilis* (7%) (Sabra 2013).

In bathrooms, microbiological contamination mostly occurs during the flushing of a toilet bowl (McFadden 2003; Barker & Jones 2005). This occurs through water droplets that are scattered in the bathroom. These water droplets are mostly faecally contaminated, but may also contain contaminants that originate from urine (Gerba et al. 1975; McFadden 2003). When the droplets settle in various areas in a bathroom; these areas may become contaminated. The most frequently affected areas include the area underneath the toilet seat, door handle, tap head and floor (McFadden 2003; Sabra 2013). Infectious diseases may be transferred to a person via direct surface-to-hand-to-mouth contact (Barker & Jones 2005). A person with an infectious disease could spread infectious microorganisms in a bathroom through droplet spraying (Barker & Jones 2005). For example, a person suffering from

acute diarrhoea may shed >10^{10} rotaviruses per ml of faeces and the droplets containing the infectious virus can be dispersed and settled in different areas in the bathroom (Anderson & Weber 2004).

2.4 Contamination of water

Water plays an important role in the life of all living organisms. The former United Nations Secretary-General, Mr Kofi Annan, stated that "contaminated water jeopardises both the physical and social health of all people and it is an affront to human dignity" (Ahmed 2010). Hence, an adequate supply of safe water must be available to all living organisms to survive (WHO, 2011a). Water usage in South Africa comprise mostly of surface water (77%), re-used of return flows (14%) and a small contribution by groundwater (9%) (DWAF 2013).

Water that is intended for human consumption, amongst other activities, must not present any significant risk to consumers. The limits of its physical, chemical and microbial properties must be within acceptable health limits (WHO 2011a). In a household, people use water for personal use, which includes water for drinking, cooking and hygiene activities. Water in the household is also used for a wide range of amenities. These include, amongst others, car washing, lawn watering and window cleaning (Howard & Bartram 2003).

The physical and chemical properties of water may affect its acceptability to consumers. Physical properties of water are regarded as those properties that contribute to the aesthetic status of the water (DWAF 2001). Physical properties contribute to the taste, odour and final appearance of the water and include properties such as temperature, pH, electrical conductivity and turbidity (WHO 2011a). Chemical properties, on the other hand, relate to the nature and concentration of dissolved chemical substances in the water. The majority of the chemical substances that are present in water occur naturally. Although chemical substances are present in domestic water; at high concentrations the water may be unpalatable and could cause life threatening illnesses (DWAF 2001). For example, the

prolonged exposure to high concentrations of fluoride can cause teeth decay and crippling skeletal fluorosis (WHO 2011a). High concentrations of arsenic may cause skin lesions, but also could be carcinogenic (WHO 2011a). The presence of nitrate and nitrite in drinking water that is used to prepare milk formula for bottle-fed infants has been implicated in the cause of methaemoglobinaemia, also known as blue baby syndrome (Fewtrell 2004; Tredoux et al. 2009; WHO 2011a, 2011b).

Water may be contaminated by various microorganisms originating from environmental sources. Runoff and wastewater discharges contribute to organic and inorganic nutrients in fresh water. Microorganisms that originate from human and animal faeces include pathogens such as *Cryptosporidium*, *E. coli* O15:H7, rotavirus, hepatitis E virus and norovirus (formerly known as Norwalk virus). Pathogenic bacteria or viruses may also originate from urine.

The presence of microorganisms in the drinking water can encourage the transmission of infectious waterborne diseases to consumers (DWAF 2001). Faecal coliforms are used as indicator organisms for microbiological contamination of drinking water and could be indicative of the presence of pathogenic microorganisms (Odonkor & Ampofo 2013). The faecal indicator bacterium *E. coli*, which is excreted in the faeces of all warm-blooded animals and some reptiles, is used to elucidate the efficacy of drinking water treatment to remove bacterial pathogens such as those responsible for cholera (*Vibrio cholerae*) and typhoid fever (*Salmonella typhi* and *S. paratyphi*) (Edberg et al. 2000; Enriquez et al. 2001). There are other enteric pathogens, which behave differently to *E. coli* in their ability to survive harsh environmental conditions and disinfection during drinking water treatment process (Ashbolt et al. 2001). Chlorine-resistant parasitic microorganisms such as the oocysts of *Cryptosporidium parvum* and various enteric viruses survive chlorine treatments for drinking water (Ashbolt 2004; Hambidge 2001). *Cryptosporidium* oocysts are especially resistant in environmental waters, and can survive for more than a year in optimal conditions (Rogers & Haines 2005).

In developed countries, water-related diseases have largely been eliminated with sporadic outbreaks still persisting. During the period from 2011 to 2012, 32 drinking water-associated outbreaks were reported in the USA, amounting to at least 431 cases of illnesses, with 102 hospitalisation and 14 deaths recorded (Beer et al. 2015). Norovirus was implicated in an outbreak involving 138 cases with no hospitalisation or death reported (Beer et al. 2015). However, in developing countries the burden of water-related diseases continues to persist (Gleick 2002). Diarrhoeal diseases remain a leading cause of illnesses and death, especially in children under the age of five years (Kosek et al. 2003). Water-related diseases include, waterborne diseases which are diseases associated with ingestion of water contaminated by human or animal faeces.

In many developing countries, including South Africa, many communities lack access to reliable sources of clean water or well developed sanitation services (Aziz et al. 2015). Particularly in rural areas, people collect drinking water from contaminated water sources shared with animals. In these communities drinking water is a major source of microbial pathogens, which exposes the communities to the risk of enteric pathogen infection (Ashbolt 2004). Human pathogenic microorganisms often detected in surface water include rotavirus (Lodder et al. 2010; Verheyen et al. 2009), *Shigella* (Faruque et al. 2002), *Vibrio* (Alam et al., 2006) and pathogenic *E. coli* (Begum et al. 2007; Ferguson et al. 2012; Momba et al. 2006). For a long time, it was believed that groundwater was safe for drinking without any further treatment. However, recent studies have revealed the presence of pathogenic microorganisms in groundwater, which include enteric viruses (Borchardt et al. 2003; Gibson & Schwab 2011; Wampler & Sisson 2011). Groundwater becomes contaminated through on-site sewage disposal, as well as through waste water generated on cattle farms, dairies and piggeries. Contamination occurs through direct infiltration of the groundwater (MOH 2007).

Many suburban settlements in South Africa obtain drinking water from municipal communal standpipes. People living in these settlements usually collect water from standpipes, which they then

store in water storage containers until used for domestic purposes. The stored water can become contaminated during the process of water collection and storage in containers (Jagals et al. 2003; Nala et al. 2003). Particularly, the people that collect water could introduce microorganisms into the water if they do not observe adequate hand sanitation (Jagals et al. 2003; Nala et al. 2003). Also, if the water storage container had not been adequately cleaned, the microbial contamination of the water could be further exacerbated (Jagals et al. 2003; Nala et al. 2003). In South Africa, water storage containers with wide openings, such as buckets, are often used. These types of storage containers increase the probability of introducing microbial contaminants into the water through contaminated hands, cups or other utensils used to remove water from the storage containers (Jagals 2004; Trevett et al. 2005a; Trevett et al. 2005b; Moabi 2006; Potgieter et al. 2009; Onigbogi & Ogunyemi 2014). Often faecal contaminants are introduced into stored water in this manner. Other factors that contribute to the risk of microbial contamination of stored water includes; exposing the stored water to high temperatures; high levels of airborne particulates, such as dust; and lengthy storage periods (WHO n.d.). Furthermore, suspended particles may harbour and possibly spread potentially hazardous microbiological contaminants such as; *E. coli* and *C. perfringens* spores (Jagals et al. 2003).

Some rural communities in South Africa use water from large rain-fed storage tanks. Studies have shown that such water may contain pathogenic microorganisms originating from faeces of birds and cats that frequent the roofs of houses from where the water is sourced (MOH 2007). Enteric pathogens, such as *Campylobacter* spp., *Giardia* spp., and *Cryptosporidium* spp. have been found in samples of tank water (Ahmed et al. 2010; Dobrowsky et al. 2014). The occurrence of gastroenteritis has been linked to consumption of untreated tank water (Ahmed et al. 2014). In a study conducted in Australia, the water quality of 72 rain-fed tanks was tested for the presence of faecal indicator bacteria, *E. coli* and *Enterococcus spp*. (Ahmed et al. 2014). *E. coli* was detected in 74% and *Enterococcus spp*. in 94% of the water samples analysed.

2.5 Water borne pathogens

2.5.1 Introduction

Worldwide, unsafe water, inadequate sanitation and poor hygiene are rated among the top ten risks to health (Ezzati et al. 2004). Unsafe water may harbour waterborne pathogens, such as parasites, viruses and bacteria. Waterborne pathogens enter water sources through faeces from infected people and animals. Water sources may also be contamination through runoff from farms and through the accidental release of sewage. Although waterborne pathogens are diluted to low concentrations when transported through the environment, they still pose a health risk, as several pathogens have extremely low infectious doses (Bridle 2014).

2.5.2 Parasites

This class of waterborne parasites comprises of protozoa and helminths. A selection of waterborne protozoan pathogens, as identified by the World Health Organization, is summarised in Table 2.1.

Table 2.1 Important waterborne protozoa, their diseases, persistence in water and transmission route (Adapted from WHO list)

Pathogen	Disease	Persistence in water	Route of transmission
Acanthamoeba spp.	Keratitis, encephalitis	May multiply	Inhalation, skin or eye contact
Cryptosporidium spp.	Cryptosporidiosis	Long	Ingestion
Cyclospora cayetanensis	Gastroenteritis	Long	Ingestion
Entamoeba histolytica	Amoebic dysentery	Moderate	Ingestion
Giardia lamblia	Giardiasis	Moderate	Ingestion
Naegleri fowleri	Primary amoebic meningoencephalitis	May multiply	Inhalation
Toxoplasma gondii	Toxoplasmosis	Long	Ingestion

Cryptosporidium is one of the most widely studied parasitic coccidian protozoan found in the intestinal tract of many vertebrates and is a major cause of gastroenteritis outbreaks worldwide (Pavli et al. 2015). Twenty-six species have been identified of which *C. parvum* and *C. hominis* are the most prevalent species that infect humans (Widerström et al. 2014). Cryptosporidiosis is transmitted mainly by the faecal-oral route, usually through oocyst-contaminated water or food or by direct contact with an infected person or animal (Widerström et al. 2014). In 2010 a waterborne cryptosporidiosis outbreak took place in Sweden and affected at least 27 000 inhabitants of Östersund (Widerström et al. 2014). In the Milwaukee outbreak in 1993, 403 000 residents became ill (Corso et al. 2003).

Giardia lamblia, which is also known as *Giardia intestinalis* or *Giardia duodenalis*, is spread by means of contaminated food or water or by direct faecal-oral transmission (Lebwohl et al. 2003). This protozoan parasite contaminates water supplies and ingestion of its cysts can cause giardiasis, an acute self-limiting gastroenteritis. Prevalence of *G. lamblia* is around 20–30% in the developing world (Bridle 2014).

2.5.3 Viruses

Viruses are the smallest of all the waterborne pathogens, and range in size from 20 to 300 nm (Bridle 2014). Many viruses can survive for lengthy periods in aqueous environments (Borchardt et al. 2007). Enteric viruses are known to cause endemic waterborne diseases in the world (Rezaeinejad et al. 2014; Gall et al. 2015). These viruses are often associated with gastroenteritis, which can lead to diarrhoea and symptoms including abdominal cramping, vomiting and fever (Gall et al. 2015). They are also capable of causing other severe illnesses, such as, encephalitis, meningitis, myocarditis, cancer and hepatitis (WHO 2011a). Enteric viruses are dangerous because of their ability to cause infection and illness at low infectious doses (Fong & Lipp 2005). When they are ingested, they replicate in the upper third of the intestine and destroy the villi covering, which results in the disruption of water

reabsorption (Bridle 2014). A selection of waterborne enteric viruses, as identified by the World Health Organization, is summarised in Table 2.2.

Table 2.2 Important waterborne viruses, their diseases, persistence in water and transmission route (Adapted from WHO list)

Pathogen	Disease	Persistence in water	Route of transmission
Adenoviruses	Gastroenteritis, respiratory infection	Long	Ingestion, inhalation
Astroviruses	Gastroenteritis	Long	Ingestion
Enteroviruses	Gastroenteritis	Long	Ingestion
Hepatitis virus A and E	Hepatitis	Long	Ingestion
Noroviruses	Gastroenteritis	Long	Ingestion
Rotavirus	Gastroenteritis	Long	Ingestion
Sapoviruses	Gastroenteritis	Long	Ingestion

The two hepatitis viruses, Hepatitis A and E, are viruses that are transmitted mainly through the faecal-oral route. They also enter the body through ingestion of faecally contaminated food, water or close contact with an infected person (Fitzgerald et al. 2014; WHO 2011a). These viruses require a low infection dose to cause infectious hepatitis (WHO 2011a). Hepatitis is characterised by nausea, malaise, abdominal pain, and jaundice; although many of these acute infections are asymptomatic or cause only mild symptoms. Once the viruses have entered the gastrointestinal tract they infect the epithelial cells of the intestine, from where they enter the liver and damage liver cells via the bloodstream (Martin & Lemon 2006). The damage to the liver may result in the inability of the liver to remove bilirubin from the bloodstream. The incubation period for Hepatitis A virus infection can range between 28 and 30 days, whilst the incubation period of Hepatitis E may be longer with an average of 40 days (WHO 2011a).

Human rotavirus is considered to be the most important single cause of infant death in the world (WHO 2011a; Bridle 2014). Typically, 50 to 60% acute gastroenteritis childhood infections have been identified to be as a result of rotaviruses infection worldwide (Bridle 2014). Human rotavirus also enters the human body via the faecal-oral route. After ingestion, this virus infects the cells in villi of the small intestine, which then causes the disruption of the sodium and glucose transport mechanisms. The onset of infection is often presented with severe watery diarrhoea, fever, abdominal pain and vomiting. Dehydration and metabolic acidosis may also develop. The infection could be fatal if not appropriately treated. An infected person can excrete human rotaviruses in numbers up to 1 011 per gram of faeces for periods of approximately 8 days.

2.5.4 Bacteria

Water borne bacterial pathogens, with the potential to infect humans, infect the gastrointestinal tract through the ingestion of contaminated food or water. These pathogens are excreted in the faeces of infected humans and other animals (WHO 2011a). A selection of waterborne bacterial pathogens, as identified by the WHO, is summarised in Table 2.3.

Table 2.3 Important waterborne pathogenic bacteria, their diseases, persistence in water and transmission route (Adapted from WHO list)

Pathogen	Disease	Persistence in water	Route of transmission
Campylobacter jejuni, C. coli	Gastroenteritis	Moderate	Ingestion
Escherichia coli (pathogenic)	Gastroenteritis	Moderate	Ingestion
E. coli 0157:H7 (enterohaemorrhagic)	Gastroenteritis, haemolytic-uremia	Moderate	Ingestion
Legionella spp.	Legionnaires' disease	May multiply	Inhalation
Pseundomonas aeruginosa	Pulmonary disease, skin infection	May multiply	Skin contact
Salmonella typhi	Typhoid fever	Moderate	Ingestion

Pathogen	Disease	Persistence in water	Route of transmission
Salmonella enterica	Salmonellosis	May multiply	Ingestion
Shigella	Shigellosis	Short	Ingestion
Vibrio cholera	Cholera	Bioaccumulates	Ingestion

Several species of *Campylobacter* are known. *C. jejuni* is the most commonly isolated species from infected patients; although *C. coli, C. laridis* and *C. f*etus have also been isolated from infected patients (WHO 2011a). In contrast to many bacterial pathogens, *Campylobacter* requires a high infection dose of about 1 000 cells (Bridle 2014). After infection, the resulting illness is self-limiting, lasting about 3–7 days presenting symptoms of abdominal pain, diarrhoea, vomiting, chills and fever (Bridle 2014). *Campylobacter*'s incubation period is usually 2–4 days (WHO 2011a). The occurrence of this pathogen in water is associated with high amounts of rainfall and high water temperature. The animal reservoirs of this pathogen are thought to be poultry, wild birds and cattle. Outbreaks have resulted from inadequate or unchlorinated water supplies or when reservoirs are faecally contaminated by bird faeces (Vandeplas et al. 2007). Millions of cases are reported annually, generally presenting with mild, self-limiting diarrhoea (Vandeplas et al. 2007).

Large numbers of harmless strains of *E. coli* exist naturally in the human and animal intestines. A limited number of enteropathogenic *E. coli* strains can cause acute diarrhoea (Bridle 2014). Enteropathogenic *E. coli* strains are classified according to their virulence, and include enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Bridle 2014). *E. coli* O157:H7 (an EHEC) is a well-known example of one of these strains. *E. coli* O157:H7 produces a Shiga toxin that can cause severe illness in infected people. *E. coli* causes contamination of water through sewage overflows, sewage systems that are not working properly and

agricultural stormwater runoff (Bridle 2014). People become infected through ingesting contaminated food, particularly raw or undercooked ground beef, as well as through direct contact with faeces of infected animals, or contact with contaminated water (CFSPH 2006). In rural areas, people often share water sources with animals and are thus in danger of becoming infected if such water sources are contaminated (CFSPH 2006). The incubation period of *E. coli* ranges from 1 to 2 days after exposure. Infected people present symptoms such as abdominal pain, cramping and watery diarrhoea with blood (Buchanan & Doyle 1997). In adults, the infection clears in about a week. Severe cases can lead to kidney problems, which can be life threatening, especially in children or the elderly (CFSPH 2006). In 2014, four states in the USA reported outbreaks of Shiga toxin-producing *E. coli* O157:H7 strains (CDC 2014). A total of 12 people were infected and 58% of the ill people were hospitalised (CDC 2014). The hospitalised people recovered without any reported death. Wolverine Packing Company recalled approximately 1.8 million pounds of ground beef products that might have been contaminated with STEC O157:H7 on 19 May 2014 to prevent further infections (CDC 2014).

Diseases that result from different species of *Salmonella* include gastroenteritis and typhoid fever. Humans usually become infected with *Salmonella* through ingestion of contaminated food or water. *Salmonella* is found in the faeces of humans and animals. Usually people who are infected with *Salmonella* do not present any symptoms, however, in some cases infected people develop diarrhoea, fever and abdominal cramps within 8 to 72 hours of infection. *Salmonella* species can be divided in two categories; those species that do not cause typhoid and those that do cause typhoid. *S. typhi* is known to be the causative agent of typhoid.

There are four *Shigella* species that can cause disease in humans. These species are, *S. dysenteriae*, *S. boydii*, *S. sonnei and S. flexineri*. These species are responsible for the disease Shigellosis. Shigellosis is caused through the infection of the gastrointestinal tract of humans via the faecal-oral route, through person-to-person contact and through ingestion of contaminated water. Shigellosis

usually develops after 24–72 hours, with symptoms including abdominal pain, fever and watery diarrhoea (Agha & Goldberg 2008). The infective dose is relatively low and can vary between 10–100 cells. An infection with *S. sonnei* usually results in relatively milder symptoms than those presented by the other species. Over two million cases of Shigellosis occur each year, resulting in about 600 000 deaths, mostly in developing countries, with most cases being children younger than 10 years of age (Bridle, 2014).

The *Vibrio* bacteria include a number of species, of which *V. cholerae* is the only pathogenic species associated with fresh water. Cholera is an acute *V. cholerae* infection of the gastrointestinal tract. *V. cholerae* serogroup O1 has been responsible for all cholera outbreaks in South Africa (Archer et al. 2009). People become infected with *V. cholerae* after eating food or drinking water that has been contaminated by the faeces of infected people. The incubation period for *V. cholerae* ranges from a hours to approximately five days (Archer et al. 2009). The onset of cholera begins with a painless watery diarrhoea, nausea and vomiting. Immune compromised people can experience more severe symptoms, which lead to profuse diarrhoea and vomiting. If an infected person is not promptly and adequately treated, the loss of large amounts of bodily fluids and electrolytes can lead to serve dehydration and death within hours (WHO 2004). Outbreaks of cholera occur sporadically in areas where water sanitation is poor and food safety, hygiene practices are lacking. Cholera remains a major public health problem, particularly in developing countries, such as South Africa, where access to safe drinking water and adequate sanitation cannot be supplied to all citizens (Ismail et al. 2013). Cholera is an important cause of diarrhoea in developing countries and frequently affects poor and overcrowded communities. Outbreaks of cholera are often associated with high mortality rates (Faruque et al. 2007; Smith et al. 2008; Mason 2009; Keddy et al. 2013).

In Africa, between January and December 2012, a total of 94 553 cases and 1 834 deaths related to cholera were reported from 25 countries (WHO 2013). Amongst those countries, the Democratic

Republic of Congo, Sierra Leone, Ghana, Guinea, Uganda and Niger accounted for 90% of the total number of cases and 70% of all deaths reported (WHO 2013). In South Africa, during the period from 15 November 2008 to 30 April 2009, diarrhoea-related cases were reported following a significant epidemic in neighbouring Zimbabwe (Archer et al. 2009). The transmission in South Africa was linked to contaminated water supplies, poor sanitation infrastructure and poor access to safe potable water sources (Archer et al. 2009). The major outbreaks were recorded in the provinces of Limpopo and Mpumalanga. Limpopo reported 5 460 cases and 26 deaths, whilst Mpumalanga reported 6 855 cases and 30 deaths (Archer et al. 2009).

2.6 Conclusion

There are many waterborne pathogens that have the potential to cause disease in humans. Often these microorganisms are of faecal origin and can be related to poor hygiene practices. In cultural rituals such as at African funerals, where hands are washed in communal hand washing bowls because of the belief that bad omens will be washed off; people are at risk of becoming infected. Several instances of mourners becoming ill after attending African funerals have been reported (Hlungwani et al. 2010; Lamond and Kinyanjui 2012; Maletsky 2007). These people typically demonstrate symptoms of diarrhoeal-related diseases. Empirical data will show to what extent these hand washing rituals may contribute to the transmission of pathogenic microorganisms and infectious disease.

Chapter 3

Materials and Methods

3.1 Introduction

In this study water was collected at African funerals in the Mangaung Metropolitan Municipality of the Free State. Accessible funerals were identified with the assistance of a local funeral undertaker. The funeral undertaker liaised with bereaved families on behalf of the researcher. The bereaved families were requested to grant permission to the researcher to collect required water samples on the day of the funeral (Letter of permission can be found as Annexure). Water samples were collected from the hand washing ritual water bowls and the water supply system. Forty two funerals were visited and samples of water collected over a period of eleven months.

3.2 Study design

Microbial water quality was determined for water samples collected at 42 funerals. Three water samples were collected at each funeral resulting in a total of 126 water samples. The respective water samples included water collected from the yard tap that was used to fill the communal hand washing bowl. This water sample acted as the control sample. The other two water samples were collected from a communal hand washing bowl of standing water. One water sample was collected from the communal hand washing bowl before the mourners had washed their hands, while the second water sample was taken from the same communal hand washing bowl after the mourners had completed the hand washing ritual. Water samples were assessed for microbial water quality and statistically analysed. A comparison between the microbial quality of the yard tap water and the microbial quality of water samples collected from the communal hand washing bowl before the commencement of the hand washing ritual, provided an indication of a “bowl effect”. The “bowl effect” indicated how

contaminated or dirty the hand washing bowl was before the water was added to the bowl. Moreover, a comparison of the microbial quality of the two water samples that were collected from the communal hand washing bowl provided an indication of the contribution of mourner's hands to the quality of the water. Residual free chlorine was also determined as an indicator of efficacy of the disinfection process in the distribution network and at point of use. Finally, a health potential index (HPI) was calculated for all the water samples. This index describes the potential health threat of a particular water sample. Supplementary demographic and environmental information was also recorded at each funeral. Figure 3.1 provides a flow diagram of the study design of the project.

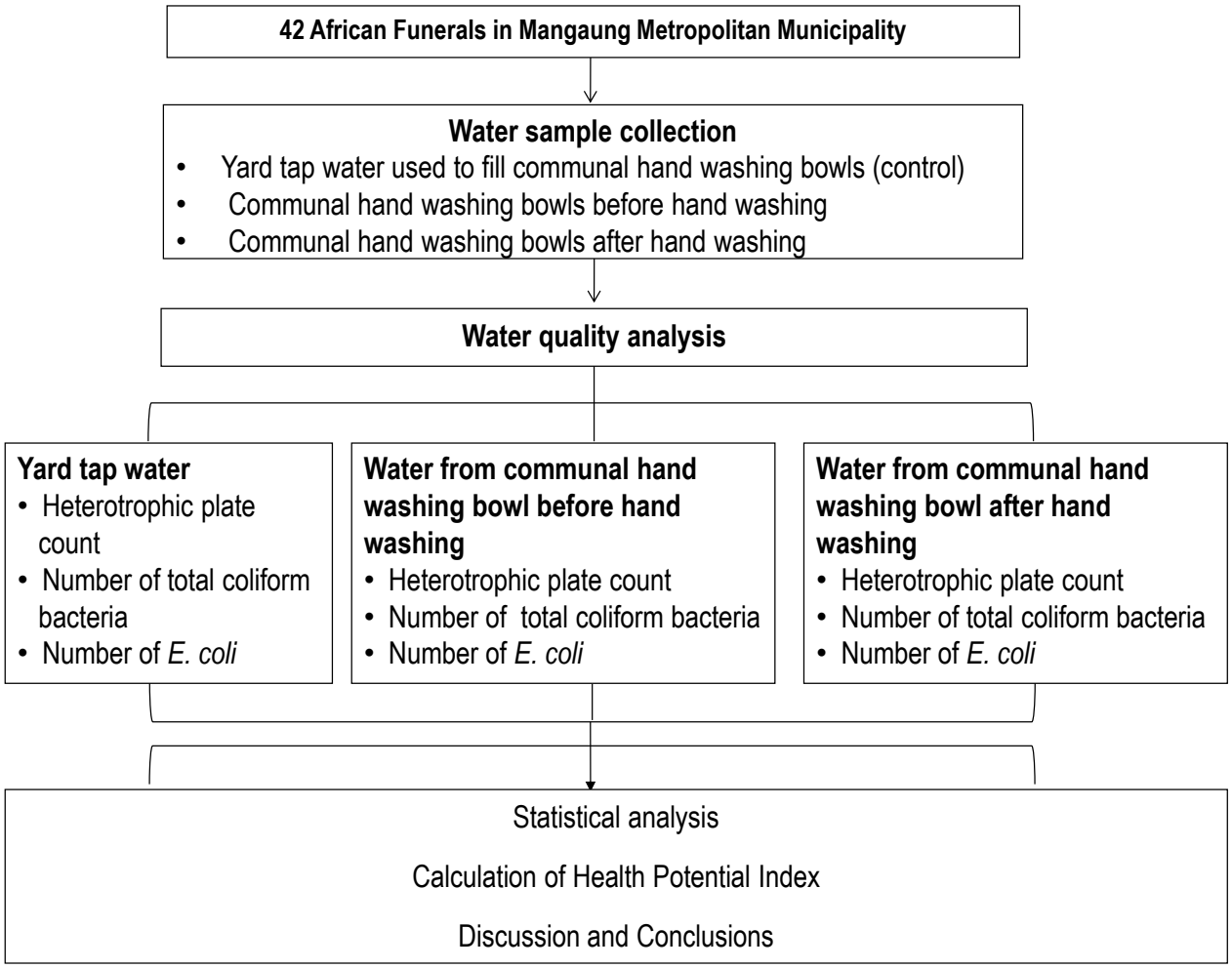


Figure 3.1 **Study design of the water quality assessment of communal hand washing water at African funerals**

3.3 Supplementary demographic and environmental information

Supplementary demographic and environmental data were recorded at each funeral. The demographic data included the number of communal hand washing bowls that were placed at the gate of the bereaved family's house and the type of bowl that was used in the hand washing ritual. Because of the possibility of winds that may transfer dirt and soil into a communal hand washing bowl and contaminate the water, the placement of the communal hand washing bowls was also recorded. These bowls were usually placed either directly on soil or on a protected pavement. Lastly, it was also noted whether the mourners ate the funeral meal using spoons or with their bare hands as is expected in the African tradition.

3.4 Collection of water samples

Usually at most African funerals two communal hand washing bowls of standing water are placed at the gate of the bereaved family's house. All the participating funerals in this study used two communal hand washing bowls in the hand washing ritual. In this study, water was collected from only one of the two bowls, because the number of mourners that washed their hands during the hand washing ritual was counted. The counting of the mourners participating in the hand washing ritual could only be performed for one communal hand washing bowl and not both simultaneously. Therefore, one of the two communal hand washing bowls was randomly selected. The random selection of a communal hand washing bowl entailed, firstly, the generation of random numbers using a Microsoft Excel spread sheet. If the right most digit of a random number was an even number, the bowl to the right of the researcher was selected as he faced the inside of the yard of the bereaved family's house. Conversely, if the digit of the random number was an uneven number, the bowl on the left of the researcher was selected (Table 3.1).

Sample 9	0.995130718	Even	Right	04 Aug 2012	✓
Sample 10	0.537432148	Even	Right	04 Aug 2012	✓
Sample 11	0.95187955	Uneven	Left	04 Aug 2012	✓
Sample 12	0.167177011	Uneven	Left	11 Aug 2012	✓

Table 3.1 Example of random numbers generated with Microsoft Excel spread sheet and selection of communal bowls

3.4.1 Water sampling at the yard tap

Yard tap water samples were collected in an aseptic manner following the standard operating procedures of Mangaung Metropolitan Municipality for collecting a drinking water sample at a yard tap. The yard tap water samples were collected in the following manner:

1. The yard tap was opened to allow the water to run for approximately two minutes to flush out the stagnant water in the pipes;
2. Thereafter, the yard tap was wiped clean using clean cotton wool dampened with 68% ethanol solution;
3. In the case of a metal tap, after the initial flushing and application of ethanol, the tap was also flamed, after which the tap was opened and allowed to run freely for a further two minutes;
4. A water sample was then collected in a 100 ml sterile plastic bottle after breaking the seal of the bottle, taking care not to touch the inside sterile surface of the bottle opening or lid;
5. The sample bottle was then closed and marked with a unique number representing the funeral and the letter “A” to denote the control sample;
6. The sample bottle was then placed in a cooler box and covered with ice cubes to keep the temperature of the water below 4°C until it reached the laboratory; and
7. Finally, the water samples were transported to the Mangaung Metropolitan Municipality laboratory in ice, where the microbial water quality was determined within six hours.

3.4.2 Water sampling at the communal hand washing bowl

Sterile glass bottles are required for the collection of water samples from the communal hand washing bowls of standing water. Thus, prior to the collection of the water samples, the water sampling bottles

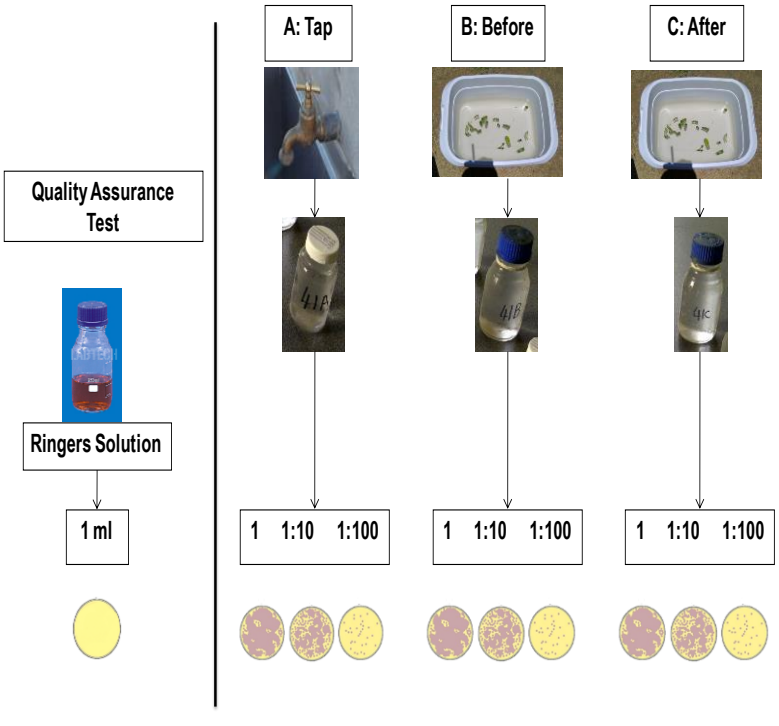
were sterilised. Water sampling bottles were sterilised, and water samples collected in the following manner:

1. One end of a cotton string was secured around the neck of a 250 ml Schott Duran glass sample bottle, while at the other end of the string a loop was knotted making it possible to hold a sample bottle when submerging it in the water of the communal hand washing bowl to collect a water sample;
2. Thereafter, the string was rolled around the neck of the bottle, and together with the lid the sample bottle was placed inside an Aseptor™ sterilisation bag;
3. The sterilisation bag was then closed and sealed with autoclave tape and autoclaved;
4. At the time of water sampling at a funeral, the sterile water sample bottle was removed from the Aseptor™ sterilisation bag, leaving the lid in the sterilisation bag;
5. While holding the string of the sample bottle, it was submerged into the water of a selected communal hand washing bowl and filled with water;
6. The sample bottle was then removed from the communal hand washing bowl and sealed with the sterile lid;
7. The sample bottle was then marked with the unique number representing the funeral and the letter "B" to indicate a sample from the communal hand washing bowl before mourners had washed their hands, or "C", for a sample collected from a communal hand washing bowl after the hand washing ritual;
8. The sample bottle was then placed in a cooler box and covered with ice cubes to keep the temperature of the water below 4°C until it reached the laboratory; and
9. Finally, the water samples were then transported to the Mangaung Metropolitan Municipality laboratory in ice, where the microbial water quality was determined within six hours.

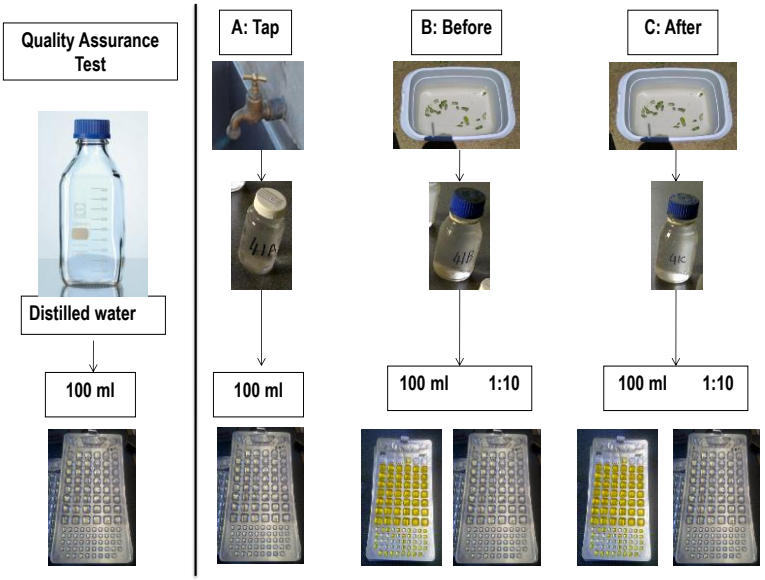
3.5 Measurement of microbiological quality of water samples

In this study, three different microbiological measurements were made. The heterotrophic plate count (HPC) was undertaken to enumerate the total aerobic bacterial population. The HPC is used as an indicator of treatment efficiency, post treatment growth in a distribution network, and adequacy of disinfectant residual (DWAF 2001). Total coliform bacteria and *E. coli* were also enumerated. Total coliform bacteria are indicative of the general hygiene quality of water, while *E. coli* is the preferred indicator of faecal pollution (SANS 2015).

As it was anticipated that some samples may contain a large number of bacteria, serial dilutions were prepared for some of the water samples. For HPC, 1:10 and 1:100 serial dilutions were prepared for all the water samples. In contrast, for total coliform bacteria and *E. coli* counts, only 1:10 serial dilutions were prepared for both types of water collected from the communal hand washing bowls. Quality assurance samples (control sample) were prepared for all the tests to establish the accuracy of the laboratory measurements. For the HPC control sample, 1 ml of Ringers solution was plated on petrifilm. In contrast, for total coliform bacteria and *E. coli* counts 100 ml of distilled water was used as control sample. This control sample was poured into a Quanti-Tray® system, which was used in the enumeration of total coliform bacteria and *E. coli*. A demonstration of the preparation of the serial dilutions for HPC, total coliform bacteria and *E. coli* counts are illustrated in Figure 3.2.



a)



b)

Figure 3.2 Demonstration of serial dilutions for; (a) heterotrophic plate count, (b) total coliform and *E. coli* enumeration

3.5.1 Heterotrophic plate count

Heterotrophic microorganisms can broadly be defined as microorganisms that require organic carbon for growth (WHO 2002). These bacteria include aerobic bacteria and facultative anaerobic bacteria in water (Verhille 2013). They are enumerated by applying a variety of methods; all referred to as heterotrophic plate counts (HPC). Only a small proportion of the metabolically active bacteria present in a water sample may grow and be detected under any given set of HPC test conditions, and the population recovered will differ substantially according to a particular method used (WHO 2002)). In this study the HPC was obtained by plating a water sample on 3M™ Petrifilm™ Aerobic Count Plates.

A total of 420 heterotrophic plate count enumerations were undertaken in this study. The enumerations consisted of plating 42 quality assurance samples and 378 water samples collected from yard taps and communal hand washing bowls. These water samples were plated on 3M™ Petrifilm™ Aerobic Count Plates under aseptic conditions in the following manner:

1. Using a pipette with a sterile tip perpendicular; 1 ml of a water sample was dispensed onto the centre of the bottom film of the 3M™ Petrifilm™ Aerobic Count Plate;

2. The top film of the Petrifilm™ was then released to cover the bottom film;
3. A plastic spreader was then used to distribute the sample evenly across the Petrifilm™ by applying gentle downward pressure and covering a circle growth area of approximately 20 cm^2;
4. The Petrifilm™ was left undisturbed for one minute for the gel to solidify;
5. The plated Petrifilm™ was then incubated at 35°C for 48 hours;
6. After incubation, a standard colony counter was used to count the red colonies, produced by the reduction of the tetrazolium indicator dye on the Petrifilm™, regardless of size or intensity; and
7. In instances where the number of colonies exceeded 250 per 1 ml, a mean number of colonies were estimated by counting the colonies in three different grid-blocks as follows:
    a. Three blocks were counted (e.g. 26 + 27 + 28 = 81 ÷ 3 = 27[Y]).
    b. Y × 20 cm^2 growth area × dilution factor (e.g. 27 × 20 × 100 = 54 000).

3.5.2 Total coliform bacteria and *E. coli* enumeration

Coliform bacteria are a commonly used as indicator organisms to indicate the sanitary quality of foods and water. Coliforms can be found in the aquatic environment, in soil and on vegetation and are generally harmless. They are also universally present in large numbers in the faeces of humans and warm-blooded animals. The presence of coliform bacteria is therefore used to indicate that other pathogenic organisms of faecal origin may be present in a sample. *Escherichia coli* (*E. coli*) is a member of the coliform group and are almost exclusively of faecal origin. Their presence in a sample is thus an effective confirmation of faecal contamination. Most strains of *E. coli* are harmless, but some can cause serious illness in humans (DWAF 2001).

In this study, a total of 252 samples were prepared. The samples consisted of 42 quality assurance samples and 210 water samples collected from yard taps and communal hand washing bowls. The Colilert-18 test kit was used for the enumeration of total coliform bacteria and *E. coli.* Colilert-18 is used for the simultaneous detection and confirmation of total coliform bacteria and *E. coli* in water samples. This test is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliform bacteria metabolise Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolises Colilert-18's nutrient indicator, MUG, the sample fluoresces. Colilert-18 can

simultaneously detect these bacteria at 1 MPN/100 ml within 18 hours, even with as many as two million other heterotrophic bacterial cells per 100 ml present. The preparation of the samples were conducted in the following manner:

1. A water sample was swirled gently and the excess water poured out of the sample bottle until 100 ml of the water sample remained in the sample bottle;
2. Colilert-18 reagent was emptied into the water sample, mixed gently and allowed to stand for a few minutes until the reagent had dissolved;
3. A Quanti-Tray® was labelled with the unique reference number of the water sample;
4. The labelled Quanti-Tray® was then carefully opened without contaminating the inside by pulling the tray tab;
5. The water sample was then poured into the Quanti-Tray® and the tray tapped slightly on a smooth surface to release trapped air bubbles;
6. The Quanti-Tray® was then mounted onto a rubber holder ensuring that the wells fitted snugly into the holes of the rubber holder;
7. The Quanti-Tray® rubber holder with the Quanti-Tray® was then placed facing downwards on the in-let point of the Quanti-Tray® Sealer and then sealed;
8. The sealed Quanti-Tray® was then incubated at 35°C for 18 to 22 hours;
9. After the incubation of the Quanti-Tray®, the wells that reacted positively were counted;
10. For total coliform bacterial enumeration, all the wells that turned any shade of yellow were counted (Figure 3.3a);
11. For *E. coli* enumeration, the Quanti-Tray® was placed under an ultra violet light and the fluorescent blue wells counted (Figure 3.3b);

(a) (b)

Figure 3.3 Positive reacting wells in the Colitert-18 Test; (a) Yellow wells indicating the presence of total coliform bacteria, and (b) blue fluorescent wells indicating the presence *E. coli*

12. For the enumeration of coliform bacteria and *E. coli*, the large wells and small wells were counted separately, after which the IDEXX Quanti-Tray®/2000 MPN Table (per 100 ml) was used to read the most probable number of bacteria (Figure 3.4).

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.5	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7

Figure 3.4 **Enumeration of total coliform bacteria and *E. coli***

3.6 Free residual chlorine concentration

Disinfection plays a significant role in the supply of safe drinking water. The concentration of free residual chlorine in drinking water in the distribution network and at point of use is indicative of the efficacy of the disinfection process and provides an idea of the probable microbiological safety of the treated water (DWAF 2001). Thus, free residual chlorine is used to measure the safety of treated drinking water. The free residual chlorine concentration of all the water samples collected were measured using a Martini instruments Mi 404 chlorine meter (Figure 3.5).

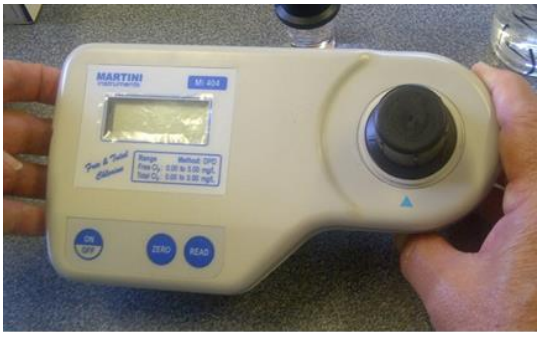


Figure 3.5 **Martini Mi 404 free chlorine meter**

Free residual chlorine concentration was measured in the following manner:

1. After switching on the Martini instrument Mi 404 chlorine meter, a water sample bottle was gently mixed, carefully opened and then a cuvette filled to the 10 ml line with the water sample;
2. After wiping the cuvette dry with a paper towel, it was placed in the instrument and the 'ZERO' button pressed to zero the instrument;
3. When '-0,0-' appeared on the screen, the cuvette was removed from the instrument;
4. A DPD No. 1 tablet was then added to the water sample and crushed with a plastic crushing rod until the tablet dissolved in the water;
5. The cuvette was then closed, shook, wiped clean and placed back in the instrument; and
6. After a few seconds the 'READ' button was pressed and the chlorine concentration reading recorded.

3.7 Data analysis

A number of statistical analyses were performed with the data. Summary statistics were calculated and included the mean, mode, range and standard deviation. Also, inferential statistical analyses were conducted to test whether the means of some groups of data demonstrated significant differences. Various other comparisons were also performed. These comparisons included the comparison of water quality measurements to respective water quality standards. These standards included the South African standard for HPC, total coliform bacteria and *E. coli* (SANS 2015) and free residual chlorine (SANS 2006).

A review of the literature was undertaken to identify an index that could be used to describe the potential health treat that any of the water samples could pose to mourners that participated in the hand washing ritual. A list of selection criteria was devised that was used to select an appropriate index. The index selection criteria were:

- Simplicity of mathematical calculations;
- Ability to be adjusted to the conditions for this study; and
- The ability to accommodate a single round of measurements.

Based upon the these criteria, the Weighted Arithmetic Index calculation method of Ramakrishnaiah et al. (2009) was selected to calculate an index that reflected the health potential threat of a water sample. This Index calculation method of Ramakrishnaiah et al. (2009) was adjusted specifically for this study to reflect the potential threat of a water sample and renamed the Health Potential Index (HPI).

3.7.1 Calculation of health potential index

The HPI was calculated in three steps. Firstly, the three parameters, HPC, number of total coliform bacteria and number of *E. coli* were each assigned a weight (w_i) based upon their relative importance in the description of the potential health threat in the index. The relative weights ranged from one to five with five indicating the highest level of importance (Table 3.2). For this study total coliform bacteria and *E. coli* were assigned a maximum weight of five, while for the HPC a weight of four was assigned. Secondly, the relative weight (W_i) was computed using the following equation:

$$W_i = \frac{w_i}{\sum_{i=1}^{n} w_i} \qquad (1)$$

Where, W_i was the relative weight, w_i the weight assigned to each parameter and n the number of parameters.

Thirdly, a quality rating (q_i) was calculated for each parameter using the concentration (C_i) of the respective parameter and the limit (S_i) (Table 3.2). Because no specific limits exist for water quality under these circumstances; the limits for food preparation and drinking water proposed by DWAF (2001) were used for total coliform bacteria and *E. coli*, while the limits of DWAF (1996) was used for HPC.

$$q_i = \frac{C_i}{S_i} \times 100 \qquad (2)$$

For computing the HPI, the sub-index (SI_i) was calculated for each parameter by multiplying the relative weight (W_i) by the quality rating scale (q_i):

$$SI_i = W_i \times q_i \quad (3)$$

Then, the HPI was calculated by summing all sub-indexes (SI_i) of the parameters for a water sample using the following equation:

$$HPI = \sum SI_i \quad (4)$$

Table 3.2 Weights, relative weights and water quality limits used for the calculation of the HPI

Parameter	Weight (w_i)	Relative weight (W_i)	Limit HPI (S_i)
Heterotrophic plate count	4	0.29	1000
Total coliforms	5	0.36	100
E. coli	5	0.36	10
	Σw_i = 14	ΣW_i = 1	

3.7.2 Health potential threat rating

Each water sample was rated in terms of its potential threat to the mourners. Because mourners traditionally eat the funeral meal with their bare hands, they may ingest pathogens that were transferred to their hands during the hand washing ritual. No applicable rating for this setting could be sourced after an extensive literature review was conducted, therefore a three-point rating scale was devised (Table 3.3). The limits chosen for the three classes of the rating scale took into account the potential threat posed by high numbers of coliform bacteria and *E. coli* in a sample of 100 ml of water and a high HPC in 1 ml of water. In this study high levels of coliform bacteria and *E. coli*, as well as and high HPCs were encountered. These high levels, which were indicative of the potential presence of harmful pathogens in the water samples, informed the decision of the limits of the three classes.

The final decision of the limits of the three classes was decided upon through discussions with Dr Elsa Potgieter, an experienced water quality specialist who works as a chief microbiologist at Mangaung Metropolitan Municipality in Bloemfontein, Free State.

Table 3.3 Health potential threat classification based on HPI values

Health potential threat	HPI value	Explanation
No	≤50	The probability for transference of microorganisms that are pathogenic to the hand and mouth is very small.
Intermediate	>50 –100	The probability for transference of microorganisms that are pathogenic to the hand and mouth is moderate.
High	>100	The probability for transference of microorganisms that are pathogenic to the hand and mouth is high.

Chapter 4

Quality of Ritual Water Used at African Funerals

4.1 Introduction

In this study, 42 African funerals were visited for the collection of water used by mourners in the hand washing rituals. At 26% of the funerals cut aloe leaves were added to the hand washing ritual water. Plastic or zinc communal hand washing bowls were equally popular containers used for the ritual water. At only three of the funerals the communal hand washing bowls were placed on a paved area. At the remainder of the funerals the communal hand washing bowls were placed directly onto the soil.

The water was collected from three different water sources at each funeral. The first sample was collected at the yard tap, which was used to fill the communal hand washing bowl. The second sample was collected from a randomly selected communal hand washing bowl that was placed at the gate outside the yard before mourners participated in the hand washing ritual. The third water sample was collected from the same communal hand washing bowl of standing water, after mourners had completed the hand washing ritual. Microbial water quality was assessed in terms of total bacterial counts (heterotrophic plate count; HPC), number of coliform bacteria and number of *E. coli*. Residual chlorine was also determined as an indicator of its disinfection strength in the distribution system.

4.2 Free residual chlorine

Free residual chlorine is an indication of the efficacy of the disinfection process of water and thus a rapid indicator of the probable microbial safety of the treated water (DWAF 2001). Disinfection of water will be sustained at a chlorine level not less than the limit of ≥0.2 mg/L specified by SANS 241:1 (SANS 2015) for bacteria indicated by the heterotrophic plate count, total coliform bacteria and *E. coli* (SANS 2006; SANS 2015). The free residual chlorine was measured for all water samples collected

at the funerals and compared to the limit of ≥0.2 mg/L for free chlorine. The residual free chlorine concentration determined for the yard tap water was for more than 50% of the samples greater than the SANS (2006) limit, while the residual free chlorine concentration of the water samples taken from the communal hand washing bowls, before the hand washing ritual, was mostly less than the prescribed limit for free chlorine SANS (2006). None of the water samples taken from the communal hand washing bowls after the hand washing ritual were compliant when compared to the limit SANS (2006) (Table 4.1).

Table 4.1 Free residual chlorine concentration for yard tap water (control) and for before and after hand washing

Funeral number	Yard tap water	Before hand washing	After hand washing
1	0.4	0.0	0.0
2	0.3	0.0	0.0
3	0.4	0.3	0.1
4	0.0	0.0	0.0
5	0.0	0.0	0.0
6	0.5	0.0	0.0
7	0.5	0.1	0.0
8	0.5	0.1	0.0
9	0.7	0.4	0.0
10	0.7	0.0	0.0
11	1.5	0.2	0.0
12	0.1	0.0	0.0
13	0.1	0.0	0.0
14	0.1	0.0	0.0
15	0.2	0.1	0.0
16	0.1	0.1	0.0
17	0.2	0.0	0.0
18	0.1	0.1	0.0
19	0.1	0.1	0.0
20	0.2	0.0	0.0

Funeral number	Yard tap water	Before hand washing	After hand washing
21	0.3	0.1	0.0
22	0.1	0.0	0.0
23	0.2	0.0	0.0
24	0.2	0.0	0.0
25	0.2	0.0	0.0
26	0.0	0.0	0.0
27	0.0	0.0	0.0
28	0.1	0.0	0.0
29	0.0	0.0	0.0
30	0.0	0.0	0.0
31	0.1	0.0	0.0
32	0.2	0.1	0.0
33	0.1	0.1	0.0
34	0.3	0.0	0.0
35	0.8	0.6	0.0
36	0.8	0.3	0.0
37	0.8	0.3	0.0
38	0.6	0.1	0.0
39	0.7	0.3	0.0
40	0.7	0.2	0.0
41	0.6	0.1	0.0
42	0.2	0.1	0.0
Minimum	0.0	0.0	0.0
Maximum	1.5	0.6	0.0
Mean	0.3	0.1	0.0
Median	0.2	0.0	0.0
Mode	0.0	0.0	0.0
Range	1.5	0.6	0.9
Standard Deviation	0.3	0.1	0.0
% Compliance	61.9	19.0	0.0

4.3 Enumeration of total aerobic bacteria

The HPC method was used to determine the total number of aerobic bacteria in all water samples collected at the 42 African funerals. The total aerobic bacteria enumerated was then compared to the HPC limit of ≤1000 colony forming units per one millilitre (cfu/1 ml), as prescribed by SANS 241:1 (SANS 2015). As it was expected, the number of aerobic bacteria in water samples collected from the yard tap was all below the prescribed limit, denoting that the quality of the water supply was safe (Table 4.2). In some instances, the recorded values of the total number of aerobic bacteria in water samples collected from the communal hand washing bowls before the commencement of the hand washing ritual, were very high. These high values, which were unexpected, indicated the possibility of contamination of the water prior to mourners washing their hands. The water samples collected after the completion of the hand washing ritual disclosed that the mourners that participated in the hand washing ritual introduced large numbers of microorganisms into the communal hand washing bowl water. The water in the communal hand washing bowls after the completion of the hand washing ritual, could thus contain a wide spectrum of pathogenic microorganisms. The compliance percentage of the water samples collected from communal hand washing bowls before the hand washing ritual commenced, declined only by approximately 5% when compared with the water samples collected from the yard tap. In contrast, the compliance percentage of the water samples collected from communal hand washing bowls after the hand washing ritual, declined by approximately 55% when compared with the water samples collected from the yard tap.

Table 4.2 **Heterotrophic plate counts for yard tap water (control and for before and after hand washing**

Funeral number	Yard tap water	Before hand washing	After hand washing
1	0	5 400	10 400
2	0	2	840
3	0	0	1 070
4	0	43	430
5	3	44	470
6	0	17	1 450
7	0	9	125
8	0	13	550
9	0	6	260
10	0	9	920
11	0	7	900
12	0	99	1 980
13	0	300	500
14	3	180	560
15	1	12 200	12 600
16	11	38	1 120
17	0	2	440
18	2	30	2 070
19	0	9	1 030
20	0	8	520
21	0	4	1 220
22	1	74	540
23	35	330	1 380
24	0	23	1 150
25	0	14	2 740
26	0	25	9 800
27	3	10	950
28	0	1	9 700
29	3	123	1 240
30	1	21	330
31	0	76	1 340

Funeral number	Yard tap water	Before hand washing	After hand washing
32	1	98	1 600
33	1	28	1 210
34	1	26	1 990
35	0	10	1 670
36	0	12	460
37	0	2	1 150
38	0	8	330
39	0	7	830
40	0	7	6 800
41	0	13	810
42	0	17	1 330
Minimum	0	0	0
Maximum	35	12 200	12 600
Mean	2	461	2 067
Median	0	16	1 095
Mode	0	2	1 150
Range	35	12 200	12 475
Standard Deviation	5.6	2 032.5	3 021.1
% Compliance	100.0	95.2	45.2

To obtain a visual perspective of the HPC distribution of the water samples collected from the yard tap, as well as from the communal hand washing bowls before and after the hand washing ritual, a histogram was constructed. The number of funerals that demonstrated compliant HPC values dropped marginally for the water samples collected from the communal hand washing bowls before the hand washing ritual; while the drop in the number of compliant funerals was substantial for the water samples collected from communal hand washing bowls after the hand washing ritual (Figure 4.1). Furthermore, the number of funerals that demonstrated noncompliant HPC values increased noticeably for water samples collected from the communal hand washing bowls after the hand washing ritual.

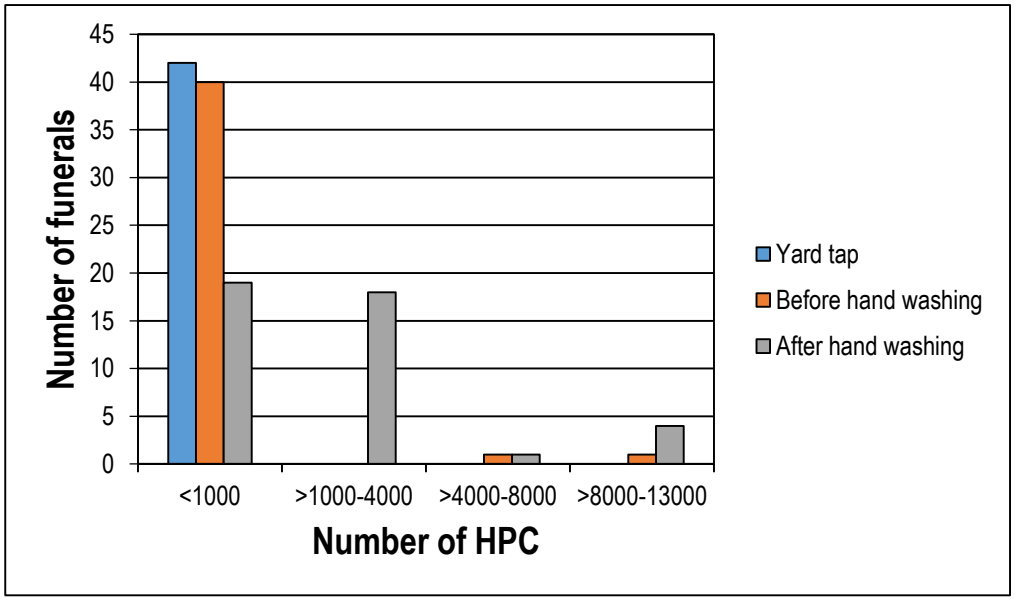


Figure 4.1 **HPC value distribution of water samples collected from the yard tap, before and after hand washing ritual**

4.4 Enumeration of coliform bacteria

The total coliform bacterial count was determined for the all the water samples collected from the three respective water sources. The recorded values of the coliform bacterial counts were then compared to the total coliform bacterial limit of ≤10 coliform/100 ml as prescribed by SANS 241:1 (SANS 2015). Generally, the total number of coliform bacteria was less than the prescribed limit of ≤10 coliform/100 ml (SANS 2015) for the yard tap water samples (Table 4.3). The compliance percentage of the water samples collected from communal hand washing bowls before the hand washing ritual commenced, declined by approximately 22%, when compared with the water samples collected from the yard tap. In contrast, the drop in the compliance percentage for the water samples collected from communal hand washing bowls after the hand washing ritual was dramatic; showing a decline in compliance of 98% when compared with the water samples collected from the yard tap.

Table 4.3 **Total coliform bacteria for yard tap water (control) and for before and after hand washing**

Funeral number	Yard tap water	Before hand washing	After hand washing
1	0	2 420	19 863
2	0	0	111
3	0	0	66
4	0	37	649
5	0	2	2 909
6	0	9	205
7	0	1	19
8	0	0	206
9	0	0	24
10	0	2	488
11	0	1	68
12	0	1	461
13	0	146	1 553
14	0	0	186
15	0	15 531	24 192
16	0	4	345
17	0	0	16
18	0	0	548
19	0	9	249
20	0	0	29
21	0	1	152
22	0	118	1 733
23	0	118	1 553
24	0	1	55
25	0	0	24
26	0	0	291
27	0	0	166
28	0	4	4 106
29	0	0	326
30	0	11	9 208
31	0	649	1 414
32	0	10	291
33	0	1	1 274

Funeral number	Yard tap water	Before hand washing	After hand washing
34	2	1	3 448
35	0	0	201
36	0	1	34
37	0	0	179
38	0	0	2
39	0	0	27
40	0	0	52
41	0	18	236
42	0	0	27
Minimum	0	0	2
Maximum	2	15 531	24 192
Mean	0	455	1 833
Median	0	1	221
Mode	0	0	24
Range	2	15 531	24 190
Standard Deviation	0.3	2 413.7	4 874.2
% Compliance	100.0	78.6	2.4

To obtain a visual perspective of the coliform bacterial count distribution of the water samples, a histogram was constructed. Similarly to the HPC values, the number of funerals that demonstrated compliant coliform bacterial counts also dropped marginally for the water samples collected from the communal hand washing bowls before the hand washing ritual. In contrast, the drop in the number of compliant funerals was more dramatic for the coliform bacterial counts when compared to the drop in HPC values for the water samples collected from communal hand washing bowls after the hand washing ritual (Figure 4.2). This resulted in a substantial increase in the number of funerals that showed noncompliant coliform bacterial counts for water samples collected from the communal hand washing bowls before, as well as after the hand washing ritual.

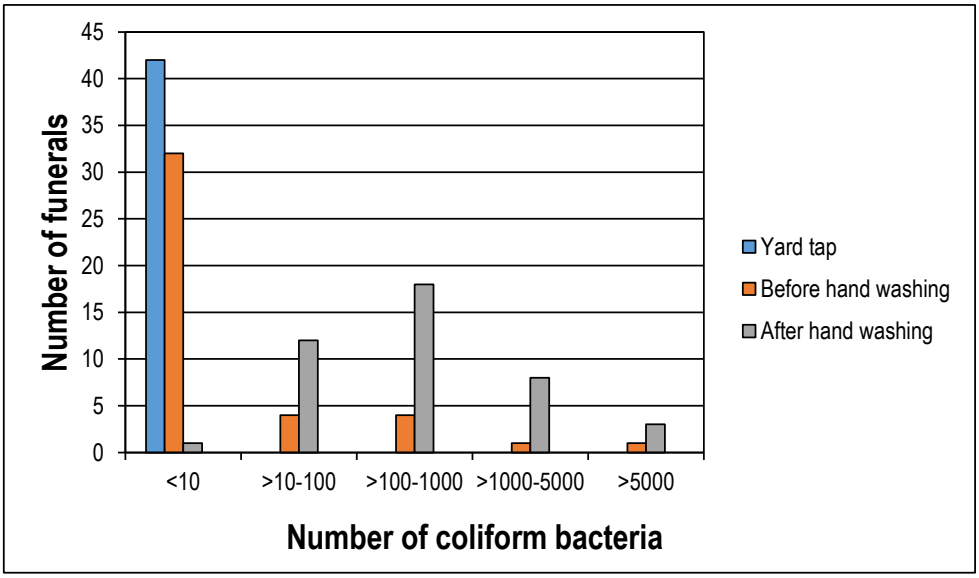


Figure 4.2 **Coliform bacteria number value distribution of water samples collected from the yard tap, before and after hand washing ritual**

4.5 Enumeration of *E. coli*

E. coli bacteria were enumerated for the all the water samples collected from the yard tap, and the hand washing ritual water sources. These enumerations were then compared to the prescribed limit of SANS 241:1 (SANS 2015), which states that *E. coli* bacteria should not be detected in a water sample. As expected, no *E. coli* were present in the yard tap water samples (Table 4.4). The pattern for the compliance percentages of the water samples collected from communal hand washing bowls before and after the hand washing ritual was similar to the patterns found for HPC and coliform bacterial counts. The compliance percentage of the water samples collected from communal hand washing bowls before the hand washing ritual commenced, declined by approximately 12%, when compared with the water samples collected from the yard tap. In contrast, the drop in the compliance percentage for the water samples collected from communal hand washing bowls after the hand washing ritual was 52% when compared with the water samples collected from the yard tap.

Table 4.4 **Measurements of *E. coli* for yard tap water (control), before and after hand washing**

Funeral number	Yard tap water	Before hand washing	After hand washing
1	0	1 120	1 553
2	0	0	4
3	0	0	5
4	0	16	411
5	0	0	0
6	0	0	47
7	0	0	2
8	0	0	1
9	0	0	1
10	0	0	0
11	0	0	0
12	0	0	3
13	0	0	4
14	0	0	1
15	0	1	3
16	0	0	0
17	0	0	1
18	0	0	0
19	0	0	6
20	0	0	0
21	0	0	0
22	0	0	0
23	0	7	4
24	0	0	0
25	0	0	0
26	0	0	19
27	0	0	0
28	0	0	30
29	0	0	0
30	0	1	1
31	0	0	0
32	0	0	0
33	0	0	1 274
34	0	0	0
35	0	0	0
36	0	0	7

Funeral number	Yard tap water	Before hand washing	After hand washing
37	0	0	2
38	0	0	0
39	0	0	0
40	0	0	0
41	0	0	1
42	0	0	1
Minimum	0	0	0
Maximum	0	1 120	1 553
Mean	0	27	81
Median	0	0	1
Mode	0	0	0
Range	0	1 120	1 553
Standard Deviation	0.0	172.7	309.8
% Compliance	100.0	88.0	47.6

A visual perspective of the *E. coli* count distribution of all the water samples is presented in a histogram. The pattern found for the *E. coli* counts was analogous to that of HPC and coliform bacterial counts. The number of noncompliant funerals rose substantially for the water samples collected from the hand washing ritual water bowls (Figure 4.3).

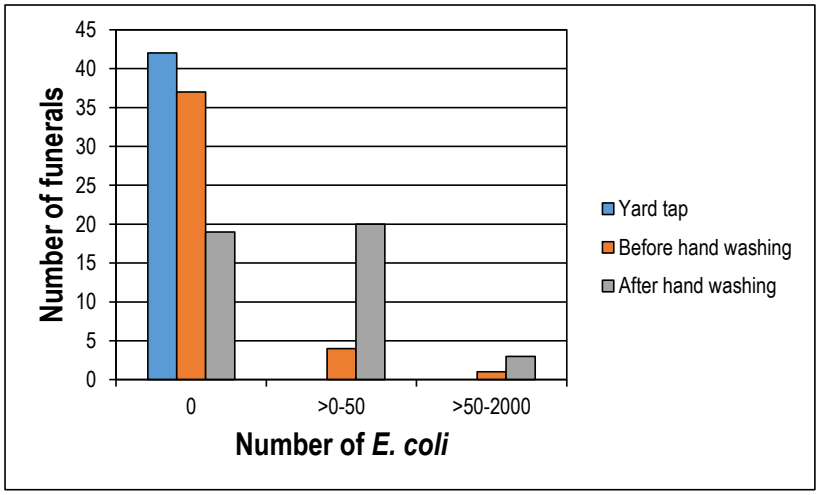


Figure 4.3 ***E. coli* bacteria number value distribution of water samples collected from the yard tap, before and after hand washing ritual**

4.6 Discussion

In this study, the yard tap water that was used to fill the communal hand washing bowls for the funeral hand washing rituals was safe; as indicated by the 100% compliance of all indicator organisms tested; when compared to the microbiological limits prescribed by SANS (2015). Furthermore, the measurements of free residual chlorine also indicated that the water that was used to fill the hand washing bowls were, for the most, safe with measurements equal or above the SANS (2006) limit at 62% of the funerals.

After the communal hand washing bowls were filled with safe water from the yard tap and placed at the gate of a deceased's house, this study revealed that in a number of incidences the water had become unsafe prior to the performance of the hand washing ritual. A number of factors could be responsible for rendering this water unsafe. These factors include contaminated communal hand washing bowls, the introduction of microorganisms from the hands of the handlers of the bowls, as well as dust from the environment. Coliform bacteria and *E. coli* were found in numbers above the limits in 21% and 12% of the funerals respectively. In particular, the water to be used in the hand washing rituals at two funerals (number 1 and 15) were highly contaminated, with exceptionally high coliform (funeral 1 = 2 420; funeral 15 = 15 531) and *E. coli* (funeral 1 = 1 120; funeral 15 = 1) counts.

The water collected after the performance of the hand washing ritual was highly contaminated in most incidences. Coliform bacterial counts, as well as *E. coli* counts were above the SANS 241:1 (2006) limits at 98% and 52% of the funerals respectively. This strongly suggests the introduction of potentially harmful pathogens through the mourner's contaminated hands. Furthermore, the absence of free residual chlorine could not provide disinfection security to the mourners participating in the hand washing ritual. Because African funerals are a community activity with many mourners participating in the hand washing ritual; pathogenic microorganisms may be introduced into the communal hand washing bowls as it is well-known that hands harbour microorganisms, which may be

of faecal origin (Bryn et al. 1995). Furthermore, mourners do not wash their hands for sanitation purposes after the hand washing ritual before eating the funeral meal and are thus at risk of ingesting pathogenic microorganisms whilst eating the funeral meal with their hands. Faecally derived microorganisms are one of the major causes of diarrhoeal type symptoms, which are particularly of concern in young children, the elderly and the immune-compromised attending a funeral. The results of this study support previous reports of mourners demonstrating symptoms of diarrhoeal related diseases after attending an African funeral (Hlungwani et al. 2010; Lamond & Kinyanjui 2012; Maletsky 2007).

Chapter 5

Health Potential Index of Ritual Water

5.1 Introduction

Water quality indexes have been used to communicate information on the quality of water for many years (Ramakrishnaiah et al. 2009; Yisa & Jimoh 2010). It was decided that a water quality assessment instrument should be developed to convey information about the potential health threat that the water sampled in this project, could pose on mourners. After reviewing the literature, the Weighted Arithmetic Index (WAI) of Ramakrishnaiah et al. (2009) was selected, revised and adjusted so that it could convey information about the potential health threat that the ritual water sampled could pose on mourners at the funerals visited in this project. This adjusted WAI is referred to as the Health Potential Index (HPI).

5.2 Calculation of HPI values

HPI values were calculated for the respective water samples collected at the 42 African funerals visited in this project. As expected, the yard tap water samples revealed, overall, low HPI values indicating that the water in the distribution system was safe (Table 5.1). The HPI values increased dramatically from the yard tap water samples to the ritual hand washing water samples collected before hand washing and after hand washing. The before hand washing water samples demonstrated a 2750× increase in the mean HPI values when compared to the HPI values of the yard tap water samples; while the after hand washing water samples showed a more than 3× increase in the mean HPI values when compared to the before hand washing water samples. These dramatic increases in HPI values are also demonstrated by the ranges in HPI values of the three water sampling groups. The maximum HPI value for the water samples collected before hand washing was approximately 6 000× greater than the maximum HPI value of the yard tap water samples; while the maximum HPI value of the water

samples collected after hand washing was more than 10 000× greater than the maximum HPI value of the yard tap water samples. Of the 42 funerals, only six (14.3%) funerals demonstrated relatively low HPI values for all three sampling groups with a difference ≤50 between the before and after hand washing sample groups.

Table 5.1 Health potential index of the water collected at the African funerals

Funerals	HPI Tap water	HPI Before hand washing	HPI After hand washing	Difference*
1	0	5 059.8	1 3043.1	7 983.3
2	0	0.1	78.7	78.7
3	0	0	72.8	72.8
4	0	72.2	1 725.7	1 653.5
5	0.1	2.0	1 060.9	1 058.9
6	0	3.7	285.1	281.3
7	0	0.6	17.7	17.1
8	0	0.4	93.7	93.3
9	0	0.2	19.8	19.6
10	0	1.0	202.4	201.4
11	0	0.6	50.6	50.0
12	0	3.2	234.2	231.0
13	0	61.3	588.0	526.7
14	0.1	5.2	86.8	81.6
15	0	5 948.6	9 085.3	3 136.8
16	0.3	2.5	156.7	154.1
17	0	0.1	22.1	22.1
18	0.1	0.9	257.3	256.4
19	0	3.5	141.1	137.6
20	0	0.2	25.5	25.3
21	0	0.5	90.1	89.6
22	0	44.6	639.5	594.9
23	1.0	77.3	613.5	536.3
24	0	1.0	53.2	52.1
25	0	0.4	88.1	87.7
26	0	0.7	457.4	456.6

Funerals	HPI Tap water	HPI Before hand washing	HPI After hand washing	Difference*
27	0.1	0.3	87.3	87.0
28	0	1.5	1 867.5	1 866.0
29	0.1	3.6	153.3	149.8
30	0	8.2	3 328.1	3 319.9
31	0	235.8	547.9	312.1
32	0	6.4	151.2	144.7
33	0	1.2	5 080.1	5 079.0
34	0.8	1.1	1 299.0	1 297.9
35	0	0.3	120.8	120.5
36	0	0.7	50.8	50.1
37	0	0.1	105.0	104.9
38	0	0.2	10.3	10.1
39	0	0.2	33.8	33.6
40	0	0.2	215.9	215.7
41	0	6.9	112.1	105.2
42	0	0.5	51.9	215.7
Mean	0.1	275.2	1 009.6	738.3
Standard deviation	0.2	1 188.1	2 509.2	1 548.1
Range	1.0	5 948.6	13 032.8	7 084.2
Minimum	0.0	0	10.3	10.3
Maximum	1.0	5 948.6	13 043.1	7 094.5

* = After hand washing minus before hand washing (indicating the increase from HPI before hand washing to HPI after hand washing)

To obtain a visual perspective of the HPI values calculated for the three water sample groups, a histogram was constructed. The histogram clearly shows that the HPI values of the yard tap water samples all fell within the interval of safe water (HPI values ≤50). The HPI values of the water samples collected before the hand washing ritual were largely safe, although a substantial number of samples did pose a health threat. This supports the earlier finding that communal hand washing bowls were contaminated before yard tap water was added for the hand washing ritual. The histogram further shows a substantial drop in the number of funerals that demonstrated HPI values for safe water in the

after hand washing water samples. This drop in the number of funerals was accompanied by an increase in the number of funerals demonstrating HPI values that are indicative of an intermediate (HPI values ≥50-100) or high (HPI values >100) health threat.

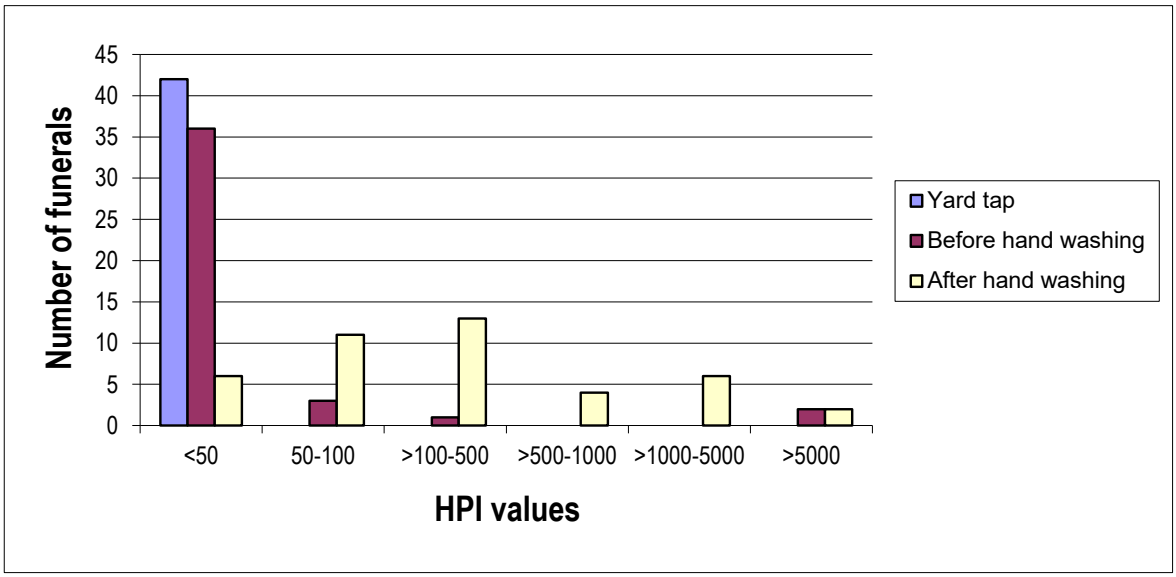


Figure 5.1 HPI value distribution of water samples collected from the yard tap, before and after hand washing ritual

The HPI values of all the water sampling groups were classified using a three-point rating scale to ascertain what the potential health threat of the water of the three water sample groups was. The health potential threat rating disclosed that all the yard tap water samples did not pose a threat and that the water was safe (Table 5.2). In contrast, the before and after hand washing ritual water samples showed an increase in the number of samples that posed an intermediate or high health threat to the mourners. However, the increase in the number of samples posing a high threat was substantially greater in the after hand washing sample group (59.5%), when compared to the before hand washing sample group (7.1%).

Table 5.2 Number African funerals categorised according to different HPI classes

Health potential threat	HPI value	Number (%) of funerals Yard tap water	Number (%) of funerals Before hand washing	Number (%) of funerals After hand washing
No	≤50	42 (100)	36 (85.7)	6 (14.3)
Intermediate	>50 –100	0 (0)	3 (7.1)	11 (26.2)
High	>100	0 (0)	3 (7.1)	25 (59.5)

5.3 Comparison of HPI values

t-Tests were performed to ascertain if significance differences existed between the HPI values of the different water sample groups. The tests revealed that the HPI values of the yard tap water sample group did not differ significantly from the before hand washing ritual water sample group. In contrast, highly significant differences were detected between the HPI values of the yard tap water sample group and the after hand washing water sample group; as well as between the two ritual water sample groups.

Table 5.3 *t*-Tests comparing HPI values of yard tap water and before and after hand washing ritual water

t-Test	df	*t*	*p* – value
Yard tap water (control) vs. before hand washing	41	-1.5	0.0706
Yard tap water (control) vs. after hand washing	41	-2.53	0.0077
Before hand washing vs. after hand washing	41	-2.94	0.0027

5.4 Discussion

The application of a HPI to describe the potential health threat of water samples successfully differentiated between water samples that did not harbour a health threat from those that did. The water sampled from the yard tap was treated water supplied by the municipality, and as expected,

demonstrated the lowest HPI values and was thus deemed to be safe for use. The HPI further revealed the potential health threat posed by some of the water samples that were collected from the communal hand washing bowls before the commencement of the hand washing ritual. These water samples were indicative of contamination of the water by means of a prior contaminated communal hand washing bowl or through the handling of the bowls by the person preparing the ritual water. When a person, who is responsible for the preparation of the ritual water did not observe proper personal hygiene practices, a communal hand washing bowl may be contaminated via the hands of the preparer. However, some contamination of the ritual water in the communal hand washing bowls may be attributed to the surrounding environment, such as dust blown into a bowl from the environment, as well as from dirt collected in the bowls prior to their use. Furthermore, the HPI made it possible to identify the potential health threat that the hand washing rituals at African funerals posed to participating mourners. It strongly indicated that many mourners were responsible for the introduction of potentially harmful microorganisms into the ritual hand washing water. These microorganisms could then be ingested whilst the funeral meal was enjoyed by mourners; who traditionally eat the meal with their bare hands.

Chapter 6

Discussion and Conclusions

6.1 Introduction

In many African cultures, rituals form a fundamental component of African life. At funerals various rituals are performed. One of these rituals involves the washing of hands in communal bowls before the funeral meal is enjoyed. Because the water used in the hand washing rituals is not replenished, it becomes a potential reservoir for the collection of microorganisms that are introduced from the hands of mourners whilst washing their hands in such communal hand washing bowls. These microorganisms may be pathogenic and could be transferred to the hands of succeeding mourners that wash their hand in the same communal hand washing bowl. Furthermore, mourners eat the funeral meal with their air dried hands that may be contaminated with pathogens and are thus at risk of ingesting pathogens whilst eating the funeral meal (Redway & Fawdar 2008; Smith & Lokhorst 2009).

Several instances of mourners becoming ill after attending African funerals have been reported (Maletsky 2007; Hlungwani et al. 2010; Lamond & Kinyanjui 2012). On the 19th of June 2010, funeral attendees of the Tshivhilwi village in the province of Limpopo, developed gastrointestinal symptoms after consuming food served at a funeral (Hlungwani et al. 2010). In Namibia, cholera was confirmed in two people after attending a funeral (Maletsky 2007). This study was thus undertaken to obtain a better understanding of the potential contribution of contaminated ritual hand washing water to disease development in mourners attending African funerals. Water quality of water samples collected from the water supply tap and the communal hand washing bowls before and after the hand washing ritual at 42 African funerals in the Mangaung Metropolitan Municipal area of South Africa, were analysed.

6.2 Potential threat facing mourners attending African funerals

A health potential index (HPI) was developed to reflect the health potential threat of water in the communal hand washing bowls at African funerals. This index was developed using the Weighted Arithmetic Index method of Ramakrishnaiah et al. (2009) as the basis of the HPI. The HPI calculates a single value indicating the potential health threat of a water sample by integrating the measurements of the water quality parameters, namely, total number of aerobic bacteria, number of coliform bacteria and number of *E. coli* present in a water sample. As expected, the treated municipal water collected from the yard taps at the 42 funerals displayed low HPI values (≤1.0), indicating that the water in the distribution systems that was used to fill communal hand washing bowls, was safe. In contrast, the HPI values of the water samples taken from the communal hand washing bowls were substantially higher than those water samples collected from the yard taps. Samples taken from communal hand washing bowls after the hand washing ritual showed much greater HPI values, ranging from approximately 10 to 13 000 when compared to the HPI values of the water samples taken before the hand washing rituals, which displayed HPI values ranging from zero to approximately 6 000. The relatively high HPI values recorded for the water samples collected from the communal hand washing bowls prior to hand washing is clearly indicative of the introduction of microorganisms via other means besides mourner's hands. These microorganisms may have originated from the bowls themselves or from the hands of the women preparing the communal hand washing water bowls, or from the environment. The substantial increase in the HPI values of the water samples collected from the communal hand washing bowls after hand washing, indicates that mourners also introduce high numbers of microorganisms into the water. Because the time lapse from the inception of the hand washing rituals to their completion was less than two hours, it can be assumed that the growth in microbial populations during this time had a negligible effect on the measurements recorded.

A rating scale of the health potential threat was developed to classify the HPI values according to their potential threat to the mourners. This three-point rating scale classifies an HPI value as having no health potential threat, intermediate health potential threat or a high health potential threat to the mourners washing their hands in the communal hand washing bowls. According to this rating scale, the municipal tap water collected at all the funerals was safe and did not pose a risk to the mourners. In contrast, the water in the communal hand washing bowls collected prior to the hand washing rituals possessed a prior health risk at 14% of the funerals before the hand washing rituals commenced. Of these funerals, half of the water samples posed a high health threat. The health potential threat of the water collected after the hand washing rituals increased dramatically. These water samples posed a health threat to the mourners at 35 of the 42 funerals, of which the health threat was high at 26 of these funerals.

In this study, two funerals (1 and 15) showed exceptionally high HPC values for their water samples that were collected from the communal hand washing bowls before the commencement of the hand washing ritual. These funerals also demonstrated high levels of coliform bacteria indicating the poor sanitary quality of the water. They also demonstrated high levels of *E. coli,* which confirmed contamination of faecal origin (Kilb et al. 2003; Fatemeh et al. 2014). Three other funerals (4, 23 and 30) also demonstrated high levels of coliform bacteria and *E. coli.* This evidence strongly supports the notion that a human factor contributed to the contamination of these ritual water samples. This contamination can only be attributed to one or more people handling the communal hand washing bowls before or during the preparation of the bowls for the hand washing ritual. The high levels of faecally derived microorganisms in these prepared ritual water samples are indicative of a lack of proper hand hygiene practices by the people handling the communal hand washing bowls (Anargh et al. 2013; Ataei et al. 2013; FitzGerald et al. 2013).

When the measurements of all the microbial analyses of the water samples collected from the communal hand washing bowls before the commencement of the hand washing ritual were compared to those collected after the hand washing ritual, dramatic increases in all measurements were encountered. For the water samples collected from the communal hand washing bowls before the commencement of the hand washing ritual, 4.8% of funerals demonstrated non-compliant HPC measurements; while 11.9% of the funerals showed non-compliant measurements for both coliform bacteria and *E. coli*. In contrast, for the water samples collected after the hand washing ritual, 54.8% of the funerals were non-compliant for HPC; 97.6% were non-compliant for coliform bacteria and 52.3% were non-compliant for the presence of *E. coli*. It can therefore be concluded that numerous microorganisms were introduced into the hand washing ritual water by the hands of mourners during the hand washing ritual. The high levels of faecally derived microorganisms are indicative of potential harmful pathogens. This evidence further supports the idea that proper hand hygiene practices were not observed by a large number of mourners.

Several studies have shown that mourners become ill after attending an African funeral. A person became ill after attending a funeral in the Tshivhilwi village of the Limpopo Province, on 19 June 2010 (Hlungwani et al. 2010). This person developed diarrhoea, nausea, vomiting, fever, headache and myalgia. In 2007, a funeral hand washing ritual in the Ohangwena Region of Namibia was linked to cholera (Maletsky 2007). Four people died and more than 20 others had to receive medical treatment after attending the same funeral. The evidence provided by this study supports the notion that poor hand hygiene practices could contaminate ritual water during hand washing rituals. Because mourners allow their hands to air dry and then eat the funeral meal with their bare hands, they may be at risk of ingesting pathogenic microorganisms, which could lead to illness.

One of the major categories of disease that can be linked to potential disease development after attending an African funeral is diarrhoea. The disease category referred to as diarrhoea includes a

number of severe diseases, such as cholera, typhoid fever and shigellosis (bacillary dysentery). Cholera is caused by the pathogen *Vibrio cholera*, typhoid fever by *Salmonella typhi* and shigellosis by *Shigella sonnei, S. flexineri, S. boydii and S. dysenteriae.* Worldwide, 88% of diarrhoeal cases can be attributed to the use of contaminated water that results in approximately 1.5 million deaths each year; most being the deaths of children, the elderly and the immune-compromised (Prüss-Üstün et al. 2008). In South Africa the high rates of immune-compromised people suffering with HIV/AIDS and tuberculosis (TB) are of particular concern (Keddy et al. 2012). The prevalence estimates of the number of people living with HIV has increased from an estimated 4.02 million people in 2002 to 6.19 million people in 2015 (Statistics South Africa 2015). The WHO estimates that about 330 000 people have both HIV and TB infection ("World TB Day 2016" 2016).

Diarrhoeal-related diseases are mainly caused by the ingestion of the pathogens, which are all related to the faecal-oral transmission pathways. Inadequate sanitation, as well as insufficient and poor hygiene promote the transmission of these pathogens via the ritual water to the hands of mourners participating in hand washing rituals at African funerals (Ezzati et al. 2004; Prüss-Üstün et al. 2008; Kortenbout et al. 2009). Although *E. coli* O157:H7, that may also cause diarrhoea, is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk; faecal contamination of water may also lead to infection (WHO 2016).

6.3 Concluding remarks

Funeral gatherings in African cultures remain a major feature in the lives of all African people. Mourners who attend African funerals come long distances to pay their respects to the dead and also console the bereaved. This study strongly suggests that hand washing ritual practices at African funerals have the potential for disease transmission convergence (Lamond & Kinyanjui 2012). It has been shown in this study that disease transmission could be linked to poor sanitation and poor personal hygiene practices. This puts mourners in a vulnerable position, thus some or other

intervention could contribute to improve the safety of mourners during their participation in these ritual practices. Because of the importance of African rituals, any intervention should take in consideration the sensitivity and importance of these rituals to African people. Some success has been achieved by community awareness campaigns highlighting the implications of poor sanitation and hygiene practices in hand washing rituals in other countries (Lamond & Kinyanjui 2012). Therefore, by devising an awareness intervention promoting good sanitation and hygiene practices could safeguard mourners against disease transmission at funerals. Such an intervention could achieve results by working through diverse media outlets, as well as through community and religious leaders. Local environmental health practitioners could also be recruited to facilitate the awareness of good sanitation and hygiene practices in their health promotion programmes.

6.4 Future prospects

This study did not focus on the potential role of food preparation and the transmission of disease at African funerals. It is thus suggested that studies should be conducted to research this aspect at African funerals. Furthermore, more studies are needed to ascertain which pathogens are mostly involved in disease development during ritual practices at African funerals. Furthermore, because this study focused mainly on funerals in and around a major town, the water used to fill the ritual bowls were in all instances treated municipal water. In the rural setting, access to treated water is limited and river water is often used to fill the bowls at African funerals. The high levels of microorganisms found in this study thus necessitates a study in a more rural setting, where untreated water is mostly used for hand washing ritual practices.

References

Abd-Elaleem, R., Bakr, W.M.K., Hazzah, W.A., Nasreldin, O., 2014. Assessment of the Personal Hygiene and the Bacteriological Quality of Butchers' Hands in some Abattoirs in Alexandria, Egypt. Food Control 41, 147–150.

Abid, R., 2014. Social Status of Girls / Women and Childbirth in African Culture. J. Soc. Obstet. Gynaecol. Pakistan 4, 208–213.

Abubakar, I., Gautret, P., Brunette, G.W., Blumberg, L., Johnson, D., Poumerol, G., Memish, Z.A., Barbeschi, M., Khan, A.S., 2012. Global Perspectives for Prevention of Infectious Diseases Associated with Mass Gatherings. Lancet Infect. Dis. 12, 66–74.

Agha, R., Goldberg, M.B., 2008. Clinical Manifestations and Diagnosis of Shigella Infection in Children.

Ahmed, R., 2010. Drinking Water Contamination and Its Effects on Human Health.

Ahmed, W., Brandes, H., Gyawali, P., Sidhu, J.P.S., Toze, S., 2014. Opportunistic Pathogens in Roof-Captured Rainwater Samples, Determined Using Quantitative PCR. Water Res. 53, 361–369.

Ahmed, W., Vieritz, A., Goonetilleke, A., Gardner, T., 2010. Health Risk from the Use of Roof-Harvested Rainwater in Southeast Queensland, Australia, as Potable or Nonpotable Water, Determined Using Quantitative Microbial Risk Assessment. Appl. Environ. Microbiol. 76, 7382–7391.

Aiello, A.E., Coulborn, R.M., Perez, V., Larson, E.L., 2008. Effect of Hand Hygiene on Infectious Disease Risk in the Community Setting: A Meta-Analysis. Am. J. Public Health 98, 1372–1381.

Al-Allak, A., Sarasin, S., Key, S., Morris-Stiff, G., 2008. Wedding Rings are Not a Significant Source of Bacterial Contamination Following Surgical Scrubbing. Ann. R. Coll. Surg. Engl. 90, 133–135.

Alam, M., Sultana, M., Nair, G.B., Sack, R.B., Sack, D.A., Siddique, A.K., Ali, A., Huq, A., Colwell, R.R., 2006. Toxigenic *Vibrio cholerae* in the Aquatic Environment of Mathbaria, Bangladesh. Appl. Environ. Microbiol. 72, 2849–2855.

Allegranzi, B., Pittet, D., 2009. Role of Hand Hygiene in Healthcare-Associated Infection Prevention. J. Hosp. Infect. 73, 305–315.

Al-Tawfiq, J.A., Gautret, P., Benkouiten, S., Memish, Z.A., 2016. Mass Gatherings and the Spread of Respiratory Infections: Lessons Drawn from the Hajj. Ann. Am. Thorac. Soc. 13, 759–765.

Alwakeel, S.S., 2007. Bacterial and Aspergillus spp. Contamination and Domestic Kitchens in Riyadh, Saudi Arabia. Saudi J. Biol. Sci. 14, 1–6.

Anargh, V., Singh, H., Kulkarni, A., Kotwal, A., Mahen, A., 2013. Hand Hygiene Practices Among Health Care Workers (HCWs) in a Tertiary Care Facility in Pune. Med. J. Armed Forces India 69, 54–56.

Anderson, E.J., Weber, S.G., 2004. Rotavirus Infection in Adults. Lancet Infect. Dis. 4, 91–99.

Annand, J.W., Bajaj, N., Sheth, A., Burgess, J., Brooke, J.S., 2009. Potential Pathogens and Effective Disinfectants on Public Telephones at a Large Urban United States University. J. Environ. Health 71, 24–48.

Archer, B.N., Cengimbo, A., De Jong, G., Keddy, K.H., Smith, A.M., Sooka, A., Ntshoe, G., Blumberg, L., 2009. Cholera Outbreak in South Africa: Preliminary Descriptive Epidemiology on Laboratory-

Confirmed Cases, 15 November 2008 to 30 April 2009. Commun. Dis. Surveill. Bull.

Ashbolt, N.J., 2004. Microbial Contamination of Drinking Water and Disease Outcomes in Developing Regions. Toxicology 198, 229–238.

Ashbolt, N.J., Gradow, W.O.K., Snozzi, M., 2001. Indicators of Microbial Water Quality. In: Fewtrell, L., Bartram, J. (Eds.), Water Quality: Guidelines, Standards and Health. IWA Publishing, London, UK, pp. 289–316.

Ataei, B., Zahraei, S.M., Pezeshki, Z., Babak, A., Nokhodian, Z., Mobasherizadeh, S., Hoseini, S.G., 2013. Baseline Evaluation of Hand Hygiene Compliance in Three Major Hospitals, Isfahan, Iran. J. Hosp. Infect. 85, 69–72.

Aziz, R.K., Khalifa, M.M., Sharaf, R.R., 2015. Contaminated Water as a Source of *Helicobacter pylori* Infection: A Review. J. Adv. Res. 6, 539–547.

Baloyi, L., Makobe-Rabothata, M., 2013. The African Conception of Death: A Cultural Implication.

Barker, J., Jones, M. V., 2005. The Potential Spread of Infection Caused by Aerosol Contamination of Surfaces After Flushing a Domestic Toilet. J. Appl. Microbiol. 99, 339–347.

Barker, J., Stevens, D., Bloomfield, S.F., 2001. Spread and Prevention of Some Common Viral Infections in Community Facilities and Domestic Homes. J. Appl. Microbiol. 91, 7–21.

Barna, Z., Kádár, M., 2012. The Risk of Contracting Infectious Diseases in Public Swimming Pools. A Review. Ann. Ist. Super. Sanita 48, 374–386.

Beer, K.D., Gargano, J.W., Roberts, V.A., Hill, V.R., Garrison, L.E., Kutty, P.K., Hilborn, E.D., Wade,

T.J., Fullerton, K.E., Yoder, J.S., 2015. Surveillance for Waterborne Disease Outbreaks Associated with Drinking – United States, 2011 - 2012, Morbidity and Mortality Weekly Report.

Begum, Y.A., Talukder, K.A., Nair, G.B., Khan, S.I., Svennerholm, A.M., Sack, R.B., Qadri, F., 2007. Comparison of Enterotoxigenic *Escherichia coli* Isolated from Surface Water and Diarrhoeal Stool Samples in Bangladesh. Can. Journal Microbiol. 53, 19–26.

Bloomfield, S.F., Aiello, A.E., Cookson, B., O'Boyle, C., Larson, E.L., 2007. The Effectiveness of Hand Hygiene Procedures in Reducing the Risks of Infections in Home and Community Settings Including Handwashing and Alcohol-based Hand Sanitizers. Am. J. Infect. Control 35, 27–64.

Boone, S.A., Gerba, C.P., 2007. Significance of Fomites in the Spread of Respiratory and Enteric Viral Disease. Appl. Environ. Microbiol. 73, 1687–1696.

Boone, S.A., Gerba, C.P., 2010. The Prevalence of Human Parainfluenza Virus 1 on Indoor Office Formite. Food Environ. Virol. 2, 41–46.

Borchardt, M.A., Bertz, P.D., Spencer, S.K., Battigelli, D.A., 2003. Incidence of Enteric Viruses in Groundwater from Household Wells in Wisconsin. Appl. Environ. Microbiol. 69, 1172–1180.

Borchardt, M.A., Bradbury, K.R., Gotkowitz, M.B., Cherry, J.A., Parker, B.L., 2007. Human Enteric Viruses in Groundwater from a Confined Bedrock Aquifer Human Enteric Viruses in Groundwater from a Confined Bedrock Aquifer. Environ. Sci. Technol. 41, 6606–6612.

Bridle, H., 2014. Overview of Waterborne Pathogens. In: Waterborne Pathogens: Detection Methods and Applications. Elsevier B.V., pp. 9–40.

Bright, K.R., Boone, S.A., Gerba, C.P., 2010. Occurrence of Bacteria and Viruses on Elementary

Classroom Surfaces and the Potential Role of Classroom Hygiene in the Spread of Infectious Diseases. J. Sch. Nurs. 26, 33–41.

Bruce, S., Yearley, S., 2006. The Sage Dictionary of Sociology, 1st ed. Sage Publications.

Bryn, J., Cochran, J., Larson, E., 1995. Hand Washing: A Ritual Revisited. Crit. Care Nurs. Clin. North Am. 7, 617–625.

Buchanan, R.L., Doyle, M.P., 1997. Foodborne Disease Significance of *Escherichia coli* O157:H7 and Other Enterohemorrhagic E. coli. Food Technol. 51, 69–76.

Castle, J., Phillips, W.L., 2003. Grief Rituals: Aspects That Facilitate Adjustment to Bereavement. J. Loss TraumaInternational Perspect. Stress Coping 8, 37–41.

CDC, 2011. CDC Estimates of Foodborne Illness in the United States CDC 2011 Estimates. Centers Dis. Control Prev.

CDC, 2014. Multistate Outbreak of Shiga Toxin-Producing *Escherichia coli* O157:H7 Infections Linked to Ground Beef. Cent. Dis. Control Prev. (Accessed 14 July 2016).

CFSPH, 2006. *E. coli* O157: H7. Cent. Food Secur. Public Heal.

Chavatte, N., Baré, J., Lambrecht, E., Van Damme, I., Vaerewijck, M., Sabbe, K., Houf, K., 2014. Co-occurrence of Free-living Protozoa and Foodborne Pathogens on Dishcloths: Implications for Food Safety. Int. J. Food Microbiol. 191, 89–96.

Choi, W.Y., Nam, H.W., Kwak, N.H., Huh, W., Kima, Y.R., Kang, M.W., Cho, S.Y., Dubey, J.P., 1997. Foodborne Outbreaks of Human Toxoplasmosis. J. Infect. Dis. 175, 1280–2.

Cocks, M.L., Dold, A.P., 2008. The Cultural Use of the Wild Olive Tree by the amaXhosa People in the Eastern Cape Province of South Africa. J. Study Relig. Nat. Cult. 3, 292–308.

Corso, P.S., Kramer, M.H., Blair, K.A., Addiss, D.G., Davis, J.P., Haddix, A.C., 2003. Cost of Illness in the 1993 Waterborne *Cryptosporidium* Outbreak, Milwaukee, Wisconsin. Emerg. Infect. Dis. 9, 426–431.

Countries and their culture, n.d. Sotho. Ctries. their Cult. (Accessed 07 July 2016).

Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014. Distribution of Indigenous Bacterial Pathogens and Potential Pathogens Associated with Roof-harvested Rainwater. Appl. Environ. Microbiol. 80, 2307–2316.

Dodrill, L., Schmidt, W.P., Cobb, E., Donachie, P., Curtis, V., de Barra, M., 2011. Male Commuters in North and South England: Risk Factors for the Presence of Faecal Bacteria on Hands. BMC Public Health 11, 1–6.

DWAF, 1996. South African Water Quality Guidelines Volume 1 Domestic Use, 2nd ed. Department of Water Affairs and Forestry, Prectoria, South Africa.

DWAF, 2001. Quality of Domestic Water Supplies Volume 1: Assessment Guide, 2nd ed. Water Research Commission, Department of Water Affairs and Forestry, Department of Health.

DWAF, 2013. Strategic Overview of the Water Sector in South Africa. Department of Water Affairs, Republic of South Africa.

Edberg, S.C., Rice, E.W., Karlin, R.J., Allen, M.J., 2000. *Escherichia coli*: The Best biological Drinking Water Indicator for Public Health Protection. Symp. Ser. Soc. Appl. Microbiol. 29, 106–116.

Enriquez, C., Nwachuku, N., Gerba, C.P., 2001. Direct exposure to Animal Enteric Pathogens. Rev. Environ. Health 16, 117–131.

Erdogrul, Ö., Erbilir, F., 2000. Microorganisms in Kitchen Sponges. Internet J. Food Saf. 6, 17–22.

Erickson, M.C., Liao, J., Cannon, J.L., Ortega, Y.R., 2015. Contamination of Knives and Graters by Bacterial Foodborne Pathogens During Slicing and Grating of Produce. Food Microbiol. 52, 138–145.

Ezzati, M., Lopez, A.D., Rodgers, A., Murray, C.J.L., 2004. Comparative Quantification of Health Risks: Global and Regional Burden of Diseases Attributable to Selected Major Risks, World Health Organization, Geneva.

Famurewa, O., David, O.M., 2009. Cell Phone: A Medium of Transmission of Bacterial Pathogens. World Rural Obs. 1, 69–72.

FAO/WHO, 2005. FAO/WHO Regional Conference on Food Safety for Africa 3-6 October 2005, Harare, Zimbabwe. Food Agric. Organ. United Nations/World Heal. Organ.

Faour-Klingbeil, D., Kuri, V., Todd, E., 2015. Investigating a Link of Two Different Types of Food Business Management to the Food Safety Knowledge, Attitudes and Practices of Food Handlers in Beirut, Lebanon. Food Control 55, 166–175.

Farahat, M.F., El-Shafie, M.M., Waly, M.I., 2015. Food Safety Knowledge and Practices Among Saudi Women. Food Control 47, 427–435.

Faruque, S.M., Khan, R., Kamruzzaman, M., Yamasaki, S., Ahmad, Q.S., Azim, T., Nair, G.B., Takeda, Y., Sack, D.A., 2002. Isolation of *Shigella dysenteriae* type 1 and *S. flexneri* Strains

from Surface Waters in Bangladesh: Comparative Molecular Analysis of Environmental Shigella Isolates Versus Clinical Strains. Appl. Environ. Microbiol. 68, 3908–3913.

Faruque, S.M., Tam, V.C., Chowdhury, N., Diraphat, P., Dziejman, M., Heidelberg, J.F., Clemens, J.D., Mekalanos, J.J., Nair, G.B., 2007. Genomic aAnalysis of the Mozambique Atrain of *Vibrio cholerae* O1 Reveals the Origin of El Tor Strains Carrying Classical CTX Prophage. Proc. Natl. Acad. Sci. U. S. A. 104, 5151–5156.

Fatemeh, D., Reza, Z.M., Mohammad, A., Salomeh, K., Reza, A.G., Hossein, S., Maryam, S., Azam, A., Mana, S., Negin, N., Reza, K.A., Saeed, F., 2014. Rapid Detection of Coliforms in Drinking Water of Arak City Using Multiplex PCR Method in Comparison with the Standard Method of Culture (Most Probably Number). Asian Pac. J. Trop. Biomed. 4, 404–9.

Fekety, R., Kim, K.H., Brown, D., Batts, D.H., Cudmore, M., Silva, J., 1981. Epidemiology of Antibiotic-associated Colitis; Isolation of *Clostridium difficile* from the Hospital Environment. Am. J. Med. 70, 906–908.

Ferguson, A.S., Layton, A.C., Mailloux, B.J., Culligan, P.J., Williams, D.E., Smartt, A.E., Sayler, G.S., Feighery, J., McKay, L.D., Knappett, P.S.K., Alexandrova, E., Arbit, T., Emch, M., Escamilla, V., Ahmed, K.M., Alam, M.J., Streatfield, P.K., Yunus, M., van Geen, A., 2012. Comparison of Fecal Indicators with Pathogenic Bacteria and Rotavirus in Groundwater. Sci. Total Environ. 431, 314–322.

Fewtrell, L., 2004. Drinking-water Nitrate, Methemoglobinemia and Global Burden of Disease: A Discussion. Environ. Health Perspect. 112, 1371–1374.

File, T.M., Marrie, T.J., 2010. Burden of Community-acquired Pneumonia in North American Adults.

Postgrad. Med. 122, 130–141.

FitzGerald, G., Moore, G., Wilson, A.P.R., 2013. Hand Hygiene After Touching a Patient's Surroundings: The Opportunities Most Commonly Missed. J. Hosp. Infect. 84, 27–31.

Fitzgerald, M., Thornton, L., O'Gorman, J., O'Connor, L., Garvey, P., Boland, M., Part, A.M., Rogalska, J., Coughlan, H., MacDiarmada, J., Heslin, J., Canny, M., Finnegan, P., Moran, J., O'Flanagan, D., 2014. Outbreak of Hepatitis A Infection Associated with the Consumption of Frozen Berries, Ireland, 2013 - Linked to an International Outbreak, Surveillance and Outbreak Reports, Euro Surveillance.

Fong, T.T., Lipp, E.K., 2005. Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection and Potential Water Quality Assessment Tools. Microbiol. Mol. Biol. Rev. 69, 357–371.

Gall, A.M., Mariñas, B.J., Lu, Y., Shisler, J.L., 2015. Waterborne Viruses: A Barrier to Safe Drinking Water. PLOS Pathog. 11, 1–7.

Gerba, C.P., Wallis, C., Melnick, J.L., 1975. Microbiological Hazards of Household Toilets: Droplet Production and the Fate of Residual Organisms. Appl. Microbiol. 30, 229–237.

Ghareeb, P.A., Bourlai, T., Dutton, W., McClellan, W.T., 2013. Reducing Pathogen Transmission in a Hospital Setting. Handshake verses Fist Bump: A Pilot Study. J. Hosp. Infect. 85, 321–323.

Gibson, K.E., Schwab, K.J., 2011. Detection of Bacterial Indicators and Human and Bovine Enteric Viruses in Surface Water and Groundwater Sources Potentially Impacted by Animal and Human Wastes in Lower Yakima Valley, Washington. Appl. Environ. Microbiol. 77, 355–362.

Girma, G., 2015. Potential Health Risks with Microbial Contamination of Mobile Phones. Glob. Res. J. Educ. 3, 246–254.

Gleick, P.H., 2002. Dirty Water: Estimated Deaths from Water-related Diseases 2000-2020, Pacific Institute Research Report.

Gorbach, S.L., 1996. Microbiology of the Gastrointestinal Tract. In: Barion, S. (Ed.), Medical Microbiology. University of Texas Medical Branch at Galveston.

Gorman, R., Bloomfield, S., Adley, C.C., 2002. A Study of Cross-contamination of Food-borne Pathogens in the DomesticKitchen in the Republic of Ireland. Int. J. Food Microbiol. 76, 143–150.

Hambidge, A., 2001. Reviewing Efficacy of Alternative Water Treatment Techniques. Health Estate 55, 23–25.

Hlungwani, N.P., Khosa, E., Tint, K., Harris, B., Khangala, E., Maimela, E., Moetlo, P., 2010. Food Poisoning Outbreak Amoung Funeral Attendees in Tshivhilwi Village, Vhembe District, June 2010. Commun. Dis. Surveill. Bull.

Howard, G., Bartram, J., 2003. Domestic Water Quantity, Service, Level and Health.

Huslage, K., Rutala, W.A., Sickbert-Bennett, E., Weber, D.J., 2010. A Quantitative Approach to Defining “High-touch” Surface in Hospitals. Infect. Control Hosp. Epidemiol. 31, 850–853.

Ismail, H., Smith, A.M., Tau, N.P., Sooka, A., Keddy, K.H., 2013. Cholera Outbreak in South Africa, 2008-2009: Laboratory Analysis of *Vibrio cholerae* O1 Strains. J. Infect. Dis. 208, S39–45.

Istenes, N., Bingham, J., Hazelett, S., Fleming, E., Kirk, J., 2013. Patients' Potential Role in the Transmission of Health Care-associated Infections: Prevalence of Contamination with Bacterial Pathogens and Patient Attitudes Toward Hand Hygiene. Am. J. Infect. Control 41, 793–798.

Jagals, P., 2004. The Effect of Engineering and Educational Interventions on the Microbiological Quality of Container-stored Water in Developing-world Households. Water Inst. South. Africa Bienn. Conf. 2-6 May 2004, Cape Town, South Africa.

Jagals, P., Jagals, C., Bokako, T.C., 2003. The Effect of Container-biofilm on the Microbiological Quality of Water Used from Plastic Household Containers. J. Water Health 1, 101–108.

Jerković-Mujkić, A., Bešta, R., Memišević, S., 2013. Bacterial Contamination of Public Telephones in the Downtown Area of Sarajevo. African J. Microbiol. Res. 7, 1664–1667.

Josephson, K.L., Rubino, J.R., Pepper, I.L., 1997. Characterization and Quantification of Bacterial Pathogens and Indicator Organisms in Household Kitchens With and Without the Use of a Disinfectant Cleaner. J. Appl. Microbiol. 83, 737–750.

Judah, G., Donachie, P., Cobb, E., Schmidt, W., Holland, M., Curtis, V., 2010. Dirty Hands: Bacteria of Faecal Origin on Commuters' Hands. Epidemiol. Infect. 138, 409–414.

Jumaa, P.A., 2005. Hand Hygiene: Simple and Complex. Int. J. Infect. Dis. 9, 3–14.

Kaltenthaler, E.C., Elsworth, A.M., Schweiger, M.S., Mara, D.D., Braunholtz, D.A., 1995. Faecal Contamination on Children's Hands and Environmental Surfaces in Primary Schools in Leeds. Epidemiol. Infect. 115, 527–534.

Kastenbaum, R., 2003. Macmillan Encylopedia of Death and Dying.

Keddy, K.H., Sooka, A., Crowther-Gibson, P., Quan, V., Meiring, S., Cohen, C., Nana, T., Sriruttan, C., Seetharam, S., Hoosen, A., Naicker, P., Elliott, E., Haffejee, S., Whitelaw, A., Klugman, K.P., 2012. Systemic Shigellosis in South Africa. Clin. Infect. Dis. 54, 1448–1454.

Keddy, K.H., Sooka, A., Parsons, M.B., Njanpop-Lafourcade, B.M., Fitchet, K., Smith, A.M., 2013. Diagnosis of vibrio cholerae o1 infection in Africa. J. Infect. Dis. 208, 23–31.

Kgatla, S.T., 2014. Rituals of Death Enhance Belief and Belonging: Analysis of Selected Elements of Northern Sotho Death Rituals. J. African Affirs 3, 81–86.

Kilb, B., Lange, B., Schaule, G., Flemming, H.C., Wingender, J., 2003. Contamination of Drinking Water by Coliforms from Biofilms Grown on Rubber-Coated Valves. Int. J. Hyg. Environ. Health 206, 563–573.

Kortenbout, W., Mtshali, N., van Dyk, A.C., van Wyk, N.C., Basson, P.M., Leech, R., Mchunu, G., 2009. Communicable Diseases in Southern Africa, First. ed.

Kosek, M., Bern, C., Guerrant, R. l., 2003. The Global Burden of Diarrhoeal Disease, as Estimated from Studies Published Between 1992-2000. Bull. World Heal. Organ. 81, 197–204.

Kusumaningrum, H.D., Riboldi, G., Hazeleger, W.C., Beumer, R.R., 2003. Survival of Foodborne Pathogens on Stainless Steel Surfaces and Cross-contamination to Foods. Int. J. Food Microbiol. 85, 227–236.

Laborde, D.J., Weigle, K.A., Weber, D.J., Kotch, J.B., 1993. Effect of Fecal Contamination on Diarrheal Illness Rates in Day-care Centers. Am. J. Epidemiol. 138, 243–255.

Lambrechts, A.A., Human, I.S., Doughari, J.H., Lues, J.F.R., 2014. Bacterial Contamination of the

Hands of Food Handlers as Indicator of Hand Washing Efficacy in Some Convenient Food Industries. Pakistan J. Med. Sci. 30, 755–758.

Lamond, E., Kinyanjui, J., 2012. Cholera Outbreak Guidelines: Preparedness, Prevention and Control. Oxfam GB.

Lebwohl, B., Deckelbaum, R.J., Green, P.H.R., 2003. Giardiasis. Gastrointest. Endosc. 57, 906–913.

Lee, Y.J., Yoo, C.G., Lee, C.T., Chung, H.S., Kim, Y.W., Han, S.K., Yim, J.J., 2013. Contamination Rates Between Smart Cell Phones and Non-smart Cell Phones of Healthcare Workers. J. Hosp. Med. 8, 144–147.

Lingaas, E., Fagernes, M., 2009. Development of a Method to Measure Bacterial Transfer from Hands. J. Hosp. Infect. 72, 43–49.

Lodder, W.J., van Den Berg, H.H.J.L., Rutjes, S.A., de Roda Husman, A.M., 2010. Presence of Enteric Viruses in Source Waters for Drinking Water Production in the Netherlands. Appl. Environ. Microbiol. 76, 5965–5971.

Lopez, G.U., Gerba, C.P., Tamimi, A.H., Kitajima, M., Maxwell, S.L., Rose, J.B., 2013. Transfer Efficiency of Bacteria and Viruses from Porous and Nonporous Fomites to Fingers Under Different Relative Humidity Conditions. Appl. Environ. Microbiol.

Macias-Rodriguez, M.E., Navarro-Hidalgo, V., Linares-Morales, J.R., Olea-Rodriguez, M.A., Villarruel-Lopez, A., Castro-Rosas, J., Gomez-Aldapa, C.A., Torres-Vitela, M.R., 2013. Microbiological Safety of Domestic Refrigerators and the Dishcloths Used to Clean Them in Guadalajara, Jalisco, Mexico. J. Food Prot. 76, 984–990.

Maletsky, C., 2007. Funeral Custom Linked to Cholera Deaths. The Namibian 1–3.

Maloka, T., 1998. Basotho and the Experience of Death, Dying and Mourning in the South African Mine Compounds, 1890-1940. Cah. Etud. Afr.

Mankga, R.W., 2013. Language Usage is some Tradititional Ritual in Northern Sotho. University of Limpopo.

Mapaya, M.G., Mugovhani, N.G., 2014. African Funeral Ceremonies as African Humanism in Action. Anthropologist.

Martin, A., Lemon, S.M., 2006. Hepatitis A Virus: From Discovery to Vaccines. Hepatology 43, 164–172.

Mason, P.R., 2009. Zimbabwe Experiences the Worst Epidemic of Cholera in Africa. J Infect. Dev. Ctries. 3, 148–151.

Mattick, K., Durham, K., Domingue, G., Jørgensen, F., Sen, M., Schaffner, D.W., Humphrey, T., 2003. The Survival of Foodborne Pathogens During Domestic Washing-up and Subsequent Transfer onto Washing-up Sponges, Kitchen Surfaces and Food. Int. J. Food Microbiol. 85, 213–226.

McFadden, R., 2003. The Usual Suspects: Microbes, Biohazards and Pathogens How Cleaning Professionals Can Protect Themselves Against Hazards Associated With Germs Transmitted by Blood, Body Fluids, Food, Air, Water and Nonliving Surfaces.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R. V., 1999. Food-related Illness and Death in the United States. Emerg. Infect. Dis. 5, 841–842.

Mela, S., Whitworth, D.E., 2014. The Fist Bump: A more Hygienic Alternative to the Handshake. Am. J. Infect. Control 42, 916–917.

Memish, Z. A., Zumla, A., Alhakeem, R.F., Assiri, A., Turkestani, A., Al Harby, K.D., Alyemni, M., Dhafar, K., Gautret, P., Barbeschi, M., McCloskey, B., Heymann, D., Al Rabeeah, A. A., Al-Tawfiq, J. a., 2014. Hajj: Infectious disease surveillance and control. Lancet 383, 2073–2082.

Mishra, B., Sarker, D., Srivastava, S., Deepthi, S., Cheta, N., Mishra, S., 2013. Hand Hygiene - Religious, Cultural and Behavioural Aspects. Univers. J. Educ. Gen. Stud. 2, 184–188.

Moabi, M., 2006. The Effect of Improving Access To Water Supplies on the Health-related Microbiological Quality of Container-stored Water in Developing-community Households. University of Johannesburg.

MOH, 2007. Pathogens and Pathways, and Small Drinking-Water Supplies. Resources for the Drinking-Water Assistance Programme.

Momba, M.N.B., Malakate, V.K., Theron, J., 2006. Abundance of Pathogenic Escherichia coli , Salmonella typhimurium and Vibrio cholerae in Nkonkobe Drinking Water Sources. J. Water Health 4, 289–296.

Moosavi, Z.B., Lofti, G., 2009. The Evaluation of Bacterial Colonisation on Skin Leasions of Hospitalised Patients in Dermatology Department of Ahvaz. Jundishapur J. Microbilogy 2, 148–151.

Munoz-Price, L.S., Arheart, K.L., Mills, J.P., Clery, T., DePascale, D., Jimenez, A., Fajardo-Aquino, Y., Coro, G., Birnbach, D., Lubarsky, D.A., 2012. Associations between bacterial contamination of health care workers' hands and contamination of white coats and scrubs. Am. J. Infect. Control

40, 1–4.

Nala, N.P., Jagals, P., Joubert, G., 2003. The Effect of Engineering and Educational Interventions on the Microbiological Quality of Container-stored Water in Developing-world Households. Water SA 29, 171–176.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J., Kruse, H., 2010. Food-borne Diseases - The challenges of 20 years ago Still Persist While New Ones Continue to Emerge. Int. J. Food Microbiol. 139, 3–15.

Nkosi, S., 2011. Lobola: Black Students' Perceptions of its Role on Gender Power Dynamics. University of Witwatersrand.

Ntsihlele, F.M., 2003. Games, Gestures and Learning in Basotho Children's Play Songs. University of South Africa.

Nwankiti, O.O., Ndako, J.A., Nwankiti, A.J., Okeke, I.O., Uzoechina, A.R., Agada, G.O., 2012. Computer Keyboard and Mouse: Etiologic Agents for Microbial Infections. Nat. Sci. 10, 162–166.

Nwankwo, E.O., Ekwunife, N., Mofolorunsho, K.C., 2014. Nosocomial pathogens Associated with the Mobile Phones of Healthcare Workers in a Hospital in Anyigba, Kogi State, Nigeria. J. Epidemiol. Glob. Health 4, 135–140.

Nyawose, T., 2000. "Living in Two Worlds" Codes and Modes of Expression at Zulu Funerals in KwaZulu-Natal at the Turn of the Millenium. University of Natal.

O'Toole, J., Sinclair, M., Leder, K., 2009. Transfer Rates of Enteric Microorganisms in Recycled Water

During Machine Clothes Washing. Appl. Environ. Microbiol. 75, 1256–1263.

Odonkor, S.T., Ampofo, J.K., 2013. *Escherichia coli* as an Indicator of Bacteriological Quality of Water: An Overview. Microbiol. Res. (Pavia). 4, 5–11.

Onigbogi, O., Ogunyemi, O., 2014. Effects of Improved Storage Containers on the Bacteriological Quality of Household Drinking Water in Low-income Urban Communities in Ibadan, Nigeria. Int. J. Public Heal. Sci. 3, 253–258.

Pavli, P., Venkateswaran, S., Bradley, M., Bridle, H., 2015. Enhancing Cryptosporidium parvum Recovery Rates for Improved Water Monitoring. Chemosphere 143, 1–7.

Potgieter, N., Becker, P.J., Ehlers, M.M., 2009. Evaluation of the CDC Safe Water-storage Intervention to Improve the Microbiological Quality of Point-of-Use Drinking Water in Rural Communities in South Africa. Water SA 35, 505–516.

Prüss-Üstün, A., Bos, R., Gore, F., Bartram, J., 2008. Safer Water, Better Health. World Heal. Organ.

Ramakrishnaiah, C.R., Sadashivaiah, C., Ranganna, G., 2009. Assessment of Water Quality Index for the Groundwater in Tumkur Taluk, Karnataka State, India. E-Journal Chem. 6, 523–530.

Redway, K., Fawdar, S., 2008. A Comparative Study of three different Hand Drying Methods: Paper Towel, Warme Air Dryer, Jet Air Dryer. In: European Tissue Symposium, Brussels. London, p. 35.

Reynolds, K. a, Watt, P.M., Boone, S. a, Gerba, C.P., 2005. Occurrence of bacteria and biochemical markers on public surfaces. Int. J. Environ. Health Res. 15, 225–234.

Rezaeinejad, S., Vergara, G.G.R. V., Woo, C.H., Lim, T.T., Sobsey, M.D., Gin, K.Y.H., 2014. Surveillance of Enteric Viruses and Coliphages in a Tropical Urban Catchment. Water Res. 58, 122–131.

Rogers, S., Haines, J., 2005. Detecting and Mitigating the Environmental Impact of Fecal Pathogens Originating from Confined Animal Feeding Operations: Review. Environ. Prot. Agency, United States 1–175.

Rusin, P., Maxwell, S., Gerba, C., 2002. Comparative Surface-to-Hand and Fingertip-to-Mouth Transfer Efficiency of Gram-positive Bacteria, Gram-negative Bacteria and Phage. J. Appl. Microbiol. 93, 585–592.

Rusin, P., Orosz-Coughlin, P., Gerba, C., 1998. Reduction of Faecal Coliform, Coliform and Heterotrophic Plate Count Bacteria in the Household Kitchen and Bathroom by Disinfection with Hypochlorite Cleaners. J. Appl. Microbiol. 85, 819–828.

Sabra, S.M.M., 2013. Bacterial Public Health Hazard in the Public Female Restrooms at Taif, KSA. Middle-East J. Sci. Res. 14, 63–68.

SANS, 2006a. SANS 241: 2006 Driniking Water. South African Natl. Stand.

SANS, 2006b. SANS 241:2006.

SANS, 2015. SANS 241-1: 2015 Drinking Water Part1: Microbiological, Physical, Aesthetic and Chemical Determinands. South Afrcan Natl. Stand.

Scott, E., Bloomfield, S.. F., 1990. The Survival and Transfer of Microbial Contamination Via Cloths, Hands and Utensils. J. Appl. Bacteriol. 68, 271–278.

Scott, E., Bloomfieldt, S.F., Barlow, C.G., 1982. An Investigation of Microbial Contamination in the Home. J. Hyg. (Lond). 279–293.

Semenya, D.K., 2014. The Practical Guidelines on the Impact of Mahadi [bride price] on the Young Basotho Couples Prior to Marriage. HTS Teol. Stud. / Theol. Stud. 70, 1–6.

Setsiba, T.H.S., 2012. Mourning Rituals and Practices in Contemporary South African Townships: A Phenomenological Study. University of Zululand.

Sklansky, M., Geffen, D., Angeles, L., Nadkarni, N., Ramirez-Avila, L., 2014. Banning the Handshake From the Health Care Setting. J. Am. Med. Assoc. 311, 5–6.

Smith, A.M., Keddy, K.H., De Wee, L., 2008. Characterization of Cholera Outbreak Isolates from Namibia, December 2006 to February 2007. Epidemiol. Infect. 136, 1207–1209.

Smith, J.M., Lokhorst, D.B., 2009. Infection Control: Can Nurses Improve Hand Hygiene Practices? Infect. Control Nurses 1 – 3.

Statistics South Africa, 2015. Mid-year Population Estimates. www.statssa.gov.za/ publications/.../ P 03022014.pdf 1–18.

Stylianou, K., Passam, A., Papoutsakis, A., Perakis, K., Kroustalakis, N., Daphnis, E., 2008. *Listeria Monocytogenes Peritonitis* in a Peritoneal Dialysis Patient with Severe Heart Failure. Kidney 17, 238–240.

Tajkarimi, M., Ibrahim, S.A., Fraser, A.M., 2013. Food Safety Challenges Associated with Traditional Foods in Arabic Speaking Countries of the Middle East. Trends Food Sci. Technol. 29, 116–123.

Tredoux, G., Engelbrecht, P., Israel, S., 2009. Nitrate in Groundwater. Why is it a Hazard and How to Control It?, Report to the Water Research Commission by CSIR, Natural Reesources and the Environment, Stellenbosch.

Trevett, A.F., Carter, R.C., Tyrrel, S.F., 2005a. The Importance of Domestic Water Quality Management in the Context of Faecal-oral Disease Transmission. J. Water Health 3, 259–270.

Trevett, A.F., Carter, R.C., Tyrrel, S.F., 2005b. Mechanisms Leading to Post - Supply Water Quality Deterioration in Rural Honduran Communities. Int. Journsl Hyg. Environ. Heal. 208, 153–161.

Tshapa, N., 2016. Ukuzila Ngendlela Yesintu - Ulwazi. Ulwazi (Accessed 13 July 2016).

Udo, E.E., Al-Bustan, M.A., Jacob, L.E., Chugh, T.D., 1999. Enterotoxin Production by Coagulase-negative Staphylococci in Restaurant Workers from Kuwait City May be a Potential Cause of Food Poisoning. J. Med. Microbiol. 48, 819–823.

Ulger, F., Esen, S., Dilek, A., Yanik, K., Gunaydin, M., Leblebicioglu, H., 2009. Are We Aware How Contaminated Our Mobile Phones With Nosocomial Pathogens? Ann. Cinical Microbiol. Antimicrob. 8, 7.

Vandeplas, S., Marcq, C., Dauphin, R.D., Beckers, Y., Thonart, P., Théwis, A., 2007. Contamination of Poultry Flocks by the Human Pathogen *Campylobacter* spp. and Strategies to Reduce Its Prevalence at the Farm Level. Biotechnol. Agron. Société Environ. 12, 317–334.

Veneman, A., Chan, M., 2009. Diarrhoea: Why children are still dying and what can be done, WHO Library Cataloguing-in-Publication Data.

Verheyen, J., Timmen-Wego, M., Laudien, R., Boussaad, I., Sen, S., Koc, A., Uesbeck, A., Mazou, F.,

Pfister, H., 2009. Detection of Adenoviruses and Rotaviruses in drinking water sources used in rural areas of Benin, West Africa. Appl. Environ. Microbiol. 75, 2798–2801.

Verhille, S., 2013. Understanding Microbial Indicators for Drinking Water Assessment: Interpretation of Test Results and Public Health Significance. Natl. Collab. Cent. Environ. Heal.

Wampler, P.J., Sisson, A.J., 2011. Spring flow, bacterial contamination and water resources in rural Haiti. Environ. Earth Sci. 62, 1619–1628.

WHO, 2002. Heterotrophic plate count measurement in drinking water safety management, World Health Organization, Geneva.

WHO, 2004. Cholera Outbreak. Assessing the outbreak response and improving preparedness. Global Task Force on Cholera Control, World Health Organization, Geneva.

WHO, 2008. Communicable Disease Alert and Response for Mass Gatherings: Key Considerations. World Health Organization, Geneva.

WHO, 2009. WHO guidelines on hand hygiene in health care first global patient safety challenge clean care is safer care. World Health Organization, Geneva.

WHO, 2011a. Guidelines for drinking-water quality, 4th ed. World Health Organization, Geneva.

WHO, 2011b. Nitrate and Nitrite in Drinking Water, Background Document for Development of WHO Guidelines for Drinking Water Quality. World Health Organization, Geneva.

WHO, 2013. Overview of Reported Outbreaks in WHO African Region. Outbreak Bull. World Heal. Organ. Off. Africa.

WHO, 2016. *E.coli* [WWW Document]. World Heal. Organ. URL http://www.who. int/mediacentre/factsheets/fs103/en/ (Accessed 04 November 2016).

WHO, n.d. Water sanitation health managing water in the home. World Heal. Organ. (Accessed 14 July 2016).

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., Ferm, M., Björkholm, B., Hansen, A., Hiltula, J., Långmark, J., Löfdahl, M., Omberg, M., Reuterwall, C., Samuelsson, E., Widgren, K., Wallensten, A., Lindh, J., 2014. Large Outbreak of *Cryptosporidium hominis* Infection Transmitted through the Public Water Supply, Sweden. Emerg. Infect. Dis. 20, 581–589.

World TB Day 2016, 2016. . South African Gov.

Yawa, S.N., 2010. A Psycho-Analysis of Bereavement in Xhosa, Zulu and Tswana Cultures. University of South Africa.

Yisa, J., Jimoh, T., 2010. Analytical Studies on Water Quality Index of River Landzu. Am. J. Appl. Sci. 7, 453–458.

Yu, Q., Zhai, L., Bie, X., Lu, Z., Zhang, C., Tao, T., Li, J., Lv, F., Zhao, H., 2016. Survey of Five Food-Borne Pathogens in Commercial Cold Food Dishes and their Detection by Multiplex PCR. Food Control 59, 862–869.

Zhong, C.B., Liljenquist, K., 2006. Washing Away Your Sins: Threatened Morality and Physical Cleansing.

Zibani, A.N., 2002. Zulu Cultural Traditions: A Draw Card for Tourims in Kwazulu Natal, with Special Refence to the Lebombo Spatial Development Initiative. University of Zululand.

Annexure

Letter of permission

Central University of Technology, Free State

Biotechnology

Celebrating 30 Years of Technological Innovation Soaring to 2020

Requesting permission to collect water samples from the yard tap and communal hand washing basin on the day of the funeral

I, Alliestar Moabi, am a registered Master's student in environmental health at the Central University of Technology, Bloemfontein. I am currently employed by Mangaung Metropolitan Municipality as an Environmental Health Practitioner. I am conducting a research project focusing on "*Microbial quality of communal hand washing water at African funerals in the Mangaung region*". In order for this project to succeed, your support and willingness in granting me permission to collect water samples at the following address will be highly appreciated.

Address: .....

.....

All contact details will be kept as confidential information and will not be made available to the public.

Your support in this research project will be appreciated.

.....
N. A. Moabi
(M. Tech. Research – Student)

13/3/2012
Date

@Fossey
Prof A. Fossey
(Supervisor)

13/03/2012
Date

.....
Dr E. Potgieter
(Co – Supervisor)

14/03/2012
Date

Our Ref: Alliestar Moabi

Your Ref: .....

Date: 12 March 2012

Cell: 082 587 7237

Faculty of Health and Environmental Sciences • Private Bag X20539 • Bloemfontein • SOUTH AFRICA • 9300 •
Tel: +27 51 507 3123 • **Fax:** +27 51 507 3354 • **E-mail:** afossey@cut.ac.za • **Website:** www.cut.ac.za

Central University of Technology, Free State

■ Biotechnology

Celebrating 30 Years of Technological Innovation Soaring to 2020

Requesting permission to collect water samples from the yard tap and communal hand washing basin on the day of the funeral

Name and Surname of the family representative		
Contact Details	**Home:**	
	Cell:	
Address (Premises / house where the samples will be collected)		
	Code	

I, ... (full names of family representative) grant permission for water samples to be collected at the abovementioned premises for the purpose of an M. Tech. research study on the day of the funeral.

....

Signature of the family representative **Date**

Thank you for contributing to the success of this project,

Mr N. A. Moabi.

Faculty of Health and Environmental Sciences • Private Bag X20539 • Bloemfontein • SOUTH AFRICA • 9300 •
Tel: +27 51 507 3123 • **Fax:** +27 51 507 3354 • **E-mail:** afossey@cut.ac.za • **Website:** www.cut.ac.za