CHARACTERIZATION OF *E. coli* STRAINS FROM RURAL COMMUNITIES IN THE VHEMBE DISTRICT (LIMPOPO SOUTH AFRICA)

by

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DECLARATION

I, Ntshunxeko Thelma Banda (Student number: 16013629) declare that this dissertation for the award of Masters' degree in Microbiology (MSc MBY) at the University of Venda is my original work and has not been previously submitted for any degree at any other University or Institution. All reference materials contained herein have been duly acknowledged.

Signed …… Date ……

DECICATION

I dedicate my work to my late uncle, Madala Fixon Ndima. You always encouraged me to further my studies. “You still young, time allows you, as long there is resources, pursue further Madam.” Sadly, you departed just as I started to follow your words.

I also dedicate this work to my precious baby girl, N’wayitelo MK Banda. Your birth timing couldn’t have been better, just before my Masters’ submission. God has the perfect time.

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ABSTRACT

Background: *Escherichia coli* is a facultative anaerobic bacterium that forms part of the gut microbiota. It is used as an indicator that confirms recent faecal contamination. *E. coli* have been identified amongst the pathogens that are mostly responsible for moderate to severe diarrheal outbreaks in the low and middle-income countries. With South Africa facing an issue in water scarcity, issues concern poor sanitation and hygiene practices results in serious public health problems and allows *E. coli* to be transmitted from infected human or animal faeces to a new susceptible host using environmental reservoirs such as soil, water, hands as the transmission pathway.

Objective: The primary objective of the study was to characterize *E. coli* strains from rural communities of Vhembe district, Limpopo, South Africa.

Methodology: Households of 7 villages in the Vhembe district were randomly selected. A total of 81 households (HHs) were part of the study. In each household, a structured questionnaire was used to background information on WASH practices. Samples taken from each HH included toilet seat swabs, floor swabs, child and mother handwash samples, stored water samples and running tap water samples. A total of 399 samples were analysed using Colilert® Quanti-trays®/2000 method to detect the presence of *Escherichia coli*. Positive *E. coli* samples were further identified using multiplex polymerase chain reaction (m-PCR) to determine the pathogenic strains of *E. coli.* Transmission pathways were established using identified strains.

Results: Data from the structured questionnaires showed common problems of availability of running tap water; lack of provision of sanitation; open practice on defaecation and very little hand hygiene practices. A total of 91 (22.81%) samples tested positive for *E. coli* with the Colilert® Quanti-trays®/2000 method. The mothers' handwash samples had the most *E. coli* prevalence followed by stored water samples. The most prevalent *E. coli* pathotype was EPEC with the virulence gene *eae*. Atypical EPEC (60.44%) outnumbered the typical EPEC (5.49%). The pathotype ETEC was detected in 41.76% samples and EHEC in 9.89% samples. Transmission pathway was observed from the different households; with *eae* gene (aEPEC) being the most detected from samples looking at the LT gene (ETEC).

Discussion: All 7 villages are facing common issues such as lacking running water, poor sanitation and improper hand hygiene practices. The mothers were the most contaminated and it was observed that its due to the daily activities that they perform around the house. It is of importance for them to practice proper hand hygiene to prevent transmission of pathogenic *E. coli* to the children via direct or indirect transmission route. The pathogenic *E. coli* was detected from all different samples collected from the households including the floor and toilet seat samples. EPEC was detected the most, and studies have shown that this strain is known to cause diarrheal infections in young children from developing countries.

Conclusion: The members of the study village households were aware of the WASH services and its importance. However, proper implementation into their day-to-day life is lacking due to the high number of TC and *E. coli* detected from handwash samples and stored water samples from the households.

Recommendation: Appropriate WASH strategies should be established to ensure good health at the village households. Further studies should be done to check possible transmission pathways from more village households.

Keywords: *Escherichia coli*, Environmental reservoirs, Diarrhoea, Transmission pathway

LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
%	Percentage
Bp	Base pairs
˚C	Degree Celsius
Ml	milliliter
AIDS	Acquired immune defiency syndrome
CDC	Center for Disease Control and Prevention
Cfu	Colony-forming unit
EE	Environmental enteropathy
EED	Environmental enteric dysfunction
aEPEC	atypical Enteropathogenic *Escherichia coli*
DEC	Diarrheagenic *Escherichia coli*
dNTP	Deoxyribonucleotide triphosphate
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ESBL	Extended spectrum β-lactamase
ETEC	Enterotoxigenic *Escherichia coli*
F	Forward
HH(s)	Household(s)
HIV	Human Immunodeficiency virus
Hr(s)	Hour(s)
LT	Heat-liable
M	Meters

Max	Maximum
MF	Membrane filtration
Min	Minimum
m-PCR	multiplex Polymerase chain reaction
MPN	Most Probable Number
MTF	Multiple-tube fermentation
MUG	4-methylumbellifery-β-D-glucoronide
ONPG	o-nitrophenyl-β-D- galactophyranoside
PCR	Polymerase Chain reaction
R	Reverse
RHRW	Rain harvested rainwater
SA	South Africa
Spp.	Species
SPA	Service Provision Assessments
STATSSA	Statistics South Africa
STEC	Shiga toxin-producing *Escherichia coli*
tEPEC	typical Enteropathogenic *Escherichia coli*
TC	Total coliform
UN	United Nations
UNICEF	United Nations Children's Fund
WASH	Water, sanitation and hygiene
WHO	World Health Organization

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Escherichia coli (*E. coli*) is a facultative anaerobic bacterium that has been comprehensively studied and is known to play a major role in the gut microbiota. This bacterial specie is easy to manipulate genetically and has become a popular laboratory workhouse (Croxen and Finlay, 2010; Drasar and Hill, 1974). However, pathogenic strains thereof have been identified and classified using their different physiological, antigenic and virulence characteristics that cause gastrointestinal diseases such as diarrhoea (Kaper et al., 2004; Nataro and Kaper, 1998).

In public health, *E. coli* is used as a faecal indicator that validates assumptions of its presence in the environment as a sign of recent faecal contamination (Goto and Yan, 2011). Pathogenic *E. coli* strains are implicated in many waterborne outbreaks, with Shiga Toxin-producing *Escherichia coli* (STEC) and Enteropathogenic *Escherichia coli* (EPEC) frequently reported to be responsible for waterborne outbreaks worldwide (Chandran and Mazumder, 2015). The presence of pathogenic *E. coli* in the environment may be due to contaminated manure, animal wastes and effluents from wastewater treatment plant (Balière et al., 2015).

Studies have challenged the use of *E. coli* as an indicator for recent faecal contamination by demonstrating its ability to survive in different environments such as sediments, beach sand and aquatic vegetations for extended period of time (Ishii et al., 2009; Ksoll et al., 2007; Ishii et al., 2006). This demonstrates that *E. coli* can survive in numerous environments that attributes to its genetic diversity. Therefore, it remains a public threat due to its high genetic diversity that demonstrates its adaptability and resistance to environmental changes (Van Elsas et al., 2011; Rauch and Bar-Yam, 2004).

Diarrheal diseases are the cause of almost 1.3 million deaths annually with the most cases occurring in limited-resource countries (Troeger et al., 2017). Despite global success of reducing all cause and diarrhoea specific mortality in the past 30 years, diarrheal infections remain to be one of leading cause of mortality among children under the age of 5 worldwide (Walker et al., 2013; Samal et al., 2008). Enterohaemorrhagic *E. coli* is one of the pathogenic strains well known to cause diarrheal infections (Page and Liles, 2013). Majowicz et al (2014) reported that serotype *E. coli* O157:H7 accounts for over 2.5 million acute illnesses annually. This serotype is usually harboured by livestock (Callaway et al, 2009). Therefore, transmission to human beings may occur when cattle manure is washed off into drinking water supply and consumed directly or any other faecal-oral transmission pathway (Thurston-Enriquez et al., 2005; Sargeant et al., 2003).

Previously, studies have indicated poor water, sanitation and hygiene (WASH) practices were a major contribution to diarrheal outbreaks (Waddington et al., 2009; Fewtrell et al., 2005; Esrey et al., 1991). Of recent, emerging evidence supports the contribution of environmental factors related to poor water, sanitation and hygiene conditions to diarrheal infection reported annually (Pickering et al., 2015; Rah et al 2015, Ngure et al., 2014). Kosek et al. (2014) elaborates on faecal contamination in the environment due to lack of sanitation that leads to high rate of diarrheal outbreaks and its hypothesized as an impact to malnutrition through environmental enteropathy.

1.2 STUDY RATIONALE

South Africa is a developing country, currently facing water scarcity issues (Nkuna et al., 2014). Many rural areas lack access to tap water services, as such an estimated 80% of rural communities rely entirely on borehole or river water for their day-to-day use (DWA, 2010). Some rural communities use contaminated water sources such as dams and rivers for domestic purposes (Majuru et al., 2011, Sobsey, 2006). The water scarcity issue also forces people to store water in different types of containers till period of use (Turton, 2008, Gundry et al., 2006; Prüss et al., 2002). Water contamination tend to occur during storing-period due to unsafe storage conditions, improper handling of storage containers and the use of dirty water-storage containers (Potgieter et al., 2009).

The Vhembe District of the Limpopo Province; the most populated district (approximately 1 393 949 people) amongst the 4 is predominantly rural, and poverty stricken (Vhembe district profile, 2017; Kyei, 2011; Obi et al., 2002). Most of the communities depend on untreated surface and groundwater for their day-to-day uses (Vhembe district profile, 2013; Obi et al., 2002). A recent study done by Traore et al (2016) in the Vhembe district, reported that environmental factors such as washing clothes and faecal run-offs tend to contaminate the water sources. Other communities without access to groundwater depend on rainwater for domestic purposes including drinking, food preparation, bathing and washing (Kahinda et al., 2010). There is a general public health perception that it is safe to drink rain-harvested water (Kahinda et al., 2007). However, Ahmed et al (2011) has reported the presence of pathogens such as *E. coli, Salmonella spp, Giardia spp, Vibro spp* and other enteric organisms in rainwater.

Resource-limited areas have domestic livestock and poultry in close proximity to humans as they serve as a primary source of income (Thumbi et al., 2015; Randolph et al., 2007; Sansoucy et al., 1995). The livestock and poultry may be left to roam around the household yard, which may lead to increase in potential faecal contamination of the soil which children usually play on (Pickering et al., 2012). This may result in zoonotic transmission of enteric pathogens harboured by the animals. Contaminated soil is problematic among young children as faecal-oral transmission may be more common to occur during time of play (Zambrano et al., 2014).

Rural community households in the Vhembe District keep domestic animals such as chickens, cattle, pigs and dogs in the yards. The people believe in traditional farming practices where the animals roam freely, eating naturally growing grass (Self-observation). Cattles are known to be a major reservoir for Enterohemorrhagic *Escherichia coli* (EHEC) infections worldwide (Beutin, 2006). Callaway et al (2009) showed that diets can affect the carriage and shedding of *E. coli* in cattle which might indirectly lead to the spread of *E. coli* to humans. Transmission may occur through

consumption of animal products, drinking contaminated water and consuming contaminated plant products (Zambrano et al., 2014).

Studies have previously focussed on the role of water, sanitation and hygiene practices as transmission of enteric bacteria that leads to diarrheal outbreaks (Mattioli et al., 2012; Craun et al., 2010; Fewtrell et al., 2005). However, recent studies have highlighted the potential importance of other transmission pathways including hands and soil (Bakker et al., 2016; Boehm et al., 2016; Mattioli et al., 2014). This supported by studies done showing evidence that both porous and non-porous surfaces and other objects can be transmission vehicles, although most studies focussed on viruses (Goodwin et al., 2012; Boone and Gerba 2007; Reynolds et al., 2005). Study done by Stauber et al (2013) detected total coliforms and *E. coli* from household toys associated with water, sanitation and hygiene conditions. Another study conducted in Harare detected *E. coli* from soil, hands, drinking water and handwashing water (Navab-Daneshmand et al., 2018). This indicates the importance of awareness on how environmental factors such as hands, surfaces and soil in households play a role in transmitting enteric bacteria.

In the Vhembe district, studies done previously provide information in detection and prevalence of *E. coli* pathogenic strains from water sources (stored, borehole, river) and stool samples (Samie et al., 2009; Obi et al., 2004; Obi et al., 2002). However, studies linking water, sanitation and hygiene practices with other aspects in households have not yet been done. Therefore, this study focusses on characterizing the pathogenic strains and potential linking of the relationship of the *E. coli* pathotypes found in rural communities of Vhembe district using samples from handwash of mother and the child; swabs from the toilet seat and floor, water samples from running taps and storage containers.

1.3 PROBLEM STATEMENT

The WASH services at rural households are poor when compared to urban areas. The knowledge may be there, however implementation is lacking. Community members from rural areas still rely on untreated water sources for water; practice open defaecation and improper hand hygiene. This leads to presence of pathogenic strains and a possible

transmission pathway between mother and child; and other environmental reservoirs. The environment might be a threat to children under the age of 5 due to the presence of pathogenic strains that may lead to diarrheal infections.

1.4 HYPOTHESIS

The EPEC, EHEC and ETEC strains are present in the rural areas of the Vhembe district due to poor level of WASH practices and animal farming in households.

1.5 OBJECTIVES OF THE STUDY

1.5.1 PRIMARY OBJECTIVE

To characterize the prevalence of pathogenic strains of *E. coli* from rural communities in the Vhembe district.

1.5.2 SECONDARY OBJECTIVES

- To detect the presence of *E. coli* using the Colilert® Quanti-tray®/2000 in several samples from visited households.
- To characterize pathogenic *E. coli* strains using a multiplex Polymerase Chain Reaction (mPCR).
- To assess of transmission pathways through Water, Sanitation and Hygiene (WASH) practices using ETEC, EPEC and EHEC virulent strains.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

Inadequate supply of water, sanitation and hygiene is estimated to contribute 5% of global burden diseases (Prüss-Ustün et al., 2016). Diarrheal infections are one of the diseases caused by faecal-oral contamination. In 2015, an estimated 2.3 billion illness and 1.3 million deaths were caused by diarrheal diseases worldwide (Vos et al., 2017). In low and middle-income countries, it is suspected that deaths associated with diarrhoea are interrelated to unsafe water, poor sanitation and unhygienic conditions (Graf et al., 2008; Manun'ebo et al., 1994; Daniels et al., 1990). Globally, one in ten death cases are attributed to diarrhoea in children under the age of 5 and the highest rates of child mortality occur in Sub-Saharan Africa and South East Asia (Liu et al., 2012).

South Africa is still considered a developing country with poverty stricken rural areas that still do not have access to potable water, proper sanitation and good hygiene practices Coovadia et al.,2009; Obi et al., 2002). Lacking access to water, sanitation and hygiene results to diarrheal outbreaks which are responsible for substantial number of child deaths in South Africa (STATSSA, 2011). Records from the health facility level estimated that incidence of diarrhoea for children under the age of 5 was 90.3 per 1 000 (District Health information system database, 2012). This may not be entirely due to health facility level records severe cases and other cases may be treated at home or by traditional healers (Friend-du-Preez et al., 2013).

Diarrhoea is reported to be interlinked to socio-economic status and has the most adverse effect in South African communities (STATSSA, 2011). Therefore, South African children living in poverty are more likely to die from diarrhoea than the ones living in privileged counterparts (STATSSA, 2011; Ataguba et al., 2011). UNICEF and WHO highlights the importance of well-known intervention for reducing the global burden reports of diarrhoea cases for young children. This include intervention of for provision of water, sanitation and hygiene (WASH) and adequate nutrition of mothers and children (WHO-UNICEF, 2014; UNICEF, 2013).

In 2015, UN reported that globally household living in poorer rural areas are less likely to have access to improved water and sanitation. Furthermore, nationally 92.5% households have gained access to improved water (STATSSA, 2016). However, in 2011 it was estimated that 3.5 million people in South Africa still did not have access to potable water and the problem is more pronounced in rural areas (Heleba, 2011). This usually forces community members to rely on untreated water sources such as dams and rivers (own observation; Majuru et al., 2011). Some rural communities have developed strategies to harvest rainwater for domestic use (Kahinda et al., 2010). Studies have indicated that even when people have an assumption of rain harvested water to be clean, contamination has been found and may be harmful (Gwenzi et al., 2015; Ahmed et al., 2011). Therefore, community members need to be given an awareness to treat rainwater before use to avoid diarrheal infections. Figure 2.1 demonstrates the roof harvesting rainwater.

Figure 2.1: Rain harvesting rainwater (RHRW) strategy

Proper sanitation and hygiene practices play a major role in stored water. Studies have identified a difference in microbiological counts in water when comparing the water in settings with different sanitation and hygiene practices (Peter, 2010; Trevett et al., 2005, Islam et al., 2011). Cleansing the storage container before storing water and keeping it

close also help reduce diarrheal causing pathogens (Onabolu et al., 2011). Infant faeces disposal practices contribute 23% risk of diarrhoea due to unsafe disposals (Mara et al., 2010). A study done by Cronin et al (2016) highlighted the importance of safe disposal for both adult and infant faeces. The use of soap prevents the spread of bacterial pathogens. A study done by Demberere et al (2016) also demonstrated the effectiveness of soap for preventing faecal-oral route for diarrheal pathogenic strains

2.2 WATER, SANITATION, HYGIENE PRACTICES AND OTHER ASPECTS AS *E. coli* TRANSMISSION PATHWAYS

Water is basic to life and health and it is reported that over 1 billion people worldwide have no access to safe drinking water (Abdul et al., 2012). It is important that adequate, clean and accessible supply of water is attained by community members daily since drinking water has been identified as the most significant single source of gastro-enteric diseases and as one of the major causes of morbidity and mortality worldwide. This is mainly due to faecal contaminated raw water, failures in treatment process or even recontamination of treated drinking water (WHO, 2004; WHO, 2003). In 2015, almost 6,5 billion people used improved sources of drinking water, however 844 million people still lacked basic drinking water services (WHO, 2017).

In South Africa, many households in rural areas still struggle with accessibility and availability of potable water to use in a daily basis (Karuaihe et al.,2014). This has been observed throughout in some rural villages during sample collection. Community members are forced to store large quantities of water in containers (Figure 2.2). During storage, water may be contaminated through hands, cups and the surrounding area of the water storage. Some community members do not close their water storage containers, some do not wash using detergents (Singh et al., 2013; Maraj et al.,2006). This demonstrates that basic hygiene conditions are still a major problem in rural communities and may serve as a transmission route of pathogenic *E. coli*. These arising

issues put the children at risk as they have lower immunity and may battle to fight against the strains leading to diarrheal outbreaks (Bloomfield et al., 2012).

Figure 2.2: Water storage containers used in rural communities of Vhembe district (taken during field work).

Lack of sanitation leads to diseases which was firstly noticed in 1842 (Chadwick, 1842). Inadequate sanitation facilities encourage people to practice open defecation and increases the risk of transmitting pathogenic *E. coli* strains leading to diarrheal outbreaks (WHO-UNICEF, 2014). In 2010, DWAF estimated that 10.5 million people in South Africa do not have access to proper sanitation, of whom, 2.5 million live in Limpopo and 0.6 million are from the Vhembe district. Inadequate sanitation causes other diseases besides diarrhoea, such as bilharzia, malaria, cholera, typhoid, eye and skin infections (Bartram and Cairncross, 2010). Figure 2.3 shows an example of pit latrine available at some village households in South Africa.

Figure 2.3: Pit latrine toilet (https://www.dailymaverick.co.za/article/2018-07-31-finally-urgent-new-plan-to-eradicate-pit-toilets-at-schools-to-be-unveiled/)[Accessed August 2018]

Sanitation intervention strive to protect human health by safely containing faecal material and preventing it from releasing into household and community environments (Prüss et al., 2002) In 2017, the Joint Monitoring Programme for water supply and sanitation updated the statistics and estimated 4.5 billion people lacking access to safely managed sanitation (WHO, 2017). In rural communities, faecal disposal is usually unsafely managed and enters the environment in high concentration since it is untreated. Multiple environmental reservoirs are contaminated and plays a role in contributing to the transmission of pathogens (Julian, 2016).

Developing countries are still facing heavy load of infectious diseases. Hands and fingers are the main sources of spreading infectious diseases since most of the daily activities are conducted by hands (Mathur et al., 2011; Luby et al., 2009; Curtis and Cairncross, 2003). Thus, hand washing is regarded as one of the most important element of infection control activities (WHO, 2010). It prevents the transmission of hand borne infection which is transmitted by the faecal-oral route. Studies done in the Vhembe district in both household and school settings evaluated the availability and accessibility of water, sanitation and knowledge of personal hygiene using (Cranston et al., 2015; Sibiya and Gumbo, 2013; Samie et al., 2012). Sibiya and Gumbo, 2013 conducted a study in rural schools of Vhembe district using a knowledge, attitude and practice (KAP) survey on water, sanitation and hygiene (WASH) practices that determined knowledge is sufficient however, implementing into action is still lacking. Figure 2.4 demonstrates the prevention of transmission due to adequate water, sanitation and hygiene practices.

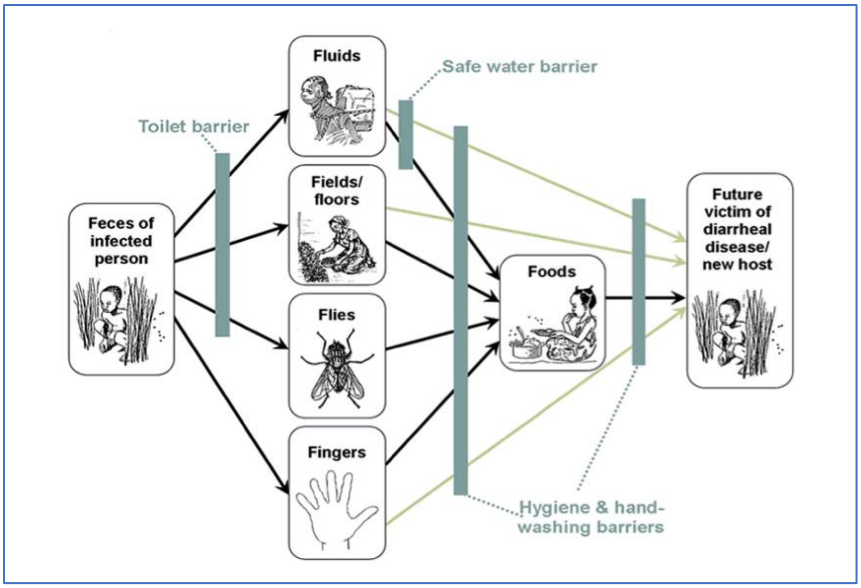


Figure 2.4: Demonstration of proper WASH practices prevents diarrheal outbreaks (http://lagoscleanbeach.wix.com/site1/apps/blog/open-defecation-sanitation-and-the-environment) (Accessed 17 July 2018)]

Studies done in low- and middle-income countries have detected high levels of microbial indicators of faecal contaminations as well as enteric pathogens on hands of community people (Schriewer et al., 2015; Mattioli et al., 2012; Pickering et al., 2010). Increased hand faecal contamination has been reported to be associated with unimproved sanitation access as well as high levels of faecal contamination in stored drinking water in households (Pickering et al., 2010) Furthermore, studies have proven that the *E. coli* contamination may increase within a short period of time even after handwashing due to typical household activities such as sweeping, preparing food and cleansing dishes (Devamani et al., 2014; Pickering et al., 2011; Ram et al., 2011). A study done in rural households in South Africa by Potgieter et al (2005) demonstrated high contamination in weaning pap that was mashed using bare hands by mothers and caregivers. This can help conclude that child diarrhoea is linked to food contamination and can be prevented by regular handwashing events (Freeman et al., 2014; Islam et al., 2012).

A study done by Ercumen et al (2017) in Bangladesh elaborates on how soil is increasingly being recognized as a reservoir for faecal organisms and is linked to faecal

contamination of drinking water, hands and food. Soil contamination could be from both human and animal. Soil faecal contamination is likely due to improper faecal disposal, such as open defecation and inadequate infant and child faecal management; inadequate wastewater disposal and inadequate animal faecal management (Penakalapati et al., 2017; Ngure et al., 2013). Faecal contaminated soil and surfaces in households are important exposure pathways for diarrheal disease in low- and middle-income countries (Boehm et al., 2016; Exum et al., 2016; Torondel et al., 2016). A study done by Pickering et al (2012) in Tanzanian households, detected high concentrations of *E. coli* in soil samples. Soil is an important factor because of potential soil ingestion by children under 5. This is corroborated by a study done by Chien et al (2017) in Taiwan that reports on children under 5 ingesting contaminated soil. Figure 2.5 demonstrates the contributors of soil contamination.

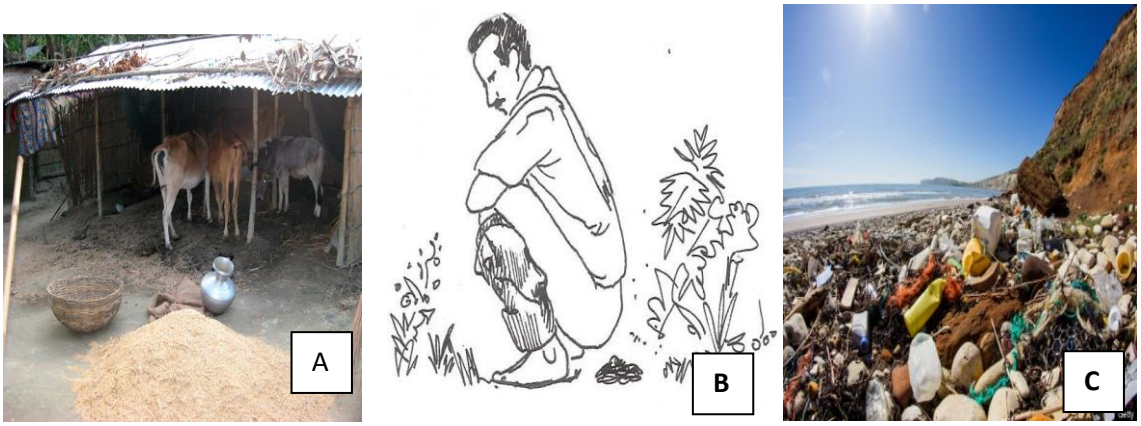


Figure 2.5: Contributors of soil faecal contamination

[A: Animal faming at households (taken from Ercumen et al., 2017); B: Open defaecation http://barakafm.org/2018/06/14/community-anti-open-defecation-project-registers-success-in-kilifi/; C: Improper waste disposal (https://www.huffingtonpost.co.uk/2013/09/09/paul-rose-marine-expert-oceans-plastic_n_3893520.html?guccounter) (Accessed 18 July 2018)]

2.3 ASSESSMENT CRITERIA FOR COLIFORMS AND *ESCHERICHIA COLI*

Total coliforms and *E. coli* are used as indicators that measures the degree of pollution and sanitary quality of well water since testing for all known pathogens may be time consume, expensive and complicated (Edberg et al., 2000). These organisms are chosen

to be indicators its due to their effectiveness since they regularly present in faecal as they part of the normal flora and appear in high numbers (DWAF, 1996). Total coliform and *E. coli* are suitable indicators since they meet criteria for an ideal indicator organism as outlined by DWAF (1996) should be as follows:

- a member of the intestinal microflora of warm-blooded animals.
- present when pathogens are present, and absent in unpolluted environments.
- present in a higher number than the pathogen.
- be resistant to the environmental factors and to disinfection in water and wastewater treatment plants
- not be able to multiply in the environment.
- be detected by easy, rapid and inexpensive methods and
- not be pathogenic and should be safe to work with in the laboratory.

Water is a basic need for life (UN, 2006). Water is regarded drinkable when the chemical properties and microbiological analysis indicates the absence of chemicals and harmful agents (total coliform and *E. coli*) respectively (Cabral, 2010). The microbiological analysis has different risk criteria that indicates when the water is of risk to drink and may results into diarrheal infection when consumed. Table 2.1 shows the summary used to analyze drinking water quality suitable for consumption.

Table 2.1. Summary of DWAF guidelines for domestic use (adapted from DWAF, 1996)

Total organisms **cfu/100 ml**	**Total coliforms** **cfu/100 ml**	***E. coli*** **cfu/100 ml**	**Guideline**
0-100	0-5	0	Negligible risk of microbial infection
100-1000	5-100	0-10	Potential risk of microbial infection with continuous use
>1000	>100	>20	Substantial risk of microbial infection

Keywords: cfu= coliform forming unit

2.4 *ESCHERICHIA COLI*

2.4.1 COMMENSAL *E. COLI*

Escherichia coli is a rod-shaped Gram-negative bacterium (Kaper et al., 2004). It is classified as a member of the *Enterobacteriaceae* within the *Gammaproteobacteria* class (Tenaillon et al., 2016). *Escherichia coli* is usually harmless and found in the microbiota of the human gut. It is predominantly facultative anaerobic and typically colonizes the infant's gastrointestinal tracts within a few hours of life and mutual benefit relationship is created (Palmer et al., 2007). Figure 2.6 shows the *E. coli* specie

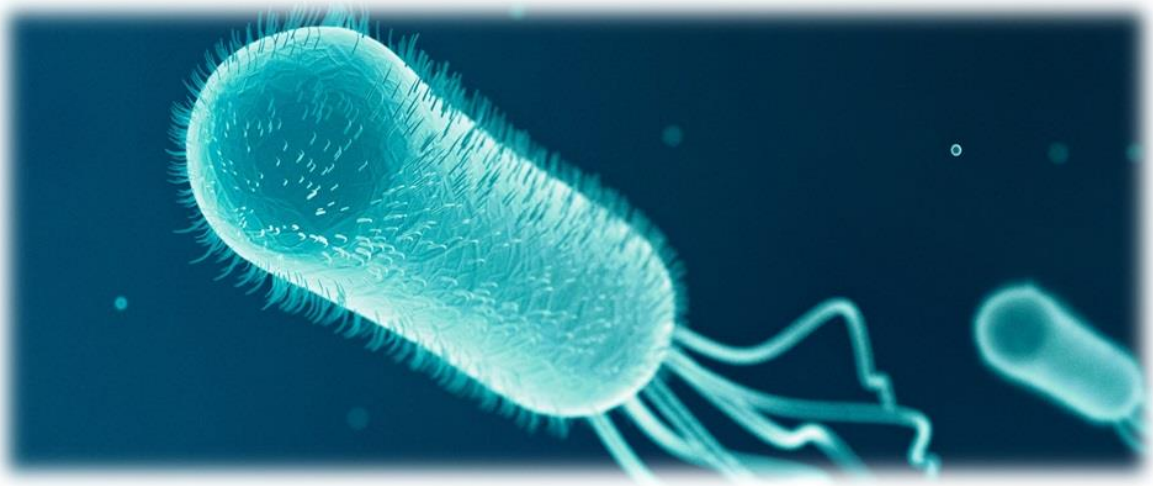


Figure 2.6: *Escherichia coli*

(https://wickhamlabs.co.uk/technical-resource-centre/fact-sheet-escherichia-coli/)

In the digestive tracts, commensal *E. coli* are situated in the large intestine, especially in the caecum and colon. It is found in the mucus layer that covers epithelial cells throughout the tract and are shed into the lumen with degraded mucus components and excreted in the faeces (Tenaillon et al., 2010). The mucus defines a nutritional ecological niche which *E. coli* metabolism has adapted (Conway and Cohen, 2015). As *E. coli* can survive in water and sediments, it is often used as an indicator of recent faecal contamination in water and other compartments (Rochelle-Newall et al., 2015).

In immunocompromised individuals, these harmless commensal strains may cause infection. Furthermore, some strains have evolved the capability to cause diseases in humans and animals by specific pathogenic mechanisms and they are known as pathogenic *E. coli* strains.

2.4.2 PATHOGENIC *E. coli* STRAINS

In developing countries, pathogenic *E. coli* has been reported to be one of the leading causes of diarrheal outbreaks in association to other bacterial and parasitic pathogens such as *Salmonella spp, Shigella spp, Citrobacter spp, Entamoeba histolica* and *Gardia lambia* (Lanata et al., 2013; Prescott et al., 2008; Smith et al., 2003). In addition, pathogenic *E. coli* has been identified as the leading etiological agent for diarrheal outbreaks for children under the age of 5 (WHO-UNICEF, 2012). Over 1800 deaths of children under the age of 5 have been reported daily from preventable diarrhoea-related diseases (UNICEF, 2013). Amongst bacterial pathogens, diarrheagenic *E. coli* are the most important cause of endemic and epidemic diarrheal outbreaks worldwide (Shetty et al., 2012; Kaper et al., 2004).

The pathogenic strains mechanisms depend on their ability to invade tissues and intestinal cells as well as the toxins that they produce. The five diarrhegenic *E. coli* (*DEC*) strains are: Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorragic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enterotoxigenic *E. coli* (ETEC) (Nataro and Kaper, 1998). EPEC and ETEC pathotypes have been recently identified as subsets of enteric pathogens that cause moderate to severe diarrhea in the low- and middle-income countries (Lanata et al., 2013).

Enteroaggregative Escherichia coli (EAEC)

In 1987, *Enteroaggregative coli was* firstly described from a child with acute diarrhoea in Lima, Peru (Nataro et al., 1987). The main source of this pathogenic strain is contaminated food and has been detected in several diarrheal foodborne outbreaks (Hedberg et al., 1997; Itoh et al., 1997). EAEC is recognized as an emerging enteric pathogen which causes persistent diarrhoea in children living where its endemic;

individuals that are HIV-positive and travellers coming from industrialized countries visiting less developed world (Huang et al., 2004; Wanke et al., 1998; Mathewson et al., 1995).

The ability for EAEC to adhere to intestinal cells, produce enterotoxins and cytotoxins as well as the ability to induce inflammation determines its pathogenicity (Okhuysen and DuPont, 2010). Genes that encode for aggregative phenotype are found in large plasmid (pAA) (Nataro et al., 1987). The aggR regulon present in pAA controls several plasmid genes coding for virulence factors and at least 2 pathogenicity islands in the EAEC chromosome. EAEC contains an antigenic anti-aggregative protein known as dispersin which is encoded by the aap gene in the pAA plasmid and regulated by the aggR (Verlade et al., 2007). This protein modulates fimbrial adhesion and facilitates the penetration of the bacteria strain through intestinal mucus. It succeeds penetration by binding into the lipopolysaccharides and altering the properties of the outer membrane surface (Jensen et al., 2014; Harrington et al., 2006).

Enterohaemorrhagic Escherichia coli (EHEC)

Enterohaemorrhagic Escherichia coli is naturally harboured in a wide range of animals and birds however the main reservoirs are cattles (Caprioli et al., 2005). This strain may be asymptomatic in their reservoirs, however in humans colonizes the colon using the fimbriae resulting in electrolyte imbalance (Hancock et al., 2001). This causes an infection which starts as watery diarrhoea and may progress to haemorrhagic colitis, haemolytic uremic syndrome or even cause death (Gyles, 2007).

EHEC succeeds in causing diseases in humans by their ability to produce one or more shiga-like toxins which inhibits protein synthesis in host cells resulting in cell death (Hunter, 2003). The toxins are encoded by *stx 1* and *stx2* with their variants (Nataro and Kaper, 1998). The other virulence factors include *eae*A gene-encoding intimin and the hlyA gene (Wang et al., 2002). These virulence factors are responsible for attaching and effacing lesions as well as a pore-forming cytolysin on eukaryotic cells respectively (Nguyen and Sperandio, 2012).

Ashbolt (2004) reported that EHEC contaminating drinking water has been associated with disease outbreaks. Study done in India by Ram et al (2008) using water samples from a river that provides water to the city indicates the presence of multi-antimicrobial resistant *E. coli* displaying virulence genes that are used to describe EHEC. Study done in Gauteng by Abia et al. 2017 using water samples from wells and boreholes that are used as water supplies for domestic use indicates the virulence factors stx 1, stx 2, *eae*A gene and flicH7 that for EHEC.

Enteroinvasive Escherichia coli (EIEC)

Enteroinvasive *Escherichia coli* was first shown in 1971 to be associated with a diarrheal outbreak a nd transmitted through faecal-oral route (DuPont et al, 1971). It is established that they closely related to *Shigella* spp with regard to their virulence, biochemical genetics and physio-pathological properties (Ud-Din and Wahid, 2014). EIEC causes dysentery using the same method of invasion as Shigella does and characterized by cramps, fever and stool that contains blood, mucus and pus (van den Beld and Reubsaet, 2012; Kaper et al., 2004). The organism invades the epithelial cells using adhesion proteins once ingested resulting to dysentery and it causes appearance of blood and mucus in stool samples of infected individuals (Martinez et al., 1999).

Enteropathogenic Escherichia coli (EPEC)

Enteropathogenic Escherichia coli is an important diarrheal pathogen in young children in developing countries (Hernandes et al., 2009; Moura et al., 2009). They have a high prevalence in both community and hospital settings (Dutta et al., 2013; Ochoa and Contreras, 2011). EPEC is the main cause of persistent diarrhoea (Abba et al., 2009). There are two subtypes namely, typical EPEC (tEPEC) and atypical *EPEC (*aEPEC). The tEPEC has a large virulence plasmid in which the bundle-forming pilus encoding gene (bfp) is present whereas the adherence factor is absent in aEPEC (Nataro and Kaper, 1998). The aEPEC has been identified in developed and developing countries as an

emerging diarrheal pathogen due to their high prevalence compared to tEPEC (Nair et al., 2010; Ochoa et al., 2008;). Furthermore, findings indicate that aEPEC may have an innate propensity to persist longer in the intestine than the other diarrheagenic *E. coli* (Nguyen et al., 2006; Afset et al., 2003).

In a systemic review of paediatric diarrhoea etiology using 266 most important pathogens between 1992-2002, EPEC was still identified as being the most important pathogen with prevalence median of 8.8% in community settings. Furthermore, study done by Nair et al (2010) in India indicated that EPEC was responsible for 3.2% of 648 diarrhoea samples in children younger than 5 years.

Enterotoxigenic Escherichia coli (ETEC)

Enterotoxigenic Escherichia coli is the prominent bacterial cause of diarrhoea in developing countries as well as a cause for traveller's diarrhoea. Study done by Gomez-Duarte et al (2013) reported on ETEC being the most frequent *E. coli* associated with childhood diarrhoea. Previously, Hunter (2003) identified ETEC as the most causative agent of waterborne diseases. ETEC uses fimbrial adhesins to colonize surfaces of the small intestine (Turner et al., 2006). It is known to produce two toxins, namely heat labile and heat stable enterotoxins which causes secretory diarrhoea through C1 secretions (Wajima et al., 2013; Kaper et al., 2004).

2.5 DIARRHEAL OUTBREAKS CAUSED BY PATHOGENIC *E. coli*

Several waterborne gastroenteritis outbreaks have been caused by diarrhoeagenic *E. coli* (EPEC, ETEC, EHEC, EIEC and EAEC) which has been detected in diverse ecological niches which range from mammalian intestines to various aquatic environments such as surface water and groundwater (Coleman et al., 2013, Lienemann et al., 2011; Swerdlow et al., 1992). Depending on type of infection, DEC can cause a wide spectrum of human diseases ranging from mild diarrhoea to severe haemorrhagic colitis (Sinclair et al., 2017). *E. coli* had serotypes that have been identified to cause diseases due to the ability to

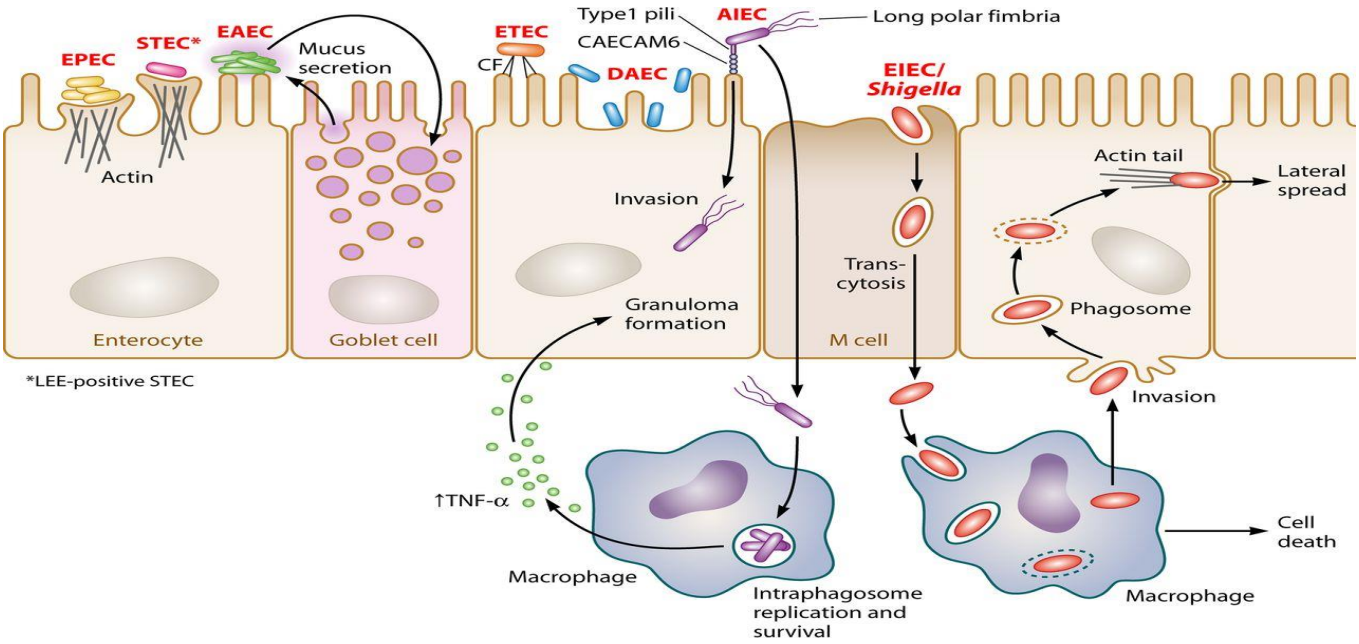
adapt to different environments such as fomites (toys, floor, etc) and the characteristics of being drug resistant (Vujcic et al., 2014; Stauber et al., 2013).

Of concern, multidrug resistant *E. coli* have been detected in the environmental samples exposed to different human activities (Jang et al., 2013; Walsh et al., 2011; Dhanji et al., 2010). A multi-drug resistant enteroaggregative *E. coli* (O44) associated with acute and persistent diarrhoea was discovered and reported in Kenya by Sang et al (1997). In Germany, a virulence shiga-toxin *EAEC* O104:H4 was identified and associated with haemolytic uremic syndrome outbreaks and contained the extended spectrum β-lactamase (ESBL) activity (Rohde et al., 2011; Rubino et al., 2011; Struelens et al., 2011). In South Africa, data on *E. coli* serotypes is scares. Tau et al.,2012 conducted a study due to the ancestral origin of the 2011 outbreak strain from Germany. The study included information from 2004-2011 and concluded that the detected *E. coli* O104 in South Africa does not produce shiga toxins and do not contain extended spectrum β-lactamase (ESBL) activity.

Enterohaemorrhagic Escherichia coli cases in outbreaks have pre-dominantly been attributed to EHEC O157:H7, however non-O157 serogroups have been reported in number of cases (O26, O103, O111 and O145) (Johnson et al., 2009). In South Africa, a study done in Eastern Cape reported on a prevalence of 10.3% of STEC O157:H7 from vegetable samples (Abong'o and Momba, 2008). EHEC O157: H7 has been detected in South African water sources that is intended for human consumptions (Műller et al., 2001). Furthermore, studies reported a prevalence of 56.5% and 43.5% from stool samples from confirmed and non-confirmed HIV/AIDS patients respectively in the Eastern Cape (Abong'o and Momba, 2009; Abong'o and Momba 2008). It was also reported that isolates from meat (7.8%) water (8.6%), vegetables (10.3%) confirmed HIV/AIDS patients (56.5%) and non -confirmed HIV/AIDS patients (43.5%) were genetically related. This shows that there is a possible transfer of pathogens between the different components of the different studies (Lupindu, 2018).

Enteropathogenic *Escherichia coli* was the common cause of infantile gastroenteritis outbreaks in Brittain from 1940s until the early 1970s. WHO originally recognized 12

serogroups that are categorized as EPEC or classical EPEC (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158) (Hernandes et al., 2009). One of the first identified EPEC serotype is O26, which was reported as the cause of pediatric EPEC diarrhoea (Orskov, 1951). However, this serotype O26 have been isolated from EHEC outbreaks in the Europe (Allerberger et al., 2003; Misselwitz et al., 2003; Werber et al., 2002). The serotype is distinguished to be either EPEC or EHEC is due to the presence of the virulence factors that characterise the pathotype (Bugarel et al., 2011). Figure 2.7 illustrate the adherence of pathogenic *E. coli* to the host cells



Adherence patterns of enteric *E. coli*. Pathogenic *E. coli* requires adherence to the host epithelium. Enteropathogenic *E. coli* (EPEC) (represented in yellow) and LEE-positive Shiga toxin-producing *E. coli* (STEC) (represented in pink) are extracellular pathogens that attach to the intestinal epithelium and efface microvilli, forming characteristic A/E lesions. Due to the presence of bundle-forming pili, EPEC is capable of forming microcolonies, resulting in a localized adherence (LA) pattern. Enterotoxigenic *E. coli* (ETEC) (represented in orange) uses colonization factors (CFs) for attachment to host intestinal cells. Enteroaggregative *E. coli* (EAEC) (represented in green) forms biofilms on the intestinal mucosa, and bacteria adhere to each other as well as to the cell surface to form an aggregative adherence pattern (AA) known as "stacked brick." Diffusely adherent *E. coli* (DAEC) (represented in blue) is dispersed over the surfaces of intestinal cells, resulting in a diffuse adherence (DA) pattern. Adherent invasive *E. coli* (AIEC) (represented in purple) colonizes the intestinal mucosae of patients with Crohn's disease and is capable of invading epithelial cells as well as replicating within macrophages. AIEC uses type I pili to adhere to intestinal cells and long polar fimbriae that contribute to invasion. Enteroinvasive *E. coli* (EIEC)/*Shigella* (represented in red) are intracellular pathogens that penetrate the intestinal epithelium through M cells to gain access to the submucosa. EIEC/*Shigella* escape submucosal macrophages by induction of macrophage cell death followed by basolateral invasion of colonocytes and lateral spread

Figure 2.7: Pathogenic *E. coli* adhering to host cells (https://cmr.asm.org/content/26/4/822/F6)

2.6 MICROBIOLOGICAL METHODS USED TO ASSESS *E. coli* IN SAMPLES

The methods commonly used to check for the presence of *E. coli* are Membrane Filtration, Multi-tube Fermentation and Colilert ®Quanti-tray®/2000. These methods differ due to their various principles and the need for media for culture or not. In addition, some methods require colony counting while others do not require colony counting.

2.6.1 MULTIPLE-TUBE FERMENTATION (MTF) TECHNIQUE

Multiple-tube was developed in the 1920's. It was used for the enumeration of coliforms and monitoring water quality (American Public Health Association, 1985). This technique requires inoculating appropriate decimal dilutions (Figure 2.8) of the water sample in a series of tubes. The solution needs to ferment lactose and produce gas, the acid formation or abundant growth in the test tubes after incubation for 48 hrs at 35°C constitutes a positive presumptive reaction (Rompré, 2002). The number of coliforms per 100 ml is then calculated from the number of tubes positive known as Most Probable Number (MPN). The MPN value calculated is an estimation of the number of bacteria in a sample based of the number of tubes positive (Eckner, 1998).

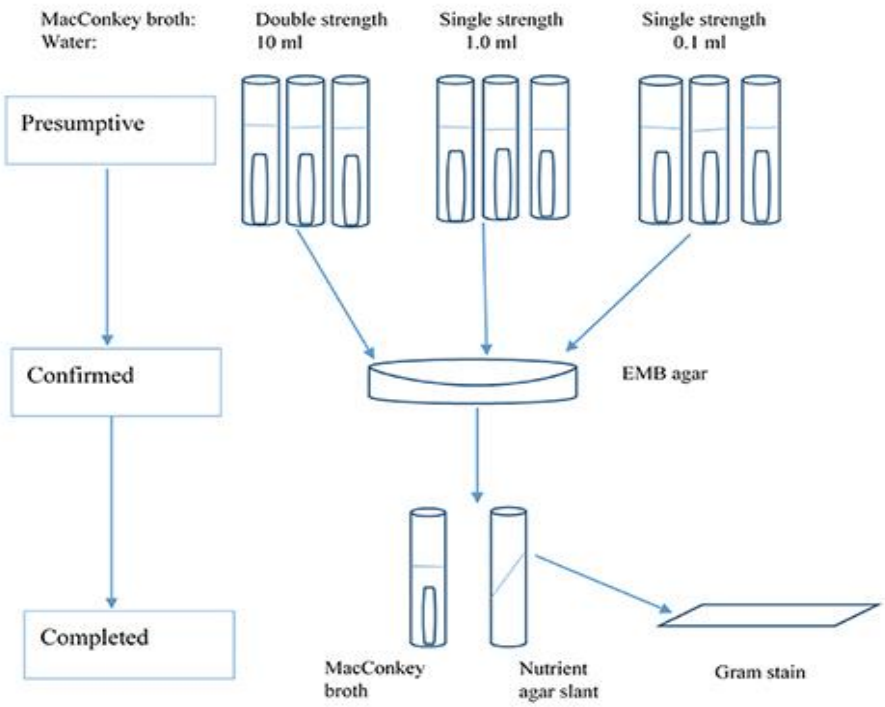


Figure 2.8: An illustration of the multiple-tube fermentation technique (taken from Akeju and Awojobi, 2015).

2.6.2 MEMBRANE FILTRATION (MF) TECHNIQUE

The Membrane filtration technique was introduced in the late 1950's as an alternative method to use other than the Multiple-tube fermentation. This technique offers an advantage of isolating discrete colonies whereas the MTF only indicated the presence or absence of the organisms (Rompré, 2002; Seidler et al., 1981). MF was accepted for Microbiological Testing of Potable water in the 11th edition of Standard Methods for examination of water and wastewater. The MF method when used for enumerating TC and *E. coli* requires the use of a primary isolation medium, mEndo or mEndo LES agar, incubated for 24 hrs at 35°C (Covert et al, 1992). This is followed by MF transfer to nutrient agar (Figure 2.9) supplemented with 4-methylumbelliferyl-b-D-glucuronide (MUG) that is incubated for an additional 4 hrs at the same temperature. The MF method has several advantages that may be considered when testing samples. This include that the method

allows a large sample volume, yields numerical results faster than multiple-tube fermentation and is effective (Rompré, 2002; Covert et al., 1992).

Figure 2.9: Steps of Membrane filtration Technique (https://www.membrane-solutions.com/News_80.htm)

2.6.3 COLILERT QUANTI-TRAY

The Colilert Quanti-tray is one of the methods that has recently adopted the detection of β-D- galactosidase and β-D-glucuronidase to detect the presence of coliforms and *E. coli* respectively in water (Fricker et al., 1997; Fricker and Fricker, 1996; Edberg et al., 1990). This method is easy, rapid and accurate having results available within 18-24hrs (Figure 2.10). There is no need for media preparations, no dilutions or colony counting required. Colilert Quanti-tray method uses nutrient indicator ortho-nitrophenyl-ß-D-glucopyranoside (ONPG) which produces a distinct yellow colour when hydrolysed by ß-D-galactosidase. This indicates the presence of Coliforms (Ditcher, 2011). The second nutrient indicator that Colilert uses is 4-methylumbelliferyl-beta-D-glucuronide that is hydrolysed by the enzyme ß-D-galactosidase to yield the 4-methylumbelliferyl moiety, which fluoresces blue

under long wavelength ultraviolet light and indicates the presence of *E. coli* (Eckner, 1998). The calculations of the observed positive wells help determine Most Probable Number (MPN) model, which provides the MPN of colony forming units (cfu) (IDEXX, 2002).

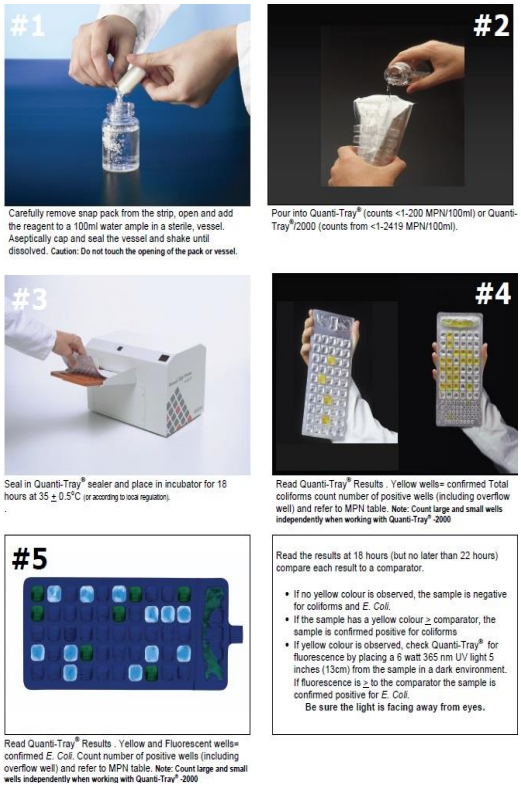


Figure 2.10: Steps of the Colilert Quanti-tray test (powerakademy.com/post/colisure)

2.7 SUMMARY OF LITERATURE REVIEW

Vhembe district in Limpopo South Africa has many rural and poverty-stricken communities which are still facing challenges with availability and accessibility of adequate water, sanitation and hygiene (WASH) services (Nkuna, 2012). Community members still depend in untreated water sources as a source of water to meet their day to day need; practice open defaecation due to lack of sanitation availability, and still practice improper hygiene practices (Hutton and Chase, 2016). Other community members have resorted to using roof-harvested rainwater as a source. Poor WASH services affect the quality of life and many cases can result in diarrheal outbreaks due to

transmission of pathogenic *E. coli* through WASH services (Kahinda et al., 2010). The unavailability of water forces community members to store water and studies have proven that stored water gets to be more contaminated at point-of -use as compared to point-of-collection (Onabolu et al., 2011; Rufener et al., 2010). This may be result to the sanitation and hygiene practices at the household. Studies have focussed on outbreaks due to WASH services and little information is available on other compartments related to WASH that have contribute to contamination of water. However, recent studies have detected pathogenic *E. coli* on compartments such handwash samples, toys, floor and other fomites (Navab-Daneshmand et al., 2018; Ercumen et al., 2017; Exum et al., 2016). Therefore, this study focussed on assessing whether the pathogenic *E. coli* that may be detected from handwash samples, floor and toilet seat swabs and water from the storage container as well as the sources

CHAPTER 3

RESEARCH METHODOLOGY

3.1 STUDY SITE

This study was conducted in seven rural villages in different municipalities of the Vhembe district in Limpopo Province (Figure 3.1). Samples were collected from 81 households (HHs) as follows: Dzingahe=10 HHs; Ngovhela= 8 HHs; Ngudza= 8 HHs; Mavambe= 8 HHs; Mphambo= 10 HHs; Phiphidi= 10 HHs; Xigalo= 25 HHs. Households in these rural areas practice livestock farming. Figure 3.2 represents community members collecting water from the water source.

Figure 3.1: Vhembe district map (https://municipalities.co.za/districts/view/29/Vhembe-District-Municipality#map)

Figure 3.2: Community members collecting water for domestic use (https://capricornreview.co.za/87897/water-users-fear-cholera/)

3.2 ETHICAL CLEARANCE

Ethical clearance was granted by the University of Venda Ethical Committee (SMNS/17/MBY/28/1212). Permission for visiting households was granted by community leaders.

3.3 HOUSEHOLD DEMOGRAPHICS

In each household, the study background was explained to participating candidates and a consent form (Appendix A) was given out for them to sign. A structured questionnaire (Appendix B) was used to conduct an interview to get the background on Water, Sanitation and Hygiene (WASH) practices.

3.4 SCHEMATIC DIAGRAM OF METHODOLOGY

Figure 3.3 provides a detailed layout of the study methodology that was followed in this study.

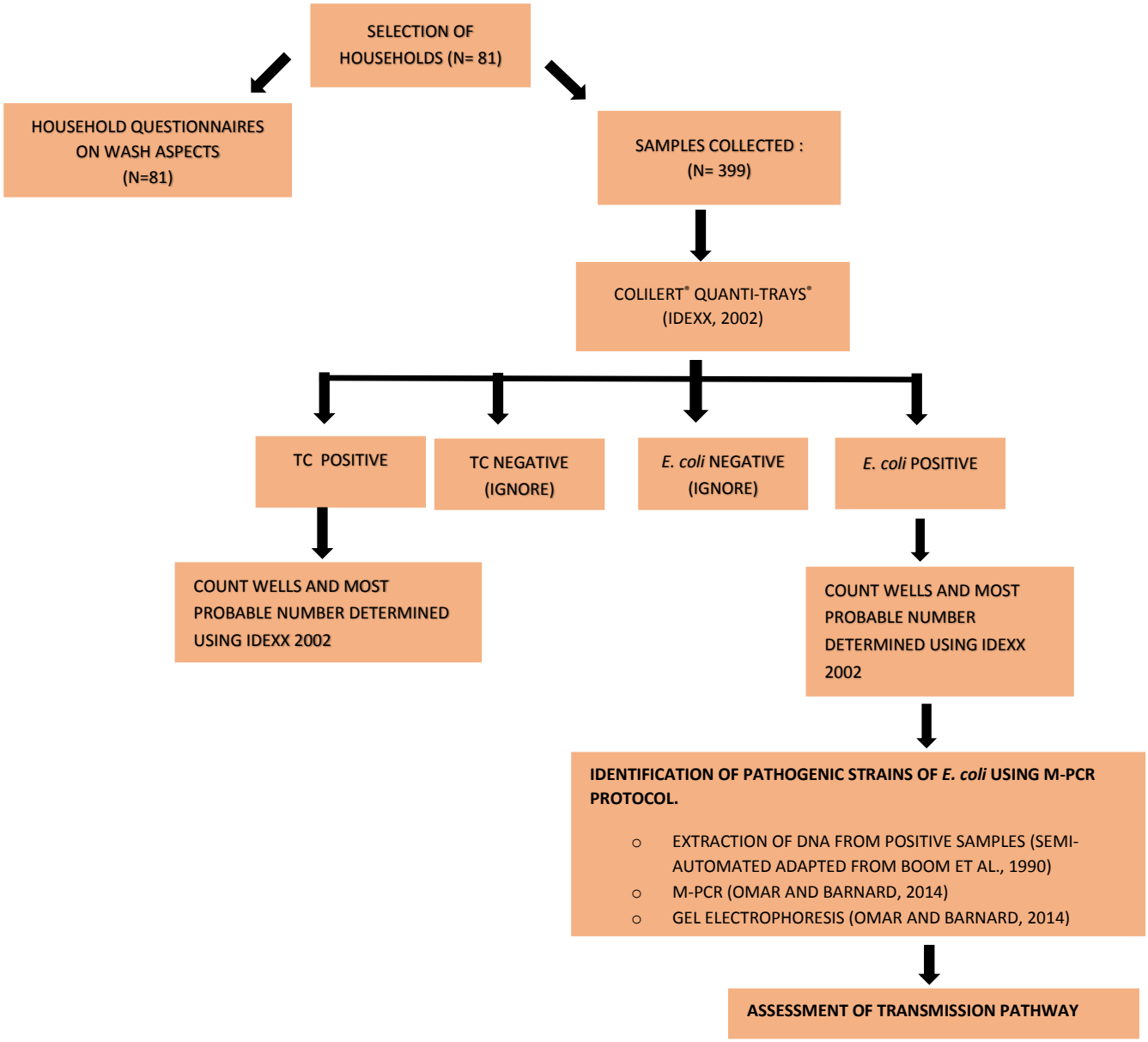


Figure 3.3: Flow chart indicating study layout

3.5 SAMPLE COLLECTION

All samples were placed in a cooler bag with ice to keep temperature at about 4℃ until arrival at the University of Venda microbiology laboratories. Samples were analysed upon arrival within 8 hrs of collection. A total number of 399 samples (Dzingahe= 45; Ngovhela= 49; Ngudza= 38; Mavambe= 41, Mphambo= 50, Phihphidi= 53, Xigalo= 123) were collected between January and June 2018.

The water used during the study was autoclaved and left to cool overnight before being packaged into bottles and Ziplock bags. In each HH the following samples were collected: (a) Tap running water, (b) Stored water sample, (c) Mothers handwash sample, (d) child handwash sample (e) Kitchen floor swab and (f) Toilet seat swab.

The samples were collected as follows:

Water samples:

(i)Tap water

Water collected from running taps which were left to run for at least 1 minute and the bottle was placed without touching any surrounding to fill up 250 ml bottles. Samples were placed into the cooler bag with ice during transportation to the laboratory

(ii) Stored water

Water collected from storage containers, the containers were shaken several times, opened and water poured into 250 ml bottles without touching the surroundings. Samples were placed into the cooler bag with ice during transportation to the laboratory

Hand samples (Mother and Child):

A total of 120 ml autoclaved distilled water was placed into a commercially available ziplock bag. The mother and child were asked to place one hand into a separate ziplock bag and wash the hand using the distilled water (dH2O). A total of 100 ml of the wash sample was poured into a sterile bottle and kept in the cooler bag with ice during transportation to the laboratory.

Toilet seat swab samples:

A total of 20 ml autoclaved distilled water (dH2O) was poured into the sterile swabs to wet the bud. The wet bud was used to swab the toilet seat. The bud was placed in a bottle with 100 ml dH20 and kept in the cooler bag with ice during transportation to the laboratory.

Kitchen floor swab samples:

A total of 20 ml autoclaved distilled water was poured into the sterile swabs to we the bud. The wet bud was used to swab the floor close the kitchen. The bud was placed in a bottle with 100 ml dH20 and kept in the cooler bag with ice during transportation to the laboratory.

3.6 MICROBIAL ANALYSIS

Each sample was assessed as follows: a total of 100 ml was poured into 120 ml of IDEXX bottles; the Colilert® 18 reagent was poured in each bottle and tilted for a few minutes to allow the solvent to dissolve; the dissolved solution was poured into Colilert® Quanti-Tray®/2000 [IDEXX, Wesbrook, Maine, United States of America (USA)] and sealed using the Quanti-Tray sealer (IDEXX, Wesbrook, Maine, USA); the Quanti-trays were incubated overnight (18-24hrs) at 35-37℃; after the incubation, the Colilert® Quanti-trays® (IDEXX, Wesbrook, Maine, USA) were examined to check if there was any colour change; the wells that had colour change into yellow indicated the presence of total coliform and were counted; the trays were examined under a long wave ultraviolet light (366nm) [(Spectroline, Thermo-fisher, Waltham, Massachuesetts, USA)] and wells that fluoresced (Figure 3.4) indicated the presence of *E. coli* and were counted. The most probable number for the counted yellow wells and fluorescend wells were determined for Total coliform (TC) and *E. coli* respectively using tables provided by manufacturer (IDEXX, 2002). The commensal *E. coli* strain was used as a positive control and *Klebsiella* was used as a negative control.

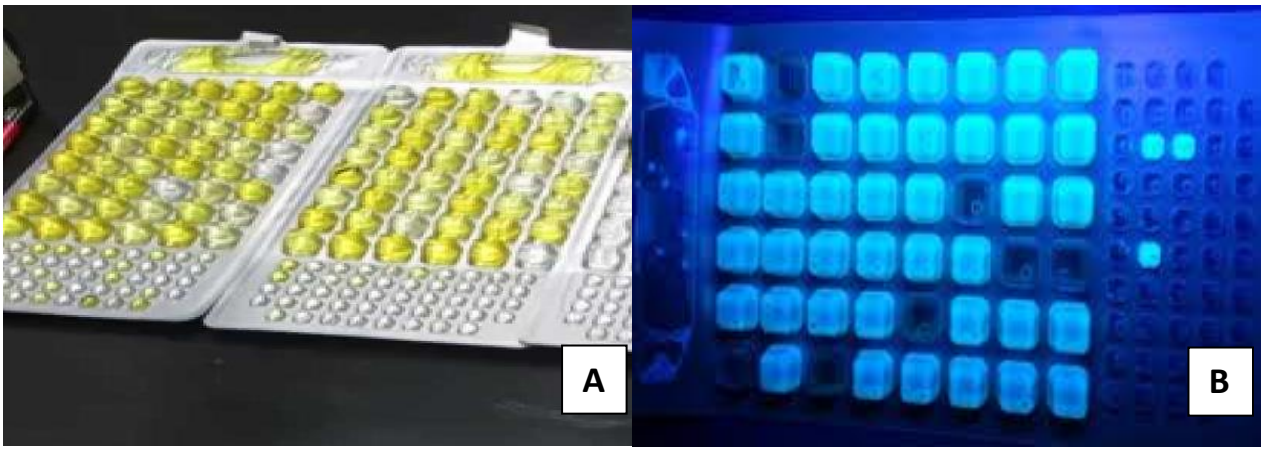


Figure 3.4: Colilert® Quanti-tray®/2000 [A: positive yellow wells B: Fluorescing wells under UV light. (Taken during lab work)]

3.7 MOLECULAR IDENTIFICATION OF *E. coli* STRAINS

3.7.1 DNA EXTRACTION FROM COLILERT® QUANTI-TRAY®/2000

Duplicate test tubes were aliquoted from the Colillert® Quanti-trays® (IDEXX, Wesbrook, Maine, USA). In each test tube, a total of 2 ml was aliquoted from 10 positive *E. coli* wells of each positive Colilert® Quanti-Tray®/2000 (IDEXX, Wesbrook, Maine, USA) using a sterile 1ml Neomadic disposal syringes mounted needles (Kendon Medical Supplies) and aliquoted into 2 ml sterile 96 well plate (Hamburg, Germany). The duplicate tubes for each sample were stored at 4˚C for further analysis. In positive Colilert® Quanti-Trays®/2000 (IDEXX, Wesbrook, Maine, USA) with less than 10 positive wells, the positive total coliform (TC) wells were used to add up to a volume of 2 ml.

The sample plate was placed in a NeoFuge-15R (Heal Force, Vacutec®) refrigerated bench-top centrifuge (Thermo-scientific, Carlsbad, California, United States) and steps were followed as demonstrated on Figure 3.4. The DNA was extracted from the collected bacterial cells using a modified method reported Boom et al. (1990) using 96 well plates.

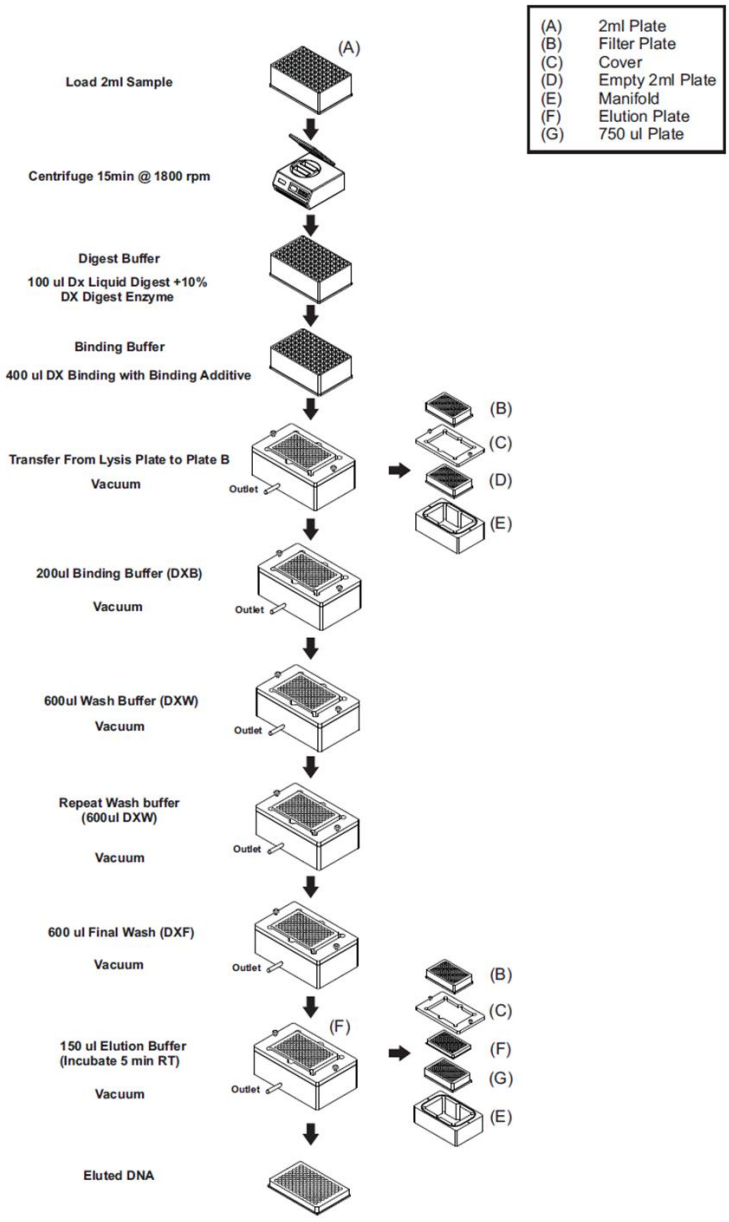


Figure 3.5: The 96 well plate DNA extraction method (Delair, 2017)

3.7.2 MULTIPLEX POLYMERASE CHAIN REACTION PROCEDURE

To assess the health implication of the WASH standards in households, molecular identification of pathogenic *E. coli* strains was performed for all *E. coli* positive samples using the multiplex PCR method published by Omar and Barnard (2014). The samples

were loaded in a 96 well plate (Greiner bio-one, Cat. No. 780285) sequential. All m-PCR reactions were performed in a Biorad Mycycler™ Thermal cycler in a total volume of 20 µl of primer mixture. A multiplex PCR kit (Qiagen®) was used for m-PCR protocol. Each reaction consisted of 1 X Qiagen® PCR multiplex mix (containing HotstartTaq® DNA polymerase, multiplex PCR buffer and dNTP mix); 2 µl of the primers mixture that detected the different strains of *E. coli* (Table 3.1), 2 µl of sample DNA and 5 µl PCR grade water. The reactions were subjected to different steps with different temperature conditions as reported by Omar and Barnard (2014).

Table 3.1: Primers and primer sequences for m-PCR (Omar and Barnard, 2014)

Pathogen	Primer	Sequence (50-30)	Size (bp)	Conc.(µM)	Reference
E. coli	*Mdh* (F)	GGT ATC GAT CGT TCC GAC CT	304	0.1	Tarr et al. (2002)
(Commensal)	*Mdh* (R)	GGC AGA ATG GTA ACA CCA GAG T			
EHEC/	*EaeA* (F)	CTG AAC GGC GAT TAC GCG AA	917	0.3	Aranda et al. (2004)
Atypical EPEC	*EaeA* (R)	GAC GAT ACG ATC CAG			
Typical EPEC	*bfpA(F)*	AAT GGT GCT TGC GCT TGC TGC	410	0.3	Aranda et al. (2004)
	bfpA(R)	TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT			Omar and Barnard (2014)
EHEC	*stx1* (F)	ACA CTG GAT GAT CTC AGT GG	614	0.5	Moses et al. (2006)
	stx1 (R)	CTG AAT CCC CCT CCA TTA TG			
	stx2 (F)	CCA TGA CAA CGG ACA GCA GTT	779	0.3	Moses et al. (2006)
	stx2 (R)	CCT GTC AAC TGA GCA CTT TG			
ETEC	lt (F)	GGC GAC AGA TTA TAC CGT GC	360	0.1	Pass et al. (2000)
	lt (R)	CGG TCT CTA TAT TCC CTG TT			
	st (F)	TTT CCC CTC TTT TACG TCA GTC AAC TG	160	0.5	Pass et al. (2000)
	st (R)	GGC AGG ATT ACA ACA AAG TTC ACA			
***E. coli* toxin**	astA (F)	GCC ATC AAC ACA GTA TAT CC	106	0.3	Kimata et al. (2005)
	astA (R)	GAG TGA CGG CTT TGT AGT C			
External control	gapdh (F)	GAG TCA ACG GAT TTG GTC GT	238	0.3	Mbene et al. (2009)
	gapdh (R)	TTG ATT TTG GAG GGA TCT CG			

Keywords: F= Forward R= Reverse

3.7.3 GEL ELECTROPHORESIS

The DNA was visualised using a 2.0% (w/v) agarose gel as reported by Omar and Barnard (2014). Using commensal *E. coli* as positive control and PCR grade water as negative control.

3.8 DETERMINING THE PATHOGENIC PATHOTYPE AND ASSESSING POSSIBLE TRANSMISSION

The samples were paired according to the pathotypes detected at the households and the virulence genes were checked to assess the possible transmission that could have occurred at the households due to possible improper WASH practices.

3.9 ANALYSIS OF DATA

The data collected from the questionnaire and results obtained during laboratory work were entered into Microsoft Excel Spread sheets (2013 Version) for analyses. The results were then used for comparison and illustration to give a detailed picture of the activities that occur in the different villages that were part of this study.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 DEMOGRAPHIC DATA OF STUDY HOUSEHOLDS

A total of 81 HHs in 7 villages were successfully sampled from the Vhembe district. In each household, the background of the study was explained to the potential participant for them to have an understanding about the study and why their households were of interest. The participants were all given a consent form (Appendix A) to sign. Thereafter, an interview was conducted using a structured questionnaire (Appendix B), to gather information on Water, Sanitation and Hygiene (WASH) practices at the household. The occupants of all [81 (100%)] households visited during the study period were of black ethnicity and their language of communication were either Xitsonga [43(53.09%)] or Tshivenda [38(46.91%)].

The household surroundings either had a cement stoep [53 (62.96%)] around the house; a cow dung stoep [18 (22.22%)] or the surrounding was just soil [10 (12.35%)]. The environment, such as soil may play a role in harbouring pathogens that are harmful for the community members (Pickering et al., 2012). At some households in this study population, freelancing animals are kept, and they practice open defaecation (self-observation).

Table 4.1 illustrates on the cleanliness of the household yards. The cleanliness of the HH yard was also accessed during the interview. Observations of no litter, long grass or faecal matter was regarded as a clean environment; whereas a dirty environment was regarded when there were signs of litter, long grass and faecal matter. Overall all (100%) households cleaned their yard with Mphambo community members indicating that they clean on a daily basis. However, 50% of the household at Mphambo village and 62.5% of the households of Ngudza were dirty at time of inspection. Mphambo village household members all (100%) said they clean the yard, however, 50% of the yards were dirty . The dirty village households may have an impact on the children's health due to chances of contaminated soil that may play as a transmission pathway. Fregonese et al (2017) reported that a contaminated environment is likely to contribute to stunting; a frequent faecal-oral transmission possibly causes environmental enteropathy (EE). This results to

a chronic inflammatory disorder that may contribute to faltering growth in children (Korpe and Petri, 2012). A study done at Bangladeshi indicates that children living in households with clean environment has less chances of severe environmental enteric dysfunction (EED) than children from contaminated households (Lin et al., 2013).

Table 4.1: Cleanliness of the yard

Variable	Overall Coverage (HH=81)	Dzingahe (HH=10)	Mphambo (HH=10)	Ngovhela (HH=10)	Mavambe (HH=8)	Ngudza (HH=8)	Phiphidi (HH=10)	Xigalo (HH=25)
Clean yard								
Yes	81 (100%)	10 (100%)	10 (100%)	10 (100%)	8 (100%)	8 (100%)	10 (100)	25 (100%)
How Often								
Everyday	64 (79.01%)	8 (80%)	7 (70%)	9 (90%)	7 (70%)	8 (100%)	10 (100%)	15 (60%)
Every 2nd day	5 (6.17%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (12%)
1/w	4 (4.94%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
2/w	5 (6.17%)	0 (0%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	3 (12%)
3/w	3 (3.70%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
Yard observation								
Clean	66 (81.48%)	8 (80%)	5 (50%)	9 (90%)	8 (100%)	3 (37.5%)	8 (80%)	25 (100%)
Dirty	15 (18.52%)	2 (20%)	5 (50%)	1 (10%)	0 (0%)	5 (62.5%)	2 (20%)	0 (0%)

Keywords: HH : Household w : week

4.1.1 AGE DEMOGRAPHIC

The study focused at households with children under the age of 5. This criterion was included due to the fact that children under age of 5 are dependent on their mother for certain activities such as for food preparation. However, transmission of pathogenic microorganisms may occur if proper hand hygiene practices are not followed. At some HHs, it was rare to find children during sampling time. Therefore, 5% of the HHs didn't have children under the age of 5. A total of 70% HHs had toddlers. This may be due to that the toddlers are left at home with their grandparents while their mothers are at school or work. The least age group that was found at the HHs was pre-scholars (3%); this is because children of age between 4-5 years are taken to pre-school to start with basic education.

Children under the age of 5 are dependent on their caregivers for their growth and development (WHO, 2004). Infants are 100% dependent since they can't perform any duties. Whereas, toddlers are at a stage where they learn to walk and try being independent. The household surroundings play a crucial role; the toddlers get to play and it's a stage where they touch and eat anything they come across (Huggins and Wickett, 2011). For example, safe faecal disposal is importance; the toddlers may touch

contaminated area and later hold something to eat and get to consume the pathogen. In this study, toddlers were the most found at the village households. Figure 4.1 indicates the overall age distribution of children in the 81 HHs.

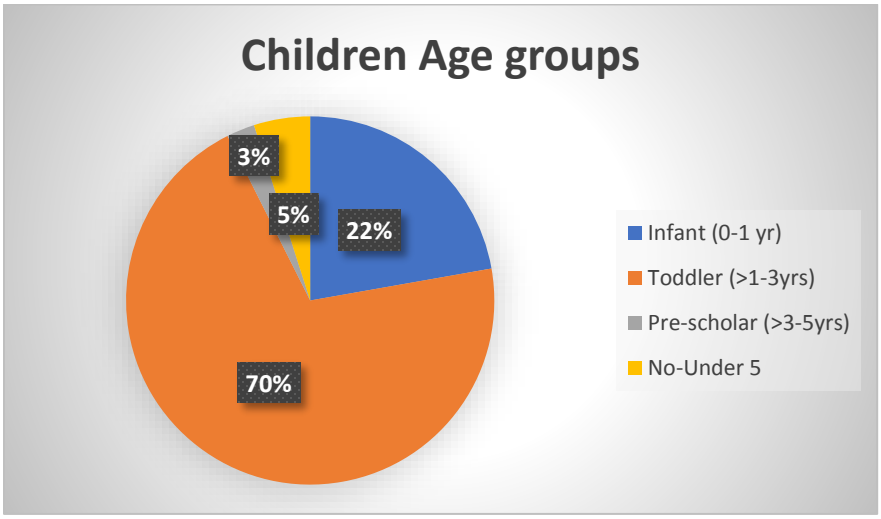


Figure 4.1 : Children Age groups of visited households

4.1.2 ANIMAL ACTIVITIES AT HOUSEHOLDS

WASH interventions are generally focused on breaking the human faecal-oral transmission route disease. However, one under recognized source of faecal contamination may be contributed by animal faeces at HHs that practice animal farming and these animals are left to roam around the yard practicing open defaecation. Animal faecal-oral transmission route is a common contaminant in the environment of children living in poor communities of low and middle-income countries (Vasco et al., 2016). A total of 51 (62.96%) households did not practice petting animals or animal farming.

The practice of animal farming at the households may be a risk to children's health due to the open defaecation of animal faeces, and faecal-oral route may occur during play-time (Penakalapati et al., 2017). Mphambo village households had many chickens and Ngudza keeps no chickens at the village households. Ngovhela village has the most households that keeps dogs and Mphambo as well as Mavambe village HHs keeps no dogs at the households. Ngudza village has the most pigs kept at households. Ngovhela

village has the most cats kept at the households when compared to the other village households. Animal faeces carry pathogens that may infect humans such as pathogenic *E. coli*, *Salmonella* and *Campylobacter* (Purohit et al., 2017). The animal faeces pose variable levels of human health risk depending on the prevalence of human-infection pathogen (Soller et al., 2015; Schoen et al., 2011; Soller et al., 2010). A study done in Ecuador isolated identical strains of *Campylobacter* from children- and chicken-faeces which suggested a zoonotic transmission (Vasco et al., 2016). A study done by Ercumen et al (2017) in Bangladesh showed that households with animals has higher levels of soil contamination as well as contamination in the stored water and food. This explains that households which keeps animals have a possible health disadvantage for children under 5's health because zoonotic transmission of pathogenic microorganisms may occur (Falkenberg et al., 2018) . Figure 4.2 indicates the type and number of animals present in the study population.

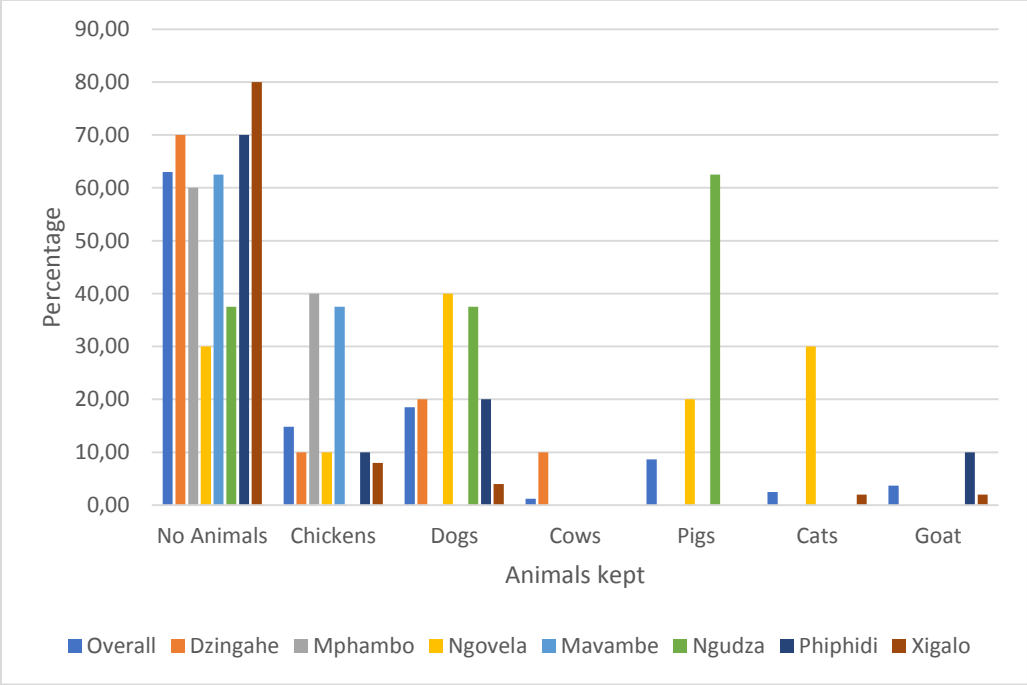


Figure 4.2: Animals kept in households' surroundings

4.1.3 WATER COVERAGE AT THE HOUSEHOLDS

Water availability in South Africa is a challenge and rural communities tend to suffer more than urban areas (Turton, 2008). The community members from the participating households relied on municipal water as the main water source. Overall 77.78% of the households are dependent on municipal water. Phiphidi and Mavambe villages rely 100% on municipal water. Dzingahe village relied the least (40%) on municipal water and reported that 60% of the HHs rely on rainwater as the main water source. The community members said there is water available, however 93.83% households stored water. Table 4.2 indicates water availability in rural communities from the rural households.

Table 4.2: Water coverage in rural communities of Vhembe district

Variable	Overall Coverage (HH=81)	Dzingahe (HH=10)	Mphambo (HH=10)	Ngovhela (HH=10)	Mavambe (HH=8)	Ngudza (HH=8)	Phiphidi (HH=10)	Xigalo (HH=25)
Main water supply								
Municipal water	63 (77.78%)	4 (40%)	8 (80%)	9 (90%)	8 (100%)	4 (50%)	10 (100%)	20 (80%)
Borehole	8 (9.88%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	1 (12.50%)	0 (0%)	5 (20%)
River water	3 (3.70%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (37.50%)	0 (0%)	0 (0%)
Rainwater	7 (8.64%)	6 (60%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Water availability								
Yes	62 (76.54%)	4 (40%)	8 (80%)	9 (90%)	8 (100%)	4 (50%)	10 (100%)	19 (76%)
Storage of water								
Yes	76 (93.83%)	10 (100%)	10 (100%)	9 (90%)	8 (100%)	8 (100%)	6 (60%)	25 (100%)
Storage duration								
<week	6 (7.41%)	0 (0%)	0 (0%)	2 (20%)	1 (12.50%)	0 (0%)	2 (20%)	1 (4%)
1 week	19 (23.46%)	0 (0%)	4 (40%)	6 (60%)	0 (0%)	4 (50%)	2 (20%)	3 (12%)
2 weeks	20 (24.69%)	2 (20%)	0 (0%)	0 (0%)	5 (62.50%)	2 (25%)	0 (0%)	11 (44%)
3 weeks	6 (7.41%)	0 (0%)	1 (10%)	0 (0%)	1 (12.50%)	2 (25%)	0 (0%)	2 (8%)
1 month	13 (16.05%)	3 (30%)	1 (10%)	0 (0%)	1 (12.50%)	0 (0%)	1 (10%)	6 (24%)
>1 month	11 (13.58%)	5 (50%)	4 (40%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (4%)
Storage condition								
25L only	19 (23.46%)	0 (0%)	1 (10%)	3 (30%)	3 (37.50%)	1 (12.50%)	3 (30%)	8 (32%)
25L and/or 100L, 200L	43 (53.09%)	4 (40%)	9 (90%)	0 (0%)	5 (62.50%)	6 (75%)	3 (30%)	16 (64%)
25L, 50L, 100L	1 (1.23%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
25L, 50L, 100L and 200L	2 (2.47%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
25L, 100L and 200 L	1 (1.23%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tank	3 (3.70%)	1 (10%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Tank, 25L and 50L	2 (2.47%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	0 (0%)	0 (0%)
25 and 50L	5 (6.17%)	0 (0%)	0 (0%)	5 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Treat water								
Yes	11 (13.58%)	5 (50%)	2 (20%)	0 (0%)	0 (0%)	3 (37.50%)	1 (10%)	0 (0%)
Type of treatment								
Salt only	1 (1.23%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Jik only	5 (6.17%)	3 (30%)	1 (10%)	0 (0%)	0 (0%)	1 (12.50%)	0 (0%)	0 (0%)
Salt and Jik	2 (2.47%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	0 (0%)	0 (0%)
Boiling	3 (3.70%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	1 (12.50%)	1 (10%)	0 (0%)

Keywords: HH : Households < : less than > : greater than L : Liters

Dzingahe, Mphambo, Mavambe and Ngudza village households all reported that households are storing water in different size storage containers. Only 13.58% of the HHs treat the stored water because they believed that the supplied water has been treated

and believed that rainwater was clean. Figure 4.3 indicates the different types of water storage containers used at the village households.

Figure 4.3: Different types of water storage [(A: 25L bottle, B: 50L bottle, C: 100L container, D: 200L container, E: Tank) (Taken during study period)]

Water availability and accessibility is of importance in everyday life (UN, 2006). However, South Africa is facing a challenge of water scarcity (Potgieter et al., 2009). This is supported by the results of this study, there was not 100% municipal water supply at the village households during sampling time. The village households resorted to use borehole water (9.88%), river water (3.70%) and rainwater (8.64%) for domestic use. Chubaka et al. (2018) have reported on the pathogens that are found in roof harvested rainwater in Australia. This is due to the type of roof materials, defaecation from animals on the roof and any other compounds (Lee et al., 2012; Mendez et al., 2011). Treating water is of importance since DWAF (2002) reported that the South African water crisis is more aligned to the water quality than quantity. Groundwater was implemented and plays a major role in meeting the basic human need for drinking water (Pietersen, 2005). However, groundwater poorly designed and installed provide users with contaminated water (Mpenyana-Monyatsi et al., 2012). River water is usually contaminated due to activities that occur around the water source, such as open defaecation; animals that drink water by the river; community members wash their clothes with river water (Potgieter et al., 2009). Therefore, village households that use the river water without treatment are exposed to pathogens that may lead to diarrheal infections (Clasen, 2015).

4.1.4 WATER STORAGE CONDITIONS AT HOUSEHOLDS

Table 4.3 indicates storage containers conditions of the households of Vhembe district. Cleaning water storage containers is one of the important factors that prevents contamination (pathogens being consumed by community members) and leads to diarrheal infections (Brick et al., 2004). Mphambo, Mavambe, Ngudza villages reported 100% in community members cleansing the storage containers while Phiphidi village only reported that 60% of HHs members clean these storage containers. Most of the households wash the storage containers once in a while, this is when they could not estimate after how long and refer to it as when we only remember or see that the containers are dirty. Dzingahe village has 1 (10%) household, Ngovhela has 2 (20%) households and Phiphidi has 4 (40%) households that reported to wash and rinse their containers on a daily basis. Most (23.46%) households in the study population reported to use water and dishwasher as cleansing material for water storage container.

In this study, a high number of households clean their water storage containers; most of the households clean the containers once in a while and only 14.81% use water only to clean the water storage container. Cleaning the water storage container is important; bacterial counts in water at source and water stored in households showed that the contamination is greater in cases where faecal counts in water at source was low (Wright et al., 2004). Inadequate cleanliness of the water storage container has been described as a key source of drinking-water contamination in many settings worldwide (Trevett et al., 2005; Ahmed et al., 1998). One possible cause of contamination of previously safe drinking water may be the presence of biofilm on inner surfaces of the container; this emphasizes the importance of frequently cleaning the water storage using proper disinfectants (Momba and Notshe, 2003).

Table 4.3: Storage container conditions

Variable	Overall Coverage (HH=81)	Dzingahe (HH=10)	Mphambo (HH=10)	Ngovhela (HH=10)	Mavambe (HH=8)	Ngudza (HH=8)	Phiphidi (HH=10)	Xigalo (HH=25)
Clean water storage								
Yes	71 (87.65%)	9 (90%)	10 (100%)	8 (80%)	8 (100%)	8 (100%)	6 (60%)	22 (88%)
No	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
N/A	7 (8.64%)	1 (10%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	4 (40%)	0 (0%)
Sometimes	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Barely	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
How often are storage containers cleaned?								
Everyday	1 (1.23%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1/w	20 (24.69%)	3 (30%)	3 (30%)	5 (50%)	1 (12.50%)	2 (25%)	3 (30%)	3 (12%)
1/ a while	30 (37.04%)	6 (60%)	5 (50%)	3 (30%)	7 (87.50%)	5 (62.50%)	1 (10%)	3 (12%)
Never/ N/A	4 (4.94%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
1/m	5 (6.17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (20%)
2/m	14 (17.28%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	11 (44%)
2/w	3 (3.70%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	1 (10%)	1 (4%)
Cleansing material								
Water only	12 (14.81%)	1 (10%)	3 (30%)	0 (0%)	5 (62.50%)	0 (0%)	1 (10%)	2 (8%)
Water +GB/BB	14 (17.28%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	3 (37.50%)	0 (0%)	9 (36%)
Water+ DW	19 (23.46%)	4 (40%)	2 (20%)	5 (50%)	3 (37.50%)	2 (25%)	3 (30%)	0 (0%)
Water+ Jik/DM	5 (6.17%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	2 (20%)
Water + DW+ Jik/DM	4 (4.94%)	2 (20%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
Water+ PS	13 (16.05%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	2 (25%)	0 (0%)	9 (90%)
Water+ HA	2 (2.47%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Water + Sand + DW	2 (2.47%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
Water+ PS+ Sand	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)

Keywords: HH: Household + : And N/A : Not Applicable w : week m=month GB : Green bar soap BB : Blue bar soap DM : Domestos DW : Dishwasher PS : Powder soap HA : Handy Andy

4.1.5 SANITATION COVERAGE AT HOUSEHOLDS

Availability of sanitation infrastructure reduces the improper faecal disposal in households and the community and reduces chances of a faecal-oral transmission (Oloruntoba et al., 2014). Dzingahe, Mphambo, Ngovhela and Phiphidi village households had 100% sanitation coverage whereas Mavambe, Ngudza and Xigalo village households only had 83.95% coverage. Ngudza is the only village with a house that used a flush toilet. The other households that had sanitation infrastructure provided, had a pit latrine with a slab with an overall of 82.75%. Overall results for the study population indicates that the sanitation infrastructure at the households are cleansed once per week using water and powder soap. Table 4.4 illustrates the sanitation coverage at households.

Table 4.4: Sanitation coverage at rural villages of Vhembe district

Variable	Overall Coverage (HH=81)	Dzingahe (HH=10)	Mphambo (HH=10)	Ngovhela (HH=10)	Mavambe (HH=8)	Ngudza (HH=8)	Phiphidi (HH=10)	Xigalo (HH=25)
Toilet available								
Yes	68 (83.95%)	10 (100%)	10 (100%)	10 (100%)	5 (62.50%)	6 (75%)	10 (100%)	17 (68%)
Type of toilet								
Pit latrine + slab	67 (82.72%)	10 (100%)	10 (100%)	9 (90%)	5 (62.50%)	6 (75%)	10 (100%)	17 (68%)
Flush	1 (1.23%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Clean toilet								
Yes	66 (81.48%)	10 (100%)	10 (100%)	10 (100%)	5 (62.50%)	6 (75%)	9 (90%)	16 (64%)
How often do you clean the toilet?								
Everyday	8 (9.88%)	0 (0%)	1 (10%)	2 (20%)	3 (32.50%)	1 (12.50%)	0 (0%)	1 (4%)
1/w	31 (38.27%)	7 (70%)	5 (50%)	2 (20%)	1 (12.50%)	2 (25%)	4 (40%)	10 (40%)
2/w	5 (6.17%)	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	1 (12.50%)	2 (20%)	1 (4%)
3/w	7 (8.64%)	0 (0%)	2 (20%)	1 (10%)	0 (0%)	1 (12.50%)	1 (10%)	2 (8%)
1/m	5 (6.17%)	2 (20%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
2/m	4 (4.94%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	1 (12.50%)	0 (0%)	2 (8%)
1/a while	6 (7.41%)	1 (10%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
Cleansing material								
Water only	3 (3.70%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
Water + DW/ PG	6 (7.41%)	0 (0%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)	1 (10%)	1 (4%)
Water + PS	18 (22.22%)	2 (20%)	4 (40%)	1 (10%)	3 (37.5%)	2 (25%)	1 (10%)	5 (20%)
Water + GB/BB	2 (2.47%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Water + Jeyes	5 (6.17%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	1 (12.5%)	2 (20%)	1 (4%)
Water + Jik/ DM	6 (7.41%)	1 (10%)	2 (20%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
Water + Jik/DM + Jeyes	4 (4.94%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)
Water + Jik/DM +PS	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
Water + Jik + PS + Jeyes	3 (3.70%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)
Water + DM + PG + Jeyes	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)
Broom (sweeping)	6 (7.41%)	1 (10%)	1 (10%)	2 (20%)	0 (0%)	1 (12.5%)	0 (0%)	1 (4%)
Water + HA	5 (6.17%)	0 (0%)	1 (10%)	1 (10%)	1 (12.5%)	0 (0%)	0 (0%)	2 (8%)
Water + PS + Jeyes	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
Water + Jeyes + PS + Ashes	1 (1.23%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Water + Vim	1 (1.23%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Water used for laundry	4 (4.94%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	3 (12%)

Keywords: HH: Household + : And w : week m: month GB : Green bar soap BB : Blue bar soap DM : Domestos DW: Dishwasher PS : Powder soap HA : Handy Andy

Improper disposal of nappies or any faecal matter allows faecal material to be released into the household and community environment (Magizvom and Mupindu, 2012). Overall 4.94% of the households have children that may use the same toilets as the adults. A total of 28.40% households allowed children under the age of 5 to practice open defaecation and dispose the faecal into the pit latrine. This does not guarantee safe removal of all faecal from the ground. This may leave the soil contaminated of pathogenic microbes.

In this study, the provision of sanitation facilities at village households is not at 100% coverage; therefore, there are community members that practice open defaecation. This increases the chances of spreading and contamination of potential health risk pathogens (WHO-UNICEF, 2012). A study done by Chola et al. (2015) in South Africa have shown that improved sanitation is effective in prevention of diarrheal diseases and can contribute

in reducing morbidity of children under five. In this study, it is observed that village household members uses pit-latrines with a slab; and only 4.94% of under 5 use the same toilet as the adults. About 28.40% of the under 5 practice open defaecation and the faeces are removed into the pit. This does not guarantee safe removal, may leave the soil faecally contaminated. It was observed that during sampling period, only 14.81% of the sanitations were clean. This is of health risk since transmission of pathogens may occur directly (direct contact) or indirectly (airborne, vectors: flies). Table 4.5 illustrates the sanitation coverage of children under 5 and the noted observation of the sanitation condition at time of inspection.

Table 4.5: Sanitation coverage for children under 5 and sanitation condition

Variable	Overall Coverage (HH=81)	Dzingahe (HH=10)	Mphambo (HH=10)	Ngovhela (HH=10)	Mavambe (HH=8)	Ngudza (HH=8)	Phiphidi (HH=10)	Xigalo (HH=25)
Under 5 use toilets								
Yes	4 (4.94%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
If not, alternative								
Nappies	32 (39.51%)	4 (40%)	3 (30%)	5 (50%)	2 (25%)	5 (62.5%)	7 (70%)	6 (24%)
Open defaecation and disposed	23 (28.40%)	2 (20%)	6 (60%)	4 (40%)	3 (37.5%)	1 (12.5%)	3 (30%)	5 (20%)
Children toilet	4 (4.94%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (16%)
Toilet observation								
Clean	12 (14.81%)	2 (20%)	1 (10%)	3 (30%)	2 (25%)	0 (0%)	1 (10%)	3 (12%)
Flies only	2 (2.47%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Faeces only	2 (2.47%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
Flies + litter	2 (2.47%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	1 (125%)	0 (0%)	0 (0%)
Faeces + litter	2 (2.47%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (4%)
Flies + odour	7 (8.64%)	3 (30%)	0 (0%)	1 (10%)	0 (0%)	1 (12.5%)	2 (20%)	0 (0%)
Odour only	3 (3.70%)	0 (0%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	1 (4%)
Odour + faeces	2 (2.47%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	1 (4%)
Slight odour	7 (8.64%)	2 (20%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (20%)	2 (8%)
Slight odour + litter	2 (2.47%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Slight odour +flies	3 (3.70%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	2 (8%)
Slight odour + faeces	2 (2.47%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
Flies + odour + faeces	4 (4.94%)	1 (10%)	3 (30%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Flies + odour + litter	4 (4.94%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (25%)	2 (20%)	0 (0%)
Flies + slight odour + faeces	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)
Flies + slight odour + faeces + litter	1 (1.23%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Flies + odour + faeces + litter	11 (13.58%)	2 (20%)	4 (40%)	0 (0%)	1 (10%)	0 (0%)	2 (20%)	2 (8%)

Keywords: HH= Households + : And

4.1.6 HYGIENE PRACTICES AT HOUSEHOLDS

Hand hygiene practices are one of the most important factors that prevents the transmission of pathogens and may contribute in the intervention of reduction of diarrheal outbreaks (Taylor et al., 2015; Pittet et al., 2006). The community members were asked whether they wash their hands after using the toilet. The follow up question was whether

they always wash their hands. The answer “Always” (Table 4.6) when both were yes answers; “sometimes” when the was both yes and no, “never” when both were no answers. Overall the results for the study population indicates that 90.12% of the members always wash their hands, with 1 (1.23%) member who reported that they don’t wash their hands. This could be that they do not have any sanitation at the household and have to walk to the bushes and practice open defaecation. The distance walked may be far that by the time they reach home, they have forgotten that they need to practice hand hygiene. All visited households have a percentage greater than 50 in practicing proper hand hygiene since they use water and soap to wash their hands. Mphambo village has 1 (10%) household that put the 2L bottles next to the toilet to remind them to always wash the hands. An overall of 87.65% have the wash station between 10 -100m away from the toilet.

Hand hygiene practices of caregivers and mothers of the study village households is not practiced at all times. The overall indicates that the caregivers and mothers that reported on practicing hand hygiene at all times were 90.12% and mostly the water source for hand hygiene practices came from stored water samples (66.67%). The caregivers and mothers that uses soap for washing hands is 62.96%. Handwashing with soap has been estimated to reduce the risk of diarrheal infection by 47% (Curtis and Cairncross, 2003). Hand hygiene improvements could reduce both transmission of pathogens through interpersonal contact as well as the risk of stored water and food are contaminated through handling (Davis et al., 2011).

Only a few caregivers and mothers of this study do not promote proper hand hygiene practices. This may lead to an assumption that children from the village households are less exposed to pathogens via the transmission route of their mothers and caregivers’ hands. Promotion of improved hand hygiene is of important public measure towards the health of children under the age of 5. However, how much hand hygiene is required to prevent transmission of pathogens is unclear. It is highlighted that using soap for handwashing is more effective in reducing pathogens. Table 4.6 illustrates the hand hygiene practiced at households.

Table 4.6: Hand hygiene practices

Variable	Overall Total HH=81	Dzingahe HH=10	Mphambo HH=10	Ngovhela HH=10	Mavambe HH=8	Ngudza HH=8	Phiphidi HH=10	Xigalo HH=25
Wash hands								
Always	73 (90.12%)	10(100%)	10 (100%)	10 (100%)	7 (87.5%)	8 (100%)	10 (100%)	18 (72%)
Sometimes	7 (8.64%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	6 (24%)
Never	1 (1.23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Handwash material								
Water only	29 (35.80%)	5 (50%)	2 (20%)	3 (30%)	4 (50%)	2 (25%)	3 (30%)	10 (40%)
Water + soap	51 (62.96%)	5 (50%)	8 (80%)	7 (70%)	4 (50%)	6 (75%)	7 (70%)	14 (60%)
Source of handwash water								
Stored water	54 (66.67%)	10 (100%)	8 (80%)	7 (70%)	3 (37.5%)	8 (100%)	1 (10%)	17 (68%)
Tap	19 (23.46%)	0 (0%)	1 (10%)	2 (20%)	5 (62.5%)	0 (0%)	9 (90%)	2 (8%)
2L bottle	1 (1.23%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tap + stored water	7 (20.99%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	6 (24%)
Wash station distance								
Inside/next to Toilet	2 (2.47%)	0 (0%)	1 (10%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
10-100m	71 (87.65%)	10 (100%)	9 (90%)	9 (90%)	7 (87.5%)	8 (100%)	10 (100%)	18 (72%)
101-200m	2 (2.47%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
201-300m	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
301-400m	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
401-500m	5 (6.17%)	0 (0%)	0 (0%)	0 (0%)	3 (37.5%)	0 (0%)	0 (0%)	2 (8%)
>500m	3 (3.70%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (12%)

Keywords: HH : Households L : Liters m : meter > : greater than

4.2 MICROBIOLOGICAL ASSESSMENT OF HOUSEHOLD SAMPLES COLLECTED

A total of 399 samples were processed using the Colilert® Quanti-tray®/2000 method. Table 4.7 provides data on samples taken during sample collection period at the 7 participated villages of Vhembe district. The Colilert® Quanti-trays® were observed after incubation at 37˚C for 24 hrs for TC (see section 4.2.1) and *E. coli* (see section 4.2.2). The positive wells for TC were determined by the colour change into yellow and the positive *E. coli* was known when wells fluorescend under UV light (366nm). The tap water samples are the least collected due to the unavailability of running tap water during the sampling period. Not many toilet seat swabs were collected due to village households that still practice open defaecation and did not have sanitation facilities.

Table 4.7: Total number of samples collected in 7 study villages (n=399)

Variable	Overall Total n=399 (%)	Dzingahe n= 45 (%)	Mphambo n= 50 (%)	Ngovhela n= 49 (%)	Mavambe n= 41 (%)	Ngudza n= 38 (%)	Phiphidi n= 53 (%)	Xigalo n= 123 (%)
Toilet seat	**68 (17.04%)**	10 (22.22%)	10 (20%)	10 (20.41%)	6(14.63%)	6(15.80%)	10(18.87%)	16 (13%)
Floor	**81 (20.30%)**	10(22.22%)	10(20%)	10 (20.41%)	8 (19.51%)	8 (21.05%)	10 (18.87%)	25 (20.33%)
Mothers hand	**81 (20.30%)**	10(22.22%)	10(20%)	10 (20.41%)	8 (19.51%)	8 (21.05%)	10 (18.87%)	25 (20.33%)
Childs hand	**73 (18.30%)**	4(8.90%)	8(16%)	8 (16.33%)	9 (21.95%)	8 (21.05%)	10 (18.87%)	26 (21.14%)
Stored water	**82 (20.55%)**	11(24.44%)	12(24%)	11 (22.45%)	8 (19.51%)	8 (21.05%)	5 (9.43%)	27 (21.95%)
Tap water	**14 (3.51%)**	0(0%)	0(0%)	0 (0%)	2 (4.8815.9%)	0 (0%)	8 (15.09%)	4 (3.25%)

Keywords: n : number of samples

4.2.1 TOTAL COLIFORM ASSESSEMNT

A total of 317 (79.44%) samples had colour change after 24 hrs incubation. Presence of Total coliform (TC) in samples indicates that there is a possibility of having pathogenic strains (Rompré et al., 2002). Running tap water was unavailable at the study village households during sampling period and village households resorted to storing water. A high prevalence of TC was detected from stored water samples [85,37% (70/82)] as well as the mothers handwash [97.53% (79/81)]. It is reported that availability of running tap water may reduce high detection of TC in stored water samples (Wright et al., 2004). Table 4.8 illustrates the TC counts of the samples collected from the study village households.

Table 4.8: Total coliform counts / 100 ml (n=317)

Variable	Overall Total n=317 (%)	Dzingahe n= 37 (%)	Mphambo n= 44 (%)	Ngovhela n= 30 (%)	Mavambe n= 36 (%)	Ngudza n= 33 (%)	Phiphidi n= 47 (%)	Xigalo n= 90 (%)
Toilet seat	**39 (12.30%)**	4 (10.81%)	7 (15.91%)	4 (13,33%)	6 (16.67%)	2 (6.06%)	10 (21.28%)	6 (6.67%)
Floor	**51 (16.09%)**	8 (21.62%)	8 (18.18%)	3 (10%)	8 (22.22%)	8 (24.24%)	10 (21.28%)	6 (6.67%)
Mothers hand	**79 (24.92%)**	10 (27.03%)	10 (22.73%)	9 (30%)	8 (22.22%)	8 (24.24%)	10 (21.28%)	24 (26.67%)
Childs hand	**70 (22.08%)**	4 (10.81%)	8 (18.18%)	7 (23,33%)	9 (25%)	8 (24.24%)	10 (21.28%)	24 (26.67%)
Stored water	**72 (22.71%)**	11 (29.73%)	11 (25%)	7 (23,33%)	5 (13.89%)	7 (21.21%)	4 (8.51%)	27 (30%)
Tap water	**06 (1.90%)**	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (6.38%)	3 (3.33%)

Keywords: n: number of samples

4.2.2 *ESCHERICHIA COLI* ASSESMENT

A total of 91 (22.81%) samples were positive for *E. coli* when observed under UV-light after 24 hrs of incubation. The presence of *E. coli* in drinking water is associated with a recent faecal contamination and its associated with increased risk of both enteric pathogens and diarrheal diseases (Ashbolt, 2015; Edberg et al., 2000). The highest *E. coli* was detected from the mothers' hands and the least was detected from the running tap water samples. Table 4.9 illustrates the type of samples detected with *E. coli.*

Table 4.9: *Escherichia coli* counts/ 100 ml (n=91)

Variable	Overall Total n=91 (%)	Dzingahe n= 10 (%)	Mphambo n= 19 (%)	Ngovhela n= 9 (%)	Mavambe n= 7 (%)	Ngudza n= 12 (%)	Phiphidi n= 11 (%)	Xigalo n= 23 (%)
Toilet seat	**10 (11%)**	1 (10%)	3 (15.79%)	1 (11.11)	0 (0%)	1 (8.33%)	3 (27.27%)	1 (4.35%)
Floor	**5 (5.49%)**	0 (0%)	3 (15.79%)	0 (0%)	0 (0%)	1 (8.33%)	0 (0%)	1 (4.35%)
Mothers hand	**29 (31.87%)**	4 (40%)	5 (26.32%)	3 (33.33%)	4 (57.14%)	2 (16.67%)	3 (27.27%)	8 (34.78%)
Childs hand	**19 (20.88%)**	0 (0%)	4 (21.05%)	3 (33.33%)	2 (28.57%)	4 (33.33%)	3 (27.27%)	3 (13.04%)
Stored water	**27 (29.67%)**	5 (50%)	4 (21.05%)	2 (22.22)	1 (14.29)	4 (33.33%)	1 (9.09%)	10 (43.48%)
Tap water	**1 (1.09%)**	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9.09%)	0 (0%)

Keywords: n : number of samples

4.3 INDIVIDUAL VILLAGE ASSESSMENT

The MPN counts/100 ml for the samples were calculated using the IDEXX table supplied by the manufacturer (IDEXX, 2002). The importance for the MPN values is to determine the health risk of community members referring WHO standards (WHO, 2008) and the DWAF guidelines (DWAF, 1996). For example, water is referred to as drinkable when chemical properties are within guideline limits and when microbiological analysis indicate absence of harmful agents such as Total coliform having an MPN count below 5 per 100 ml and *E. coli* having an MPN count of <1 per 100 ml (DWAF, 1996). Table 4.10 to Table 4.16 represent the MPN counts for TC and *E. coli* for each sample at the 7 different villages. For calculation purposes the <1 MPN/ 100 ml was replaced with Zero (0 MPN/ 100 ml) and counts >2416.6 MPN/ 100 ml was rounded off to 2420 MPN/ 100 ml.

4.3.1 DZINGAHE VILLAGE

Dzingahe village is one of the mountainous villages that didn't have running tap water during sampling period. Therefore, the MPN counts for TC and *E. coli* counts for this village couldn't be determined. The household with the highest TC counts for toilet seat samples was at HH2 (240.0 MPN/ 100 ml) and the highest *E. coli* counts detected was at HH3 while the other HHs had no *E. coli* detected. The floor samples had the highest TC counts detected at HH10 and no *E. coli* was detected for all households for this type of sample. The mothers hand samples had the highest TC counts detected at HH1 and the highest *E. coli* at HH3. The were no hand samples from children's hands, only sampled from 4 HHs because the children were at pre-school. The highest (104.6 MPN/ 100 ml) TC counts was detected at HH2 and no *E. coli* was detected from the sampled children's hand. In HH3, HH4, HH6 and HH9 TC was detected the highest (2420 MPN/ 100 ml) and highest (613.1) *E. coli* was detected from HH3.

Children from HH3,6 and 9 have chances of getting a diarrheal infection due to the high level (2420 MPN/ 100 ml) of TCs from the samples. According to the DWAF (1996) guidelines, the risk criteria shows that drinking water with > 100 MPN/ 100 ml TC is of potential risk for infection. The stored water sample from HH5 is of greatest high risk if used for drinking. The MPN count for *E. coli* is 613.1 MPN/ 100 ml which can cause infection when consumed. There was no child at this HH, therefore contamination of the stored water may be assumption due to the type of water used, during of storage and cleanliness of the storage container. There were no high *E. coli* counts detected from any other samples at the HHs that can allow assumption of transmission pathways. Table 4.10 shows the TC counts and *E. coli* counts from Dzingahe village.

Table 4.10: MPN counts for TC and *E. coli* counts for Dzingahe village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	3.1	0	1.0	0	2420	0	-	-	1.0	0	-	-
HH2	240.0	0	1.0	0	307.6	0	104.6	0	179.3	0	-	-
HH3	16.0	2.0	240.0	0	307.6	4.1	72.6	0	2420.0	27.2	-	-
HH4	0	0	0	0	3.1	0	41.4	0	2420	0	-	-
HH5	0	0	4.1	0	195.6	2.0	-	-	16.1	613.1	-	-
HH6	1.0	0	1.0	0	344.8	0	12.0	0	2420.0	12.1	-	-
HH7	70.3	0	2.0	0	18.3	0	-	-	1.0	0	-	-
HH8	0	0	16.9	0	167.0	1.0	-	-	1203.3	0	-	-
HH9	0	0	0	0	1119.9	0	-	-	2420.0	1.0	-	-
HH10	0	0	2420.0	0	1553.1	34.5	-	-	238.2	1.0	-	-
MEAN	33.04	0.2	268.5	0	643.7	4.16	57.65	0	1131.89	65.44	-	-
MEDIAN	0.5	0	1.5	0	307.6	0	57	0	720.75	0.5	-	-
MIN	0	0	0	0	3.1	0	12	0	1	0	-	-
MAX	240	2	2420	0	2420	34.5	104.6	0	2420	613.1	-	-
STDV	75.93	0.63	759.63	0.00	799.57	10.74	39.90	0.00	1161.94	192.63	-	-

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.2 MPHAMBO VILLAGE

During sample collection, the Mphambo village had no running water. Therefore, results for TC and *E. coli* could not be determined for tap water. The toilet seat sample of HH2 had the highest count (2420 MPN/ 100 ml) for TC and *E. coli* whereas HH10 had no TC and *E. coli* was detected. The mean value for all households was 491.4 MPN/ 100 ml for TC and 242.85 MPN/ 100 ml for *E. coli*. The mothers' hands sample in HH5, HH7 and HH 9 had the highest value (2420 MPN/ 100 ml for TC and less count for *E. coli* ranging from 0 – 10.8 MPN/ 100 ml. The mother's hand from HH3 had the lowest count (8.5 MPN/ 100 ml). The mean value of mothers' hands is 1029.01 MPN/100 ml. The children's hand samples from HH7 and HH8 had the highest TC counts however the *E. coli* detected was very low. There were no children to sample at HH4 and HH6 , they were off to pre-school. The storage water from HH3, HH4, HH5, HH6, HH9 had the highest TC and HH10 has the lowest count.

Stored water from HH1, 8 and 10 have MPN counts lower than 100 MPN/ 100 ml when looking at the TC counts. According to DWAF guidelines (1996) continuous use of water with MPN count of greater than 5 MPN/ 100 ml has a potential risk to cause infection. The

child's hand at HH10 indicate a recent faecal contamination, the MPN count of *E. coli* shows substantial risk since its >20 MPN/ 100 ml. At this HH, transmission pathway is possible due to detection of TC counts from the mother and child's handwash samples as well as the stored water sample. The floor sample from HH4 is highly contaminated (TC and *E. coli*), assumption is that contamination may be caused by the animals kept at the yard or even the type of stoep (cow-dung) surrounding the compound. The toilet seat from HH2, has high counts of both TC and *E. coli*; this indicates possibility of improper use of the toilet. Table 4.11 shows the TC and *E. coli* counts from Mphambo village.

Table 4.11: MPN counts for TC and *E. coli* counts for Mphambo village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	11.0	1.0	980.4	0	133.3	0	93.4	0	43.2	0	-	-
HH2	2420.0	2420.0	101.4	44.3	1553.1	2.0	816.4	1.0	344.8	1.0	-	-
HH3	0	0	648.8	0	8.5	0	12.1	0	2420.0	2.0	-	-
HH4	1.0	0	2420	727.0	152.3	7.5	-	-	2420.0	15.2	-	-
HH5	1.0	0	34.1	0	2420.0	0	4.1	0	2420.0	0	-	-
HH6	2420.0	0	135.4	4.1	261.3	2.0	-	-	2420.0	0	-	-
HH7	34.1	0	1.0	0	2420.0	0	2420.0	0	261.3	0	-	-
HH8	23.3	7.5	0	0	770.1	2.0	2420.0	3.1	13.4	0	-	-
HH9	0	0	10.9	0	2420.0	10.8	980.4	2.0	2420.0	1.0	-	-
HH10	0	0	0	0	151.5	0	52.1	52.9	11.0	0	-	-
MEAN	491.04	242.85	433.2	77.54	1029.01	2.43	849.81	7.38	1277.37	1.92	-	-
MEDIAN	6	0	67.75	0	515.7	1	454.9	0.5	1382.4	0	-	-
MIN	0	0	0	0	8.5	0	4.1	0	11	0	-	-
MAX	2420	2420	2420	727	2420	10.8	2420	52.9	2420	15.2	-	-
STDV	1016.72	764.98	774.01	228.62	1060.29	3.74	1040.24	18.43	1208.98	4.72	-	-

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.3 NGOVHELA VILLAGE

Ngovhela village had no running water during sample collection water. Therefore, TC and *E. coli* counts/ 100 ml for tap could not be determined. Toilet seat and floor samples from this village had no high MPN determined for both TC and *E. coli*. The TC counts were 20 MPN/100 ml and lower while *E. coli* highest MPN calculated was 1.0 MPN/ 100 ml for both toilet seat and floor samples. The mean value for TC is 2.24 MPN/ 100 ml and 0.2

MPN/ 100 ml for toilet seat and floor samples respectively. The mothers hand samples from HH1 and HH8 had the highest (2420) MPN count for TC . The child hand sample from HH7 had the highest 2420 MPN/ 100 ml count for TC and 21.8 MPN/ 100 ml for *E. coli* while HH2 had no TC and *E. coli* detected from collected samples. HH9 and HH10 had no samples taken due to children that were at pre-school during sampling time. The stored water from HH9 had the highest TC count, however no *E. coli* was detected in the same sample.

The microbiological indicators used in this study (TC and *E. coli*) were not detected in most households. The TC and *E. coli* was detected at HH5, however is not of high risk for the children living at this household. At HH1, TC and *E. coli* were detected from the mother's handwash sample, the child's handwash sample and the stored water sample; a possible transmission may have occurred. The water at HH1 is only of risk when its continuously used (DWAF, 1996). At HH7, the child's handwash and stored water sample have *E. coli* detected, this allows assumption that water storage container could have been left open and the child contaminated the water with their contaminated hands. Table 4.12 shows the TC and *E. coli* counts from Ngovhela village.

Table 4.12: MPN counts for TC and *E. coli* counts for Ngovhela village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	17.3	0	0	0	2420.0	344.8	1533.1	1.0	1119.9	1.0	-	-
HH2	0	0	0	0	5.2	0	0	0	0	0	-	-
HH3	0	0	0	0	1119.9	0	22.8	0	0	0	-	-
HH4	0	0	0	0	93.3	0	275.5	0	0	0	-	-
HH5	2.0	1.0	0	0	172.0	0	187.2	11.0	1.0	0	-	-
HH6	0	0	0	0	32.7	0	34.1	0	196.8	0	-	-
HH7	0	0	0	0	1119.9	0	2420.0	21.8	1119.9	4.0	-	-
HH8	0	0	1.0	0	2420.0	21.6	9.8	0	0	0	-	-
HH9	0	0	1.0	0	131.4	12.1	-	-	2420.0	0	-	-
HH10	3.1	0	0	0	0	0	-	-	4.1	0	-	-
MEAN	2.24	0.1	0.2	0	751.44	37.85	560.31	4.23	486.17	0.5	-	-
MEDIAN	0	0	0	0	151.7	0	110.65	0	2.55	0	-	-
MIN	0	0	0	0	0	0	0	0	0	0	-	-
MAX	17.3	1	1	0	2420	344.8	2420	21.8	2420	4	-	-
STDV	5.40	0.32	0.42	0.00	979.30	108.10	910.88	8.06	819.21	1.27	-	-

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.4 MAVAMBE VILLAGE

Mavambe village is situated next to the road and has a Primary healthcare facility within the village. HH1 and HH2 of Mavambe village had tap running water available during sampling time and no TC and *E. coli* were detected from this samples. The toilet seat and floor samples from HH1 and HH2 had the highest TC detected. There was no (0 MPN/ 100 ml) *E. coli* was detected for all samples taken for the toilet seat and floor samples. There is no TC and *E. coli* determined for HH5 and HH8 due to the household had no toilets available; members of the households practice open defaecation. The mean value for TC were 846.27 MPN/ 100 ml and 645.55 MPN/ 100 ml for toilet seat and floor samples respectively with 0 mean calculated for *E. coli* for both types of samples. The mothers hand samples from HH1, HH2 and HH7 have the highest (2420 MPN/ 100 ml) TC; having only HH2 with the highest (2420 MPN/ 100 ml) *E. coli* detected and the others at lower counts 2.0 MPN/ 100 ml (HH1) and 0 MPN/ 100 ml (HH7). The mean for the mothers' hands is 1009.5 MPN/ 100 ml TC and 303.39 MPN/100 ml for *E. coli*. The samples from the children hand samples in HH1, HH2 and HH3 had the highest count (2420 MPN/ 100 ml) for TC and only in HH2 the sample had the highest (2420 MPN/ 100 ml) *E. coli* detected. HH3 had twins and it was noted that the counts for TC and *E. coli* were different when compared. Storage water samples seemed to be same with only HH3 having *E. coli* (1.0 MPN/ 100 ml) detected.

In comparison of the household samples from Mavambe village (Table 4.13), the samples from HH2 (Ts, F, M_H and C_H) have the same count of TC (2420 MPN/ 100 ml); the Ts and F sample has no *E. coli* detected. However, M_H and C_H have 2420 MPN/ 100 ml of *E. coli* detected. Assumption of possible transmission may have occurred. The mothers handwash and stored water samples have the same count of MPN detected (2420 MPN/ 100 ml), however no *E. coli* is detected. This does not mean that the water is safe to drink. According to the DWAF (1996) guidelines categories this level of MPN detected as substantial risk for use. The children's handwash samples at HH3 have different counts of TC and *E. coli*. The one twin has TC (2420 MPN/ 100 ml) and *E. coli* (4.1 MPN/ 100 ml) detected that may cause infection when the child eats before practicing any proper hand hygiene. Table 4.13 shows the TC and *E. coli* counts from Mavambe village.

Table 4.13: MPN counts for TC and *E. coli* counts for Mavambe village households

	T_S		F		M_H		C_H		S		T	
	TC	***E. coli***	**TC**	***E. coli***	**TC**	***E. coli***	**TC**	***E. coli***	**TC**	***E. coli***	**TC**	***E. coli***
HH1	2420.0	0	2420.0	0	2420.0	2.0	2420.0	62.0	0	0	0	0
HH2	2420.0	0	2420.0	0	2420.0	2420.0	2420.0	2420.0	214.2	0	0	0
HH3	58.4	0	36.9	0	201.4	3.1	25.0	0	1732.9	1.0	-	-
							2420.0	4.1				
HH4	58.3	0	59.4	0	488.4	2.0	85.7	0	0	0	-	-
HH5	-	-	65.0	0	17.5	0	770.1	0	36.4	0	-	-
HH6	75.4	0	46.4	0	78.9	0	488.4	0	0	0	-	-
HH7	45.5	0	57.3	0	2420.0	0	275.5	0	2420.0	0	-	-
HH8	-	-	59.4	0	29.8	0	49.6	0	52.9	0	-	-
MEAN	846.27	0	645.55	0	1009.5	303.39	994.92	276.23	557.05	0.13	0	0
MEDIAN	66.9	0	59.4	0	344.9	1	488.4	0	44.65	0	0	0
MIN	45.5	0	36.9	0	17.5	0	25	0	0	0	0	0
MAX	2420	0	2420	0	2420	2420	2420	2420	2420	1	0	0
STDV	1219.05	0	1095.25	0	1177.35	855.24	1094.21	804.17	958.19	0.35	0	0

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.5 NGUDZA VILLAGE

During sampling period, Ngudza village households had no running water available. Therefore, no TC and *E. coli* could be determined. The swab samples from toilet seat and floor had low counts for TC having the calculated counts for all households at 0.5 MPN/ 100 ml and 5.41 MPN/ 100 ml for toilet seat and floor samples respectively. Only at HH1 had *E. coli* detected for toilet seat and at HH2 for floor sample with MPN count of 2.0 MPN/ 100 ml for both. The *E. coli* mean counts calculated for all households was 0.33 MPN/ 100 ml (toilet seat) and 0.25 MPN/ 100 ml (floor). The mothers hand samples had the highest MPN counts for TC (290.9 MPN/ 100 ml) and *E. coli* (129.6 MPN/ 100 ml) at HH2. Most of the HHs had no *E. coli* detected for the toilet seat and floor samples. The children's hand samples had the highest (2420 MPN/ 100 ml) TC detected at HH4 and HH5 with the highest (12.1 MPN/ 100 ml) *E. coli* detected at HH4. The mean is 710.8 MPN/ 100 ml for TC and 2.01 MPN/ 100 ml for *E. coli*. The storage water from HH1 and HH3 had low TC counts and the other HHs had the highest count of 2420 MPN/ 100 ml and HH5 with 2420 MPN/ 100 ml count for *E. coli* determined.

In comparison of the household samples from Ngudza village (Table 4.14), the stored water samples from HH1 and HH3 are the ones that are safe for drinking, whereas the

water samples from the other HHs are of substantial risk for consumption. The MPN count are 2420 MPN/ 100 ml for TC. The water sample from HH5 has *E. coli* also detected, which shows high recent faecal contamination. The contamination of the water samples from this village can allow an assumption that the water from the water source may be contaminated. The toilet seat and floor samples have low MPN/ 100 ml (<25 MPN/ 100 ml). Transmission from this sample type are of less risk for infection. The children's handwash samples from HH4 and HH5 have high MPN (2420 MPN/ 100 ml) detected for TC, this is of substantial risk for the children. Table 4.14 shows the TC and *E. coli* counts from Ngudza village.

Table 4.14: MPN counts for TC and *E. coli* counts for Ngudza village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	0	2.0	1.0	0	12.2	0	3.1	2.0	0	0	-	-
HH2	2.0	0	3.1	2.0	290.9	129.6	224.7	0	2420.0	179.3	-	-
HH3	0	0	21.8	0	101.9	0	435.2	0	5.2	0	-	-
HH4	1.0	0	1.0	0	275.5	95.9	2420.0	12.1	2420.0	0	-	-
HH5	0	0	13.4	0	204.6	0	2420.0	1.0	2420.0	2420.0	-	-
HH6	0	0	1.0	0	146.4	0	135.5	0	2420.0	1.0	-	-
HH7	-	-	1.0	0	1.0	0	18.7	1.0	2420.0	1.0	-	-
HH8	-	-	1.0	0	18.9	0	29.2	0	2420.0	0	-	-
MEAN	0.5	0.33	5.41	0.25	131.43	28.19	710.8	2.01	1815.65	325.16	-	-
MEDIAN	0	0	1	0	124.15	0	180.1	0.5	2420	0.5	-	-
MIN	0	0	1	0	1	0	3.1	0	0	0	-	-
MAX	2	2	21.8	2	290.9	129.6	2420	12.1	2420	2420	-	-
STDV	0.84	0.82	7.88	0.71	117.43	52.96	1064.38	4.14	1119.04	848.76	-	-

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.6 PHIPHIDI VILLAGE

Phiphidi had only two households that didn't have water during sampling period. The highest TC (2420 MPN/ 100 ml) was detected at HH4, HH5 and *E. coli* was detected at HH4 (2420 MPN/ 100 ml) and HH5(1299.7 MPN/ 100 ml) with the others having no *E. coli* detected. The highest TC was detected at HH2 (2420 MPN/ 100 ml) and lowest count at HH3 (49.6 MPN/ 100 ml) for the floor samples. No *E. coli* was detected from all floor samples from this village. The highest TC (2420 MPN/ 100 ml) was detected from HH1,

HH8 and HH9 for the mothers' hands and the lowest count was detected at HH 6 (18.3 MPN /100 ml). *E. coli* was detected from HH1, HH8 and HH10 and no *E. coli* was detected at the others HHs. Five HHs didn't store water because they always have running tap water. From the 5 HHs that were sampled, 3 HHs had the highest TC counts detected and *E. coli* was detected from HH3 and HH4 (20.1MPN/ 100 ml). Two HHs had no running tap water during sampling period. The highest TC counts was detected at HH2 (866.4 MPN/ 100 ml) and the highest (1.0 MPN/ 100 ml) *E. coli* was detected at the same HH.

In comparison of the household samples from Phiphidi village (Table 4.15), toilet seat samples from HH4 and HH5 have high counts of TC and *E. coli*. This shows improper use; faeces may have been present on the seat and contamination had recently occurred. The floor samples have TC, but no *E. coli* detected at all households visited; this means other bacteria are present. The mother and child handwash samples from HH8 and HH9 show possible pathway transmission due to the TC and *E. coli* detected; other possible contamination may be from same source if transmission had not occurred. Stored water from HH4 and HH5 are not suitable for drinking due to the high TC (2420 MPN/ 100 ml) and number of *E. coli* (2420 MPN/ 100 ml) detected. The water samples have potential for causing diarrheal infection (DWAF, 1996). The tap water samples from the households are clean; only HH2 that has high TC counts. This may be influenced by the environmental factors. Table 4.15 shows the TC and *E. coli* counts from Phiphidi village.

Table 4.15: MPN counts for TC and *E. coli* counts for Phiphidi village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	15.8	0	93.3	0	2420.0	1.0	344.8	0	-	-	3.1	0
HH2	85.7	0	2420.0	0	204.6	0	2420.0	0	2420	0	866.4	1.0
HH3	166.4	0	49.6	0	1413.6	0	204.6	13.3	2420	20.1	-	-
HH4	2420.0	2420.0	88.4	0	1413.6	0	648.8	39.3	2420	20.1	-	-
HH5	2420.0	1299.7	142.1	0	80.5	0	146.4	0	0	0	0	0
HH6	131.4	1.0	109.2	0	18.3	0	26.2	0	24.9	0	0	0
HH7	204.6	0	187.2	0	30.9	0	61.3	0	-	-	0	0
HH8	261.3	0	166.4	0	2420.0	3.0	1299.7	1.0	-	-	1.0	0
HH9	325.5	0	1732.9	0	2420.0	0	2420.0	0	-	-	0	0
HH10	248.1	0	307.6	0	1986.3	2.0	344.8	0	-	-	0	0
MEAN	627.88	372.07	529.67	0	1240.78	0.6	791.66	5.36	1456.98	8.04	108.81	0.13
MEDIAN	226.35	0	154.25	0	1413.6	0	344.8	0	2420	0	0	0
MIN	15.8	0	49.6	0	18.3	0	26.2	0	0	0	0	0
MAX	2420	2420	2420	0	2420	3	2420	39.3	2420	20.1	866.4	1
STDV	948.72	827.40	834.15	0	1062.77	1.07	934.60	12.63	1318.70	11.01	306.11	0.35

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.3.7 XIGALO VILLAGE

Xigalo is a huge village and the chief requested we sampled from more than 20 HHs. In two consecutive days, 25 HHs were successfully sampled. From the 25 HHs, 9 HHs didn't have toilets available. Therefore, no samples could be taken, and the household members practice open defaecation. The highest TC for toilet seat samples was detected at HH23 (111.9 MPN/ 100 ml) and this HH is the only one with *E. coli* detected (1.0 MPN/ 100 ml). The lowest count (2.0 MPN/ 100 ml) was detected from HH13 for the toilet seat sample. The floor samples had the highest TC detected from HH5 (435.2 MPN/ 100 ml) and it's the only HH with *E. coli* detected. The lowest TC counts was detected from HH19. The mothers hand samples had the highest TC (2420 MPN/ 100 ml) was detected from several HHs (HH2, HH4, HH9, HH13, HH20 and HH24) and the lowest count (5.0 MPN/ 100 ml) was detected from HH23. The highest *E. coli* was detected from HH2 (118.7 MPN/ 100 ml) and the lowest count was detected from HH22 and HH23 (1.0 MPN/ 100 ml). The children's' hand samples had the highest TC detected from several HHs (HH3, HH6, HH9, HH20, HH22 and HH24) with 2420 MPN/ 100 ml and the highest *E. coli* was detected from HH6 (5.0 MPN/ 100 ml). There were two kids at HH4 of different age. The

youngest had the most TC (1553.1 MPN/ 100 ml) and the eldest with the lowest count (12.0 MPN/ 100 ml). Both the kid's samples had *E. coli* detected at 1.0 MPN/ 100 ml. The stored water samples had the highest TC detected from HH4, HH8 and HH 17 with 2420 MPN/ 100 ml and *E. coli* was the highest detected at HH20 (2420 MPN/ 100 ml). During the sampling period, only 6 HHs had running tap water. The highest TC was detected at HH23 (145.5 MPN/ 100 ml) and no *E. coli* was detected from all collected samples.

In comparison of the household samples from Xigalo village (Table 4.16), at this village, not all HHs had access to toilets. The HHs with toilets have no *E. coli* detected or the MPN/ 100 ml calculated was very low. The floor samples from the households have low MPN/ 100 ml but no *E. coli*, except for HH5 which had 1 MPN/ 100 ml of *E. coli* detected. This shows that the environment in this study village is faecal free and safe for the children to play. The mothers handwash samples from HH2, HH4, HH9, HH13, HH20 and HH24 have MPN value of 2420 MPN/ 100 ml and only a few have *E. coli* detected. This means that those with no *E. coli*, other bacteria were present but no recent faecal contamination had occurred. In the children handwash samples, the majority had no *E. coli* detected, however a high number of samples TC counts detected. This may be from fomites that they play with around the yard. The most contaminated stored water is from HH20, this may be due to a biofilm created inside the container due to lack of cleaning the container or a contaminated utensil or hands have contaminated the water during handling (Trevett et al., 2005; Momba and Notshe, 2003). Most of the HHs had no running tap water and those HHs with running tap water showed to be safe to drink due to no *E. coli* was detected from the samples (DWAF, 1996). Table 4.16 shows the TC and *E. coli* counts from Xigalo village.

Table 4.16: MPN counts for TC and *E. coli* counts for Xigalo village households

	Ts		F		M_H		C_H		S		T	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
HH1	-	-	0	0	72.7	0	25.9	0	25.9	0	-	-
HH2	-	-	7.5	0	2420.0	118.7	111.9	0	40.8	2.0	-	-
HH3	-	-	18.7	0	7.4	1.0	2420.0	0	99.1	0	-	-
HH4	-	-	1.0	0	2420.0	27.7	1553.1 12.0	1.0 1.0	2420.0	0	-	-
HH5	0	0	435.2	1.0	118.7	0	1.0	0	50.4	0	-	-
HH6	11.0	0	1.0	0	78.5	0	2420.0	5.0	241.3	1.0	-	-
HH7	-	-	0	0	66.3	7.4	248.9	1.0	461.1	0	-	-
HH8	-	-	0	0	13.2	0	3.0	0	2420.0	3.1	-	-
HH9	14.5	0	30.9	0	2420.0	0	2420.0	0	35.0	0	-	-
HH10	52.1	0	0	0	60.2	0	165.0	0	0	0	-	-
HH11	26.5	0	20.3	0	18.9	0	7.3	0	36.8	0	16.0	0
HH12	0	0	0	0	9.7	0	165.0	0	1.0	0	8.6	0
HH13	2.0	0	44.6	0	2420.0	0	98.5	0	344.8	0	3.1	0
HH14	0	0	0	0	344.8	0	0	0	235.9	1.0	-	-
HH15	0	0	3.1	0	115.3	0	204.6	0	435.2	2.0	-	-
HH16	0	0	29.2	0	151.5	0	187.2	0	185.0	0	-	-
HH17	-	-	0	0	9.8	0	0	0	2420.0	2.0	-	-
HH18	-	-	0	0	18.5	0	31.5	0	579.4	1.0	-	-
HH19	0	0	1.0	0	160.7	0	3.1	0	66.3	0	0	0
HH20	0	0	0	0	2420.0	9.7	2420.0	0	272.3	2420.0	-	-
HH21	0	0	0	0	325.5	0	2.0	0	2.0	0	-	-
HH22	-	-	0	0	125.9	1.0	2420.0	0	816.4	0	10.8	0
HH23	111.9	1.0	0	0	5.0	1.0	7.4	0	47.3	2.0	145.5	0
HH24	0	0	42.0	0	2420.0	0	2420.0	0	6.3	0	-	-
HH25	0	0	0	0	0	0	1.0	0	5.2	1.0	-	-
MEAN	13.63	0.06	25.38	0.04	648.90	6.66	667.25	0.31	449.9	97.4 0	30.67	0
MEDIAN	0	0	0	0	115.3	0	105.2	0	99.1	0	9.7	0
MIN	0	0	0	0	0	0	0	0	0	0	0	0
MAX	111.9	1.0	435.2	1.0	2420	118.7	2420	5.0	2420	2420	145.5	0
STDV	29.78	0.25	86.53	0.20	1019.58	24.07	1023.57	1.01	771.32	483.88	56.54	0

Keywords: HH : Household Ts : Toilet seat F : Floor M_H : Mothers handwash
C_H : Children handwash S :Storage water T : Tap water Min : Minimum Max : Maximum
STDV : Standard deviation

4.4 COMPARISON OF WASH SERVICES AT STUDY VIILLAGES

In comparison of the WASH practices from all 7 villages using the mean calculated in Table 4.10 to Table 4.16. Mavambe village households' toilet seat samples had the highest TC counts (846.27 MPN/ 100 ml) detected but no *E. coli* was detected. Phiphidi village households' toilet seat samples had the second highest TC counts (627.88 MPN/ 100 ml) detected and the highest *E. coli* (372.07 MPN/ 100 ml) was detected from toilet

seat samples. Xigalo village households had the lowest TC counts (13.63 MPN/ 100 ml) and *E. coli* (0.06 MPN/ 100 ml) detected from the toilet samples. The floor samples had the highest TC (645.55) detected and no *E. coli* was detected from Mavambe village households. Mphambo village households had the highest *E. coli* (77.54 MPN/ 100 ml) detected from the floor swab samples. The lowest TC count (0.2 MPN/ 100 ml) was detected from Ngudza village households and the lowest *E. coli* count (0.04 MPN/ 100 ml) was detected from Xigalo village households for floor samples. The samples from the mothers' hands had the highest TC (1240.78 MPN/ 100 ml) detected at Phiphidi village households and the lowest *E. coli* count (0.6 MPN/ 100 ml) detected. The samples from children's hand had the most TC (994.92 MPN/ 100 ml) and *E. coli* (276.23 MPN/ 100 ml) detected from Mavambe village households. The lowest TC count (57.65 MPN/ 100 ml) was detected from Dzingahe village households and the lowest *E. coli* count (0.31 MPN/ 100 ml) was detected from Xigalo village households. The water samples from storage containers had the highest TC (1815.65 MPN/ 100 ml) and *E. coli* (325.14 MPN/ 100 ml) detected from Ngudza village households. A total of 4 (57.14%) out of 7 villages didn't have any running tap water during the sampling period. From the 3 villages (Phihphidi, Mavambe, Xigalo) , Phihidi village households had the most TC detected, and the *E. coli* detected is below 1 (0.13 MPN/ 100 ml).

The availability and accessibility of water, sanitation and hygiene plays an important role in preventing the transmission of pathogens (Armah, 2014; Pullan et al., 2014). Proper coverage of WASH services tends to be lower at rural communities when compared to urban area communities (Prűss-Ustűn et al., 2016). In this study, community members tend to report that water is available. However, with observation, taps at households and communal taps have been placed but there is no running water at all times. This is supported by the number of samples taken during sample collection (See section 4.2). According to Service Provision Assessments (SPA), water should be available year-round and be accessible within 500 meters (Cronk et al, 2015). The observation in this study does not comply since water was not available in most households during sample collection. Some communities have adapted to using rainwater as a water source and most do not treat the water. The presence of TC and *E. coli* in samples shows recent faecal contamination. Drinking water with high *E. coli* is not safe for drinking and the

children under 5 are at risk of getting diarrheal infections (DWAF, 1996). Stored water samples from the 7 villages had high TC and *E. coli* counts detected. In 2004, Wright et al. detected a high faecal count from stored samples compared to water source at households in developing countries. Consequently, this compromises the health benefits gained by improved community water supply. Storage period may be one of the factors contributing to *E. coli* being detected in the stored water samples. The longer period of storage may allow more exposure to contamination due to mishandling and improper storage. For example, lids of the storage container may be left open (Hoque et al., 2006) where kids have access to throwing soil or utensils in container or even play with the water while the hands are containing enteric pathogens (Heitzinger et al., 2015). Other factors such as the toilet seat and floor swabs support the recent concern of how fomites are able to harbor *E. coli* which may be of danger for under 5 as they will serve as a potential transmission pathway for diarrheal infections (Bakker et al., 2016; Stauber et al., 2013). Observation and data collected from household members of the 7 different villages, it can be said that intervention is needed at the rural communities referring to the WASH practices such as treating stored water before use, especially drinking water; frequency of washing hands, availability of sanitation for both adults and children under the age of 5 and to highlight the effectiveness of using soap when washing hands.

4.5 PREVALENCE OF PATHOGENIC *E. coli* STRAINS IN HOUSEHOLD SAMPLES

To determine the pathogenic strains of *E. coli* that may be present in this study population sites, the multiplex PCR was performed using primers indicated under Chapter 3. The PCR-products were run in 2.0% agarose gel to distinguish the amplified band sizes to be able to determine the band sizes. A total of 91 (22.86%) samples were positive for *E. coli* under UV-light and were further analysed to determine the pathogenic strains. The band sizes help to categorize the pathogenic strains (Figure 4.4). The different pathogenic strains of *E. coli* were able to be identified from 80 (87.91%) samples, 6 (6.59%) samples were only commensal *E. coli* and 5 (5.49%) samples were not identified. All DEC strains namely EPEC, ETEC, EHEC, EAEC and EIEC were detected. Figure 4.5 is an agarose

gel picture of the loaded samples that allows visualization of the band sizes to determine which strains were present in the samples that were tested.

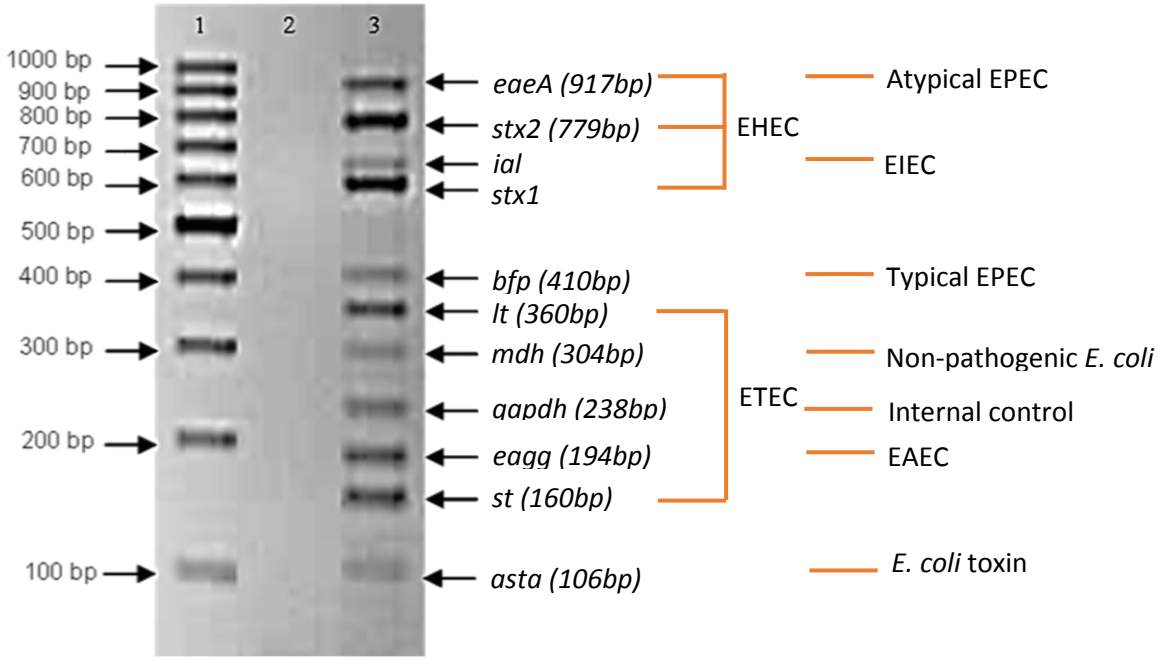
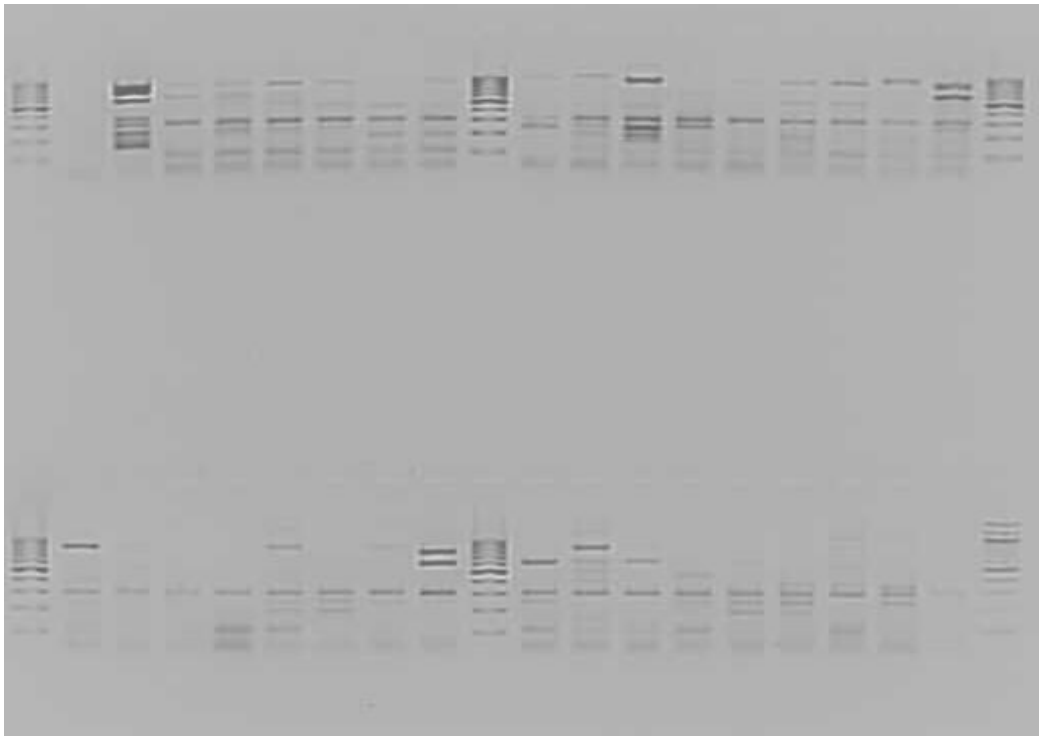


Figure 4.4: Reference agarose gel for determining pathogenic *E. coli* strains (Hoorzook, 2018)



Top wells	Bottom wells
Lane1: DNA ladder	Lane1: DNA ladder
Lane2: NC	Lane2: T16
Lane3: PTC	Lane3: T17
Lane4: T1	Lane4: T18
Lane5: T2	Lane5: T19
Lane6: T3	Lane6: T20
Lane7: T4	Lane7: T21
Lane8: T5	Lane8: T22
Lane9: T6	Lane9: T23
Lane10: DNA ladder	Lane10: DNA ladder
Lane11: T7	Lane11: T24
Lane12: T8	Lane12: T25
Lane13: T9	Lane13: T26
Lane14: T10	Lane14: T27
Lane15: T11	Lane15: T28
Lane16: T12	Lane16: T29
Lane17: T13	Lane17: T30
Lane18: T14	Lane18: T31
Lane19: T15	Lane19: T32
Lane20: DNA ladder	Lane20: DNA ladder

Figure 4.5: Agarose gel picture of *E. coli* positive samples after mPCR

There are different types of diarrheagenic *E. coli* stains, however in this study, the pathotypes of interest were EPEC, EHEC and ETEC strains. The three pathotypes were chosen due to studies that have reported EPEC and ETEC as common pathotypes found in diarrheal cases of children under the age of 5 (Vidal et al., 2016; Nguyen and Sperandio, 2012; Ochoa and Contreras 2011). The EHEC pathotype is due to studies that have reported that it is harboured by cattles ; study population practice animal farming (self- observation). Therefore, only samples that had these 3 interested pathotypes are reported on showing which pathotype was detected from which type of sample. The mothers' hands are most samples (n=29) that had *E. coli* detected and tap water had the least (n=1) detected. Atypical EPEC (aEPEC) is the most prevalent pathogenic *E. coli* that was detected from the samples , whereas typical EPEC (tEPEC) was the least detected. Table 4.17 shows the prevalence of the interested pathogenic *E. coli* strains.

Table 4.17: Prevalence of pathogenic *E. coli* strains

	Pathotypes			
Samples	Atypical EPEC	Typical EPEC	EHEC	ETEC
Ts (n=10)	7 (70%)	0 (0%)	0 (0%)	4 (40%)
F (n=5)	4 (80%)	0 (0%)	0 (0%)	1 (20)
M_H (n=29)	16 (55.17%)	0 (0%)	4 (13.79%)	14 (48.27%)
C_H (n=17)	10 (58.82%)	3 (17.65%)	3 (17.65%)	7 (41.18%)
S (n=27)	17 (62.96%)	2 (7.41%)	2 (7.41%)	11 (40.74%)
T (n=1)	1 (100%)	0 (100%)	0 (0%)	1 (100%)
Total n= 91	55 (60.44%)	5 (5.49%)	9 (9.89%)	38 (41.76%)

Keywords: Ts= Toilet seat F= Floor MH= Mothers hands CH=Childrens hand
S= Storage water T= Tap water

Studies have reported EPEC being the significant cause of diarrhea in low-income countries which is responsible for the high rates reported on infant morbidity and mortality (Rocha et al., 2014; Moreno et al., 2010; Ochoa et al., 2008). Additionally, atypical EPEC is considered as an emerging pathogen in developing countries (Bueris et al., 2007). In this study, high prevalence of EPEC [65.93% (60/91)] was detected. When subtypes, high prevalence of atypical EPEC was detected [60.44% (55/91)] and followed by typical EPEC [5.49% (5/91)]. The results of this study relate to a study done by Zhou et al (2018) in China that detected EPEC to be dominant in DEC infections of children under the age

of 5. Atypical EPEC is becoming more dominant subtype of EPEC, outnumbering tEPEC as the common type being found in studies as a causative diarrheal agent (Araujo et al., 2007; Franzolin et al., 2005).

Previous studies identified ETEC as one of the etiological agents that accounts to an estimated 1.5 million deaths per year in endemic outbreaks of all developing countries (Qadri et al., 2005; Kosek et al., 2003). In this study, prevalence of 41.76% (38/91) was detected which is a bit higher than what has been detected in other developing countries, regardless of the study type and sample type. Studies from Bangladesh, Mexico, Peru, Egypt, Argentina and India indicate the prevalence of 18-38% in symptomatic cases of children under the age of 5 (Hien et al., 2008; Al-Gallas et al., 2007; Bueris et al., 2007; Qadri et al., 2005). This highlights the importance of ETEC as an etiological agent in developing countries (Iseri et al., 2011).

The least detected strain from the three interested pathotype was EHEC [9.49% (9/91)]. This pathogenic strain is reported to be transmitted to humans by shedding the pathogen in their faeces (Rice et al., 2003). This means that the practice of animal farming at households may be of risk to young children under the age of 5, since the pathogen was detected in several samples. A possibility of high prevalence detection in future studies is possible, since some households are still using cow dung for making the stoep and practicing animal farming as food source. EHEC has the most popular serotype known as O157:H7 which is responsible for bloody diarrhoea and hemolytic uremic pathogen worldwide (Nguyen and Sperandio, 2012). A study done by Goldwater and Bettelheim (2012) had reported that no treatment was available for EHEC infections. In addition, a study reported that children on antibiotic therapy for hemorrhagic colitis caused by EHEC pathotype are likely to develop HUS (Tarr et al., 2005; Safdar et al., 2002). This highlights the risk of practicing animal farming at households with young children.

4.6 ASSESMENT OF POTENTIAL TRANSMISSION PATHWAYS IN RURAL HOUSEHOLDS

A study done by Cumming and Cairncross (2016) highlights on the need to understand the dominant environmental exposure of enteric pathogens infections during childhood and infancy particularly with regard to water, sanitation and hygiene which may contribute to an effective intervention in prevention of transmission of diarrheal infections. In this study, the results of mPCR was analysed by checking the common virulence factors that were found in different sample types that were collected in each household. The most common virulence factor detected was *eae* which was found under EPEC pathotype. Table 4.18 shows the *E. coli* virulence genes detected at the village households.

Table 4.18: *Escherichia coli* virulence genes detected at households of the 7 study villages.

VILLAGE NAME	HH#	SAMPLE	EPEC		ETEC	
			Bfp	*Eae*	Lt	St
DZINGAHE	HH3	Ts		√		
		M_H		√	√	
		S		√	√	
	HH5	M_H		√	√	
		S		√		
MPHAMBO4	HH2	Ts		√	√	
		F		√	√	
	HH6	F		√		
		M_H		√		
	HH8	Ts		√	√	
		M_H		√		
NGOVHELA	HH7	C_H	√	√		
		S		√		√
MAVAMBE	HH1	M_H		√		√
		C_H	√	√		
	HH3	M_H		√		
		C_H		√		
PHIPHIDI	HH4	Ts			√	
		S		√	√	
		C_H		√		
	HH8	M_H		√	√	
		C_H		√		
NGUDZA	HH2	F		√		
		M_H		√		
		S		√		
	HH4	M_H		√	√	
		C_H		√	√	
	HH5	C_H			√	
		S		√		√
	HH7	C_H		√	√	
		S				
XIGALO	HH4	M_H		√		√
		Ch1	√	√		
		CH2	√	√		
	HH7	M_H		√	√	√
		C_H			√	
	HH23	Ts		√		
		M_H		√		
		S		√		√

Keywords: Ts : Toilet seat F : Floor MH : Mothers hands CH :Childrens hand
S : Storage water T : Tap water HH : Household
EPEC: Enteropathogenic *Escherichia coli* EHEC : Enterohaemorrhagic *Escherichia coli*

The detected pathotypes were paired in each household, for example, when both water sample and the storage water had the sample pathotype they were grouped together to assess a possible transmission. Most samples are paired under the pathotype EPEC. In the samples which had EHEC detected, no samples could be paired. Table 4.19 shows the matching pathotypes per household in each village as a summary of Table 4.18.

Table 4.19 : Matching pathotypes detected at households

VILLAGE NAME	HH #	PATHOTYPES	
		ETEC	EPEC
DZINGAHE	HH3	M_H + S	Ts + M_H + S
	HH5		M_H + S
MPHAMBO	HH2	Ts + F	Ts + F
	HH6		F + M_H
	HH8		Ts + MH
NGOVHELA	HH7		CH + S
MAVAMBE	HH1		MH + CH
	HH3		MH + CH
PHIPHIDI	HH4	Ts + S	CH + S
	HH8		MH + CH
NGUDZA	HH2		F + MH + S
	HH4	MH + CH	MH + CH
	HH5	CH + S	
	HH7	CH + S	CH + S
XIGALO	HH4		MH + CH1 + CH2
	HH7	MH + CH	
	HH23		Ts + MH + S

Keywords: Ts : Toilet seat F : Floor MH : Mothers hands CH :Childrens hand
S : Storage water T : Tap water HH : Household
EPEC: Enteropathogenic *Escherichia coli* EHEC : Enterohaemorrhagic *Escherichia coli*

Looking at Tables 4.18 and 4.19, the most detected pathotypes was EPEC and almost every household had a hand sample that paired with other samples such as the toilet seat swab, floor swab and stored water sample. The most detected gene of EPEC in this study was *eae*. The *eae* gene was detected in three samples (toilet seat, mothers handwash and storage water sample) from Dzingahe village HH3. This may conclude that hand hygiene in this household played a role in transmitting the diarrheal causative gene that may infect children under the age of 5 at the household. Water sample was contaminated, there is a chance that the children under the age of 5 consume the water and have diarrheal infection.

Ngovhela village HH7 and Ngudza HH7 have samples from a child handwash linking to stored water. This may indicate that the child has access to playing with the stored water. Containers may be left open and the children can put their hands inside the container with water resulting to having contaminated water (Jensen et al., 2002).

The following households have mother and child handwash pairing and having the *eae* genes in both samples: Mavambe village HH1 and HH3; Phiphidi HH8, Ngudza HH4 and Xigalo HH4. This may allow conclusion that direct contacted has occurred between the mother and child, resulting in transmission of the *eae* gene from one individual to another.

The EPEC strains are known to be a significant cause of acute and persistent infant diarrhea in developing countries (Nair et al., 2010; Ochoa et al., 2008). In industrialized countries, EPEC is very limited to occasional outbreaks in nurseries and day-care centres. However, in developing countries it occurs at a high incidence for children under the age of 5 (Tilak and Mudaliar, 2012). The most detected *eae* gene in this study characterizes the subtype of EPEC known as aEPEC (Hernandes et al., 2009). This gene is necessary for the attachment of this strain to the epithelial cells which results in diarrheal outbreaks (Donnenberg et al., 1993). Ocha and Contreras (2011) reported that aEPEC was becoming more prevalent than tEPEC in both developed and developing countries. Furthermore, aEPEC was reported as an important factor for both paediatric endemic diarrhoea and diarrhoea outbreaks (Nakhjavani et al., 2013).

The heat-liable (LT) virulence gene that characterizes the ETEC pathotype (Nataro and Kaper, 1998). In this study, LT was detected in a few households such as Mphambo village HH2 having toilet seat and floor swab having the LT gene; Phiphidi HH4 having the toilet seat swab and storage water and Ngudza having the mother and child handwash samples linking together. This shows a possible transmission by vector or direct contact.

In developing countries and semitropical areas such as Latin America, the Caribbean and Africa, ETEC is known to be a major cause of traveler's diarrhea and the childhood diarrhea pathogen (de la Cabada Bauche and Dupont, 2011). In developed countries diarrheal outbreaks caused by ETEC are rare, however occasional outbreaks have been reported at Norway (MacDonald et al., 2015). The LT gene is closely related to cholera enterotoxin (CT). LT causes diarrheal disease by deregulating host adenylate cyclase

and enhances ETEC adherence to intestinal cells (Wang et al., 2012; Johnson et al., 2009).

In this study, virulence genes that characterize EHEC did not show potential transmission route. However, possibility of transmission may occur in future or in different study sites. A study conducted by Gorham et al. (2017) in the rural settlement of North Cameroon using a structured questionnaire to get background on WASH practices and collecting water samples detected a high prevalence of Shiga toxin producing *E. coli* (stx 1 44.9% and stx 2 31.9%) which indicated the EHEC strain is circulating due to poor WASH practices.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The primary objective of this study was to characterize the *E. coli* strains from rural households of the Vhembe district. To achieve the primary objective, samples were collected and analysed to detect the presence of *E. coli* by using the Colilert® Quanti-tray® /2000. To further characterize the type of pathotype that could be present in the sample, multiplex polymerase chain reaction (m-PCR) was used. The possible transmission route was also assessed by pairing the pathotypes that were detected in each village household with positive *E. coli* looking specifically at the ETEC, EPEC and EHEC pathotypes.

The objectives were achieved with the prevalence of *E. coli* 91 (22.81%) detected from a total of 399 collected samples. The most prevalent pathotype that was circulating within the villages of Vhembe district was EPEC. This pathotype is known as the leading cause of morbidity and mortality of children under the age of 5. The prevalence was very high with atypical EPEC (60.44%) outnumbering the typical EPEC (5.49%) strains. Prevalence of EHEC (9.89%) due to animal farming practices at village households were also seen.

In many households, EPEC has been detected in handwash samples, regardless of the sample coming from the mother or child. This means that hand hygiene practices awareness's are needed in these communities. Availability of running tap water may help reduce transmission of these strains. Overall the study demonstrated the need of WASH awareness to help reduce the prevalence of *E. coli* in the village households and stop fomites playing a role in the transmission pathways for *E. coli*.

Limitation

The study had limitations:

- This was a pilot study to test structured questionnaires and techniques. Therefore, the study did not look at the social aspects, only limited social data were gathered and used to strengthen what needs to be done for a bigger study.

- Getting community leaders to agree in allowing the study to0 be conducted at the village households.
- Once community leaders agreed, the community members were difficult to partake in the study once they heard we needed handwash samples. Some had issues with potential witchcraft practices.
- The lack of running water during sample collection which limited the number of samples collected.

5.2 RECOMMENDATION

In this study, diarrheal outbreaks records from either the households or data from local clinic was not obtained to link the results with diarrheal episodes as well as checking if there were recent diarrheal cases treated for children under the age of 5 at the household.

- The first recommendation for future studies is to gather diarrheal samples and number of diarrheal episodes in study households to make a connection between WASH and diarrhea.
- The second recommendation is to take handwashing samples from before and after use of a proper handwashing event.
- The third recommendation is to use different methods on the detection of enteric pathogens to compare the effectiveness. This study used Colilert® Quanti-tray® /2000 which may have decreased the detected in swabs whereas direct swabbing to selective media such as Hi Chrome may detect higher counts.
- The fourth recommendation due to high prevalence of aEPEC detected in this study, is to check if the circulating strain does not contain any
- antibiotic resistance. Canizalez-Roman et al (2016) conducted a study that detected high level of antibiotic resistance of aEPEC.
- Lastly, we recommend that a bigger study should be conducted with a social scientist involved for social aspects. Statistical analysis for determining the significance of WASH conditions and the prevalence of organisms should be one of the aspects looked into.

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APPENDIX A

CONSENT FORM

PROJECT TITTLE

Characterization of *E. coli* strains from rural communities of Vhembe district, Limpopo, South Africa.

PROJECT IDEA AND CONCEPT

Escherichia coli is a facultative anaerobic bacterium that plays a major role as a member of the gut microbiota. However, pathogenic *E. coli* strains have been identified and classified using their different physiological, antigenic and virulence characteristics. *E. coli* is worldwide used as a faecal indicator organism that validates the assumption of its presence in the environment in results of a recent faecal contamination. It is discovered that it can survive in numerous environments attribute to its genetic diversity. The high genetic diversity improves the species adaptability and resistance to environmental changes. This contributes to *E. coli* being a public health treat due to their ability to survive in different environments such as the soil and sediments that may lead to entering the water bodies. Therefore, it is of importance to investigate the diversity that may be present in communities.

WHY HAVE YOU BEEN INVITED TO PARTICIPATE?

Because you are in the rural community of interest.

WHAT WILL YOUR RESPONSIBILITY BE?

Participation in this study is completely voluntary. You have the right not to participate and provide water or utensil samples for the study and withdraw completely from the study at any time.

PARTICIPANTS CONSENT

I understand that the study is voluntary and that I may withdraw from the study anytime.

Full Name: _____

Signature _____ Date: _____

Thumb

DECLARACTION BY INTERPRETER:

Full Name: _____

I declare that:

- I have interpreted the information using the language of preference
- I am satisfied that the participant understands all aspects of the research, as discussed above.

Signature: _____ Date: _____

STATEMENT BY RESEARCHER

I gave the participant background details at the best of my knowledge

Full Name: _____

Cell number: _____ Student number: _____

Signature: _____ Date: _____

APPENDIX B

SECTION A: HOUSEHOLD PROFILE

Question 1: Household location

District		**Village**	
Municipality			

Question 2: Household members

2.1 What is the ethnicity of the household (HH)?

Ethnic group	**Black**	**Coloured**	**Asian**	**White**	**Other**
Tick					

Other:_____

2.2 What language does the HH predominantly speak?

Language	**Xitsonga**	**Tshivenda**	**Sesotho**	**Other**
Tick				

Other:_____

2.3 How many people live in the HH for the past month/permanent basis? ☐

2.4 Are any children under the age of five? If yes, how old?

Yes	**No**

Age	**0-12**	**12-24**	**24-26**	**36<**
Tick				

Question 3: Settlement

3.1 How many years have you been living in the settlement? ☐

3.2 What type of housing

Categories	**Tick**
Mud hut	
Shack	
RDP house	
Big house (more than 5 units)	

3.3 The type of surrounding

Categories	**Tick**
Stoep made from cow dung	
Stoep made of cement	
Soil	
Grass	
Any animals kept in the yard	
Specify types of animals:	

Additional information:

_____

_____

SECTION B: WATER, SANITATION AND HYGIENE

Question 1: WATER SUPPLY

1.1 What is the main type of water?

Water source	Tick
Piped water into compound	
Public tap/stand pipe	
Tubewell/borehole	
Protected dug well	
Unprotected dug well	
Other	

Other: _____

1.2 Distance from HH to water source

Distance	Tick
At HH	
Less than 200m	
Less than 500m	
1km from HH	
More than 1km	

1.3 Do you store water? If yes, the type of storage container and duration

Water stored	Tick
Yes	
No	

Storage container	Tick
25 L buckets/Jerry can	
50 L Buckets/Jerry can	
100 L drums	
200 L drums	
Other:	

Other:_____

Duration (days)	Tick
1-7	
8-14	
15-21	
22-28	
Other	

Other: _____

1.4 Do you treat the stored water? If yes, how to you treat the water?

Treat water	Tick
Yes	
No	

Type of treatment	Tick
Chlorinating	
Filtration	
Boiling	
Distilling	
Other	

Other:_____

1.5 Do you clean the containers? ☐

1.6 How often do you clean the containers?

Cleaning time	Tick
Once a week	
Twice a week	
Once a month	
Twice a month	
Other	

Other:_____

1.7 What do you use to clean them?

Cleansing material	Tick
Water only	
Jik/Bleach/Domestos	
Dishwasher	
Other	

Other:_____

OBSERVATIONS

Condition of storage container outside	Tick	Condition of storage container inside	Tick	Container mouth size (diameter in cm)	Tick	Lid condition at time of inspection	Tick
Dirty		Dirty		>10cm		Open	
Clean		Clean		10-30cm		Half open	
				>30cm		Closed	

Notes:_____

_____

Question 2: SANITATION

2.1 Do you have a toilet? If yes, which one is the main excreta?

Toilet available	Tick
Yes	
No	

Type of excreta	Tick
Flush	
Ventilated improved pit	
Pit latrine with slab	
Pit latrine without slab	
Other:	

Other:_____

2.2 Do you clean the toilet? If yes, how often do you clean the toilet in a week

Clean toilet	Tick
Yes	
No	

Often cleaned	Tick
Every day	
Every second day	
Three days a week	
Once a week	
Other	

Other:_____

2.3 What do you use to clean the toilet?

Material used	Tick
Water only	
Jeyes	
Jik	
Soap (liquid/powder)	
Other	

Other:_____

2.4 Do you children use the toilet? If so, which age group?

Children use toilet	Tick
Yes	
No	

Age group	Tick
1-2	
3-5	
>5	

If answer on 2.4 is NO, then what is the alternative sanitation used by children?

Alternate toilet	Open space out in bush	Open space in the yard	Children's toilet	Other
Tick				

Other: _____

2.4 OBSERVATIONS

Condition observed at time of inspection	Comment
Flies present	
Odour present	
Faeces visible	
Plastic and feathers	
Estimation distance from HH	

Notes

_____

_____

Question 3: HYGIENE

3.1 Do you wash your hands after visiting the toilet?

Wash hands	Tick
Yes	
No	

3.2 If the 3.1 is YES, do you always wash your hands after visiting the toilet?

Always wash	Tick
Yes	
No	

3.3 What do you use to wash the hands?

Cleansing method	Tick
Water only	
Water with handwashing soap	
Water with dishwashing soap	
Other	

Other: _____

3.3 Where do you get the water?

Water source	Tap	Stored container	Other
Tick			

Other:_____

3.4 How far is the washing point from toilet.

Distance	Next to toilet	500m	Other
Tick			

Other:_____

Question 4: FACILITY COMPOUND

4.1 Do you clean the compound? If so, how often is the compound cleaned?

Clean facility	Tick
Yes	
No	

Often cleaned	Tick
Every day	
Every second day	
Three days a week	
Once a week	
Other	

Other:_____

4.2 OBSERVATIONS

Condition observed at time of inspection	Comment
Dirty (plastic and plastics, long grass)	
Clean (no litter, grass cut)	

Notes_____

_____