Chapter 2

LITERATURE REVIEW

2.1 INTRODUCTION

The United Nations (UN) set a goal in their Millennium Declaration to reduce the amount of people without safe drinking water by half in the year 2015 (UN, 2000). Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Kirkwood, 1998). However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrhoeal diseases (WHO, 2002a; WHO, 2002b). Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation (Kosek *et al.*, 2003; Obi *et al*., 2003; Lin *et al*., 2004; Obi *et al*., 2004).

Political upheaval, high numbers of refugees in some developing countries, and the global appearances of squatter camps and shanty rural towns, which lack proper sanitation and water connections, have contributed to conditions under which disease causing microorganisms can replicate and thrive (Leclerc *et al*., 2002; Sobsey, 2002; Theron and Cloete, 2002). The people most susceptible to waterborne diseases include young children, the elderly, people suffering from malnutrition, pregnant woman, immunocompromised individuals, people suffering from chemical dependencies and persons predisposed to other illnesses like diabetes (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Furthermore, an increasing number of people are becoming susceptible to infections with specific pathogens due to the indiscriminate use of antimicrobial drugs, which have lead to the selection of antibiotic resistant bacteria and drug resistant protozoa (WHO, 2002c; NRC, 2004).

In developing countries, many people are living in rural communities and have to collect their drinking water some distances away from the household and transport it back in various types of containers (Sobsey, 2002). Microbiological contamination of the water may occur between the collection point and the point-of-use in the household due to unhygienic practices causing the water to become a health risk (Sobsey, 2002; Gundry *et al*., 2004; Moyo *et al*., 2004).

To improve and protect the microbiological quality and to reduce the potential health risk of water to these households, intervention strategies is needed that is easy to use, effective, affordable, functional and sustainable (CDC, 2001; Sobsey, 2002). Many different water collection and storage systems have been developed and evaluated in the laboratory and under field conditions (Sobsey, 2002). In addition, a variety of physical and chemical treatment methods to improve the microbiological quality of water are available (Sobsey, 2002). The aim of this study was to improve the microbiological quality of drinking water in rural households by the implementation of intervention strategies which include the use of traditional storage containers as well as the CDC safe storage container, with or without the addition of a sodium hypochlorite solution at the point-of-use.

2.2 WATERBORNE DISEASES

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide (Leclerc *et al*., 2002; Theron and Cloete, 2002). Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites (Table 2.1) that are transmitted by the faecal oral route (Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Waterborne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year (Deetz *et al*., 1984; Leclerc *et al*., 2002; Theron and Cloete, 2002).

Table 2.1 **Waterborne pathogens and their associated diseases (Bifulco *et al.*, 1989; Grabow, 1996; WHO, 1996a; Guerrant, 1997; Leclerc *et al.*, 2002; Theron and Cloete, 2002; Yatsuyanagi *et al.*, 2003; NRC, 2004)**

Pathogen		Diseases
Bacteria	*Campylobacter* spp.	Diarrhoea and acute gastroenteritis
	Enteropathogenic *Escherichia coli*	Diarrhoea
	Escherichia coli O157:H7	Bloody diarrhoea and haemolytic uremic syndrome
	Salmonella spp.	Typhoid fever, diarrhoea
	Shigella spp.	Dysentery, diarrhoea
	Vibrio cholera	Cholera, diarrhoea
	Yersinia spp.	Diarrhoea, gastrointestinal infections
Viruses	Adenoviruses	Diarrhoea, respiratory disease, conjunctivitis
	Astroviruses	Diarrhoea
	Coxsackie viruses (Enterovirus)	Respiratory, meningitis, diabetes, diarrhoea, vomiting, skin rashes
	Echoviruses (Enterovirus)	Meningitis, diarrhoea, myocarditis
	Enteroviruses 68-71	Meningitis, diarrhoea, respiratory diseases, rash, acute enteroviral haemorrhagic conjunctivitis
	Hepatitis viruses (A, E)	Hepatitis (jaundice), gastroenteritis
	Caliciviruses	Diarrhoea, vomiting
	Poliovirus (Enterovirus)	Poliomyelitis
	Rotaviruses	Diarrhoea, vomiting
	Small Round Structured viruses	Diarrhoea, vomiting
Protozoan parasites	*Cryptosporidium parvum*	Cryptosporidiosis, diarrhoea
	Entamoeba hystolytica	Amoebic dysentery
	Giardia	Giardiasis, diarrhoea
Helminths	*Dracunalis medinensis*	Guinea worm (Dracunculiasis)
Emerging opportunistic pathogens	*Actinobacter* spp.	Septicemia, meningitis, endocarditis
	Aeromonas spp.	Diarrhoea, gastroenteritis
	Cyclospora spp.	Diarrhoea, abdominal cramping, fever
	Isospora spp.	Diarrhoea
	Legionella spp.	Legionnaires disease, Pontiac fever
	Microsporidia spp.	Gastrointestinal infections, diarrhoea
	Nontuberculosis *Mycobacteria*	Skin infections, cervical lymphadenitis, nontuberculosis mycobacterium disease
	Pseudomonas aeruginosa	Septicaemia, wound and eye infections

The survival of microorganisms such as bacteria in water environments depends on the presence of nutrients and the water temperature (Edberg *et al*., 2000; Leclerc *et al*., 2002). The infectious dose of some bacteria range between 10^7 to 10^8 cells, with some enteric bacteria able to cause infections at doses as low as 10^1 cells (Edberg *et al*., 2000; Leclerc *et al*., 2002). Viruses cannot replicate outside living cells, but can survive for extended periods in the water (Raphael *et al*., 1985; Leclerc *et al*., 2002). The infectious dose of viruses has been established to be as low as 1 to 10 infectious particles (Raphael *et al*., 1985; Leclerc *et al*., 2002). Enteric protozoa such as *Giardia* and *Cryptosporidium* cannot replicate in water and are highly resistant to most disinfectants and antiseptics used for water treatment (Leclerc *et al*., 2002; Masago *et al*., 2002). The infectious dose for parasites depends on host susceptibility and strain virulence (Leclerc *et al*., 2002; Masago *et al*., 2002). The infectious dose for *Giardia* might be as low as 10 oocysts and for *Cryptosporidium* the presence of 30 oocysts might cause an infection (Leclerc *et al*., 2002; Masago *et al*., 2002; Carlsson, 2003).

Although waterborne pathogens are distributed worldwide, outbreaks of cholera, Hepatitis E and *Dracunculiasis* tend to be subjected to geographical factors (Sacks *et al*., 1986; Alarly and Nadeau, 1990; Kukula *et al*., 1997; Kukula *et al*., 1999; Hänninen *et al*., 2003; Hrudey *et al*., 2003). In the last number of years several outbreaks of pathogenic diseases have appeared that cannot be prevented by traditional water treatment. In 1981 a community waterborne outbreak in Colorado, USA, could be traced to Rotavirus (Hopkins *et al*., 1984). In 1983 and in 1987 two community outbreaks of waterborne *Campylobacter* spp were reported in the USA and Canada, respectively (Sacks *et al*., 1986; Alarly and Nadeau, 1990). In 1993 in Milwaukee, USA, 400 000 people fell ill with 54 deaths from using drinking water that was contaminated by *Cryptosporidium* cysts (Hoxie *et al*., 1997). In 1998, Calici-like viruses in municipal water were responsible for an acute gastroenteritis outbreak in Heinävesi, Finland, affecting approximately 3 000 people (Kukkula *et al*., 1997; Kukkula *et al*., 1999). In 2000, *E. coli* O157:H7 was responsible for 2 300 people falling ill in Walkerton, Canada (Hrudey *et al*., 2003). Recent flooding in Bangladesh has lead to 67 718 reported cases of diarrhoea and 9 people died due to waterborne diseases (International Water Association, 2004)

Consequently, during the past 5 years in rural communities in South Africa, severe outbreaks of cholera in the KwaZulu Natal, Limpopo, Eastern Cape and Mpumalanga have been reported with confirmed cases of mortality (DOH, 2000; DOH, 2002; DOH, 2003; NICD, 2004a; NICD, 2004b). In addition, typhoid cases have been reported in the Limpopo and the Mpumalanga Provinces during 2004 and 2005 with cases of mortality (NICD, 2004b). Rotaviruses have been found during 2005 to be the responsible agent in a large outbreak of watery diarrhoea in the Northern Cape (Laprap, 2005). A report compiled by the Department of Water Affairs and Forestry (DWAF) focussed on the waterborne diseases currently reported in South Africa by the Department of Health (DOH), the National Laboratory Services, DWAF and Rand Water (DWAF, 2005). In summary this report found that records in some provinces are not well kept and although information on waterborne diseases such as Hepatitis A, *Shigella* spp, cholera and typhoid fever is available, it is not reported. The report found that the number of people infected with Hepatitis A in South Africa was 231 in 2003 and 9 503 in 2004 indicating an increase in the rate of infection (DWAF, 2005). The report further showed that during 2003, 761 people and during 2004, 894 people were infected with *Shigella* spp. However, the data for *Shigella* spp are underreported because it is not on the list of notifiable diseases (DWAF, 2005). All these statistics confirm the need for the implementation of a national surveillance system to monitor waterborne disease outbreaks in South Africa.

2.3 THE MICROBIOLOGICAL QUALITY OF WATER

Water supplies in developing countries are devoid of treatment and the communities have to make use of the most convenient supply (Sobsey, 2002; Moyo *et al*., 2004). Many of these water supplies are unprotected and susceptible to external contamination from surface runoff, windblown debris, human and animal faecal pollution and unsanitary collection methods (Chidavaenzi *et al*., 1998; WHO, 2000; Moyo *et al*., 2004).

Detection of each pathogenic microorganism in water is technically difficult, time consuming and expensive and therefore not used for routine water testing procedures (Grabow, 1996). Instead, indicator organisms are routinely used to assess the

microbiological quality of water and provide an easy, rapid and reliable indication of the microbiological quality of water supplies (Grabow, 1996).

In order for a microorganism to be used as an indicator organism of pollution, the following requirements should be fulfilled (Grabow, 1986; WHO, 1993; NRC, 2004):

- The concentration of the indicator microorganism should have a quantitative relationship to risk of disease associated with exposure (ingestion/recreational contact) to the water;
- The indicator organism should be present when pathogens are present;
- The persistence and growth characteristics of the indicator organism should be similar to that of pathogens;
- Indicator organisms should not reproduce in the environment;
- The indicator organism should be present in higher numbers than pathogens in contaminated water;
- The indicator organism should be at least as resistant to adverse environmental conditions, disinfection and other water treatment processes as pathogens;
- The indicator organism should be non-pathogenic and easy to quantify;
- The tests for the indicator organism should be easy, rapid, inexpensive, precise, have adequate sensitivity, quantifiable and applicable to all types of water;
- The indicator organism should be specific to a faecal source or identifiable as to the source of origin of faecal pollution.

Although many microorganisms have desirable features to be considered as possible indicators of faecal pollution, there is no single microorganism that meets all of these requirements (Moe *et al*., 1991; Payment and Franco, 1993; Sobsey *et* al., 1993; Sobsey *et al*., 1995). Several studies have showed the limitations of some of the current indicator organisms, which include the following:

- Indicator organisms may be detected in water samples in the absence of pathogens (Echeverria *et al.*, 1987).
- Some pathogens may be detected in the absence of indicator organisms (Seligman and Reitler, 1965; Thompson, 1981). Echeverria and co-workers

(1987) have showed that *Vibrio cholera* (*V. cholera*) persists in water exposed to solar disinfection well after *E. coli* was inactivated. El-Agaby and co-workers (1988) have showed that potable water supplies in Egypt contained bacteriophages, with zero total and faecal coliform counts, which indicated the possible risk of the presence of human enteric viruses.

- Thompson (1981) has showed that *E. coli* bacteria have a short die-off curve with temperature playing an important role.
- McFeters and co-workers (1986) have showed that injured coliform bacteria can be undetected due to several chemical and physical factors and were unable to grow on commonly used media.
- LeChevallier and co-workers (1996) have showed that improper filtration, temperature, inadequate disinfection and treatment procedures, biofilms and high assimilable organic carbon (AOC) levels, could all be responsible for the regrowth of coliform bacteria in water samples.
- Regli and co-workers (1991) and Hot and co-workers (2003) have showed that the prevalence of viruses in water may differ from that of indicator organisms. Low numbers of viruses are present in water samples compared to indicator organisms, viruses are only excreted for short periods of time while coliform bacteria is excreted continuously, and the structure, size, composition and morphological differences between viruses and bacteria also had an influence on behavioural and survival patterns of these microorganisms (Regli *et al*., 1991; Hot *et al*., 2003).

In spite of the shortcomings of indicator microorganisms, it is better to use a combination of indicator microorganisms to give a more accurate picture of the microbiological quality of water (DWAF, 1996; NRC, 2004). In general, every country has its own set of guidelines for drinking water. However, most of these guidelines are similar for different countries and the same indicator microorganisms to indicate the presence of pathogenic microorganisms are used. The water quality guidelines for South Africa are shown in Table 2.2.

Table 2.2 Microbiological requirements for domestic water in South Africa (Kempster *et al*., 1997; SABS, 2001)

Indicator organism	Units	Allowable compliance
Heterotrophic plate count	Colony forming units.1 ml^{-1}	100
Total coliform bacteria	Colony forming units.100 ml^{-1}	10
Faecal coliform bacteria	Colony forming units.100 ml^{-1}	1
Escherichia coli	Colony forming units.100 ml^{-1}	0
Somatic bacteriophages	Colony forming units.10 ml^{-1}	1
Enteric viruses	Plaque forming units.100 l^{-1}	1
Protozoan parasites (*Giardia/Cryptosporidium)*	Count.100 l^{-1}	0

The most commonly used indicator microorganisms include heterotrophic plate counts, total coliform bacteria, faecal coliform bacteria, *E coli*, faecal enterococci, *C. perfringens* as well as somatic and male specific F-RNA bacteriophages (WHO, 2000). Each of these indicator microorganisms has advantages and disadvantages which will be discussed in more detail in the following sections.

2.3.1 Heterotrophic plate counts

Heterotrophic microorganisms or heterotrophs are naturally present in the environment and can be found in soil, sediment, food, water and in human and animal faeces (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). Broadly defined, heterotrophs include bacteria, yeasts and molds that require organic carbon for growth (WHO, 2002c). Although generally considered harmless, some heterotrophic microorganisms are opportunistic pathogens, which have virulence factors that could affect the health of consumers with suppressed immune systems (Lye and Dufour, 1991; Bartram *et al*., 2003). Heterotrophic microorganisms can also survive in biofilms inside water distribution systems, water reservoirs and inside household storage containers (Momba and Kaleni, 2002; Jagals *et al*., 2003). Therefore, heterotrophic plate counts can also be used to measure the re-growth of organisms that may or may not be a health risk (WHO, 2002c).

Heterotrophic Plate Count, also known as Total or Standard Plate Count includes simple culture based tests intended to recover a wide range of heterotrophic microorganisms from water environments (Bartram *et al*., 2003). Enumeration tests for heterotrophic plate counts are simple and inexpensive giving results within 48 h to 5 days, depending on the method, type of media and the incubation temperature used (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). The pour plate, membrane filtration or spread plate methods are used routinely in various laboratories, with either Yeast-extract agar, Plate Count Agar (PCA), Tryptone Glucose agar or R2A agar, and incubation periods either at room temperature (25ºC) for 5 to 7 days, or at 35°C to 37°C for 48 h (Collin *et al*., 1988; Olson *et al*., 1991; Standard Methods, 1995; Lillis and Bissonnette, 2001). Heterotrophic plate counts alone cannot indicate a health risk and additional studies on the presence of *E. coli* or other faecal specific indicator microorganisms need to be conducted to establish the potential health risk of the water analysed (WHO, 2002c).

2.3.2 Total coliform bacteria

Total coliform bacteria are defined as aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, which ferments lactose and produce gas at 35°C (Standard Methods, 1995). Total coliforms include bacteria of known faecal origin such as *E. coli* as well as bacteria that may not be of faecal origin such as *Klebsiella* spp, *Citrobacter* spp, *Serratia* spp and *Enterobacter* spp which are found in nutrient rich water, soil decaying vegetation and drinking water with relatively high levels of nutrients (Pinfold, 1990; Ramteke *et al*., 1992; WHO, 1996a). The recommended test for the enumeration of total coliforms is membrane filtration using mEndo agar and incubation at 35°C to 37°C for 24 h to produce colonies with golden-green metallic shine (Standard Methods, 1995).

In water quality studies, total coliform bacteria are used as a systems indicator, which provides information on the efficiency of water treatment (Standard Methods, 1995). The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water environments and pathogenic pathogens such as *Salmonella* spp,

Shigella spp, *V. cholera*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *E. coli* may be present (DWAF, 1996; Grabow, 1996). These pathogens and opportunistic microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis to consumers (DWAF, 1996; Grabow, 1996). In particular, individuals who suffer from HIV/AIDS related complications are more at risk of being infected by these microorganisms (DWAF, 1996).

2.3.3 Faecal coliform bacteria

Faecal coliform bacteria are Gram negative bacteria, also known as thermotolerant coliforms or presumptive *E. coli* (Standard Methods, 1995). The faecal coliform group includes other organisms, such as *Klebsiella* spp, *Enterobacter* spp and *Citrobacter* spp, which are not exclusively of faecal origin (Standard Methods, 1995). *Escherichia coli* are specifically of faecal origin from birds, humans and other warm blooded animals (WHO, 1996a; Maier *et al*., 2000). Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faeces (Maier *et al*., 2000).

The recommended test for the enumeration of faecal coliforms is membrane filtration using mFC agar and incubation at 44.5°C for 24 h to produce blue colored colonies (Standard Methods, 1995). Faecal coliforms are generally used to indicate unacceptable microbial water quality and could be used as an indicator in the place of *E. coli* (SABS, 2001). The presence of faecal coliforms in a water sample indicates the possible presence of other pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli, V. cholera*, *Klebsiella* spp and *Campylobacter* spp associated with waterborne diseases (DWAF, 1996). Unfortunately faecal coliform bacteria exhibit species to species variations in their respective stability and resistance to disinfection processes; do not distinguish between faeces of human and animals origin; have low survival rates and have been detected in water sources thought to be free of faecal pollution (Goyal *et al.*, 1979; Fujioka *et al.*, 1988).

2.3.4 *Escherichia coli* bacteria

Globally *E. coli* is used as the preferred indicator of faecal pollution (Edberg *et al.*, 2000). It is a Gram negative bacterium and predominantly an inhabitant of the intestines of warm blooded animals and humans, which is used to indicate recent faecal pollution of water samples (Rice *et al.*, 1990; Rice *et al.*, 1991; WHO, 1996a; Edberg *et al.*, 2000). Confirmation tests for *E. coli* include testing for the presence of the enzyme β-glucuronidase, Gram staining, absence of urease activity, production of acid and gas from lactose and indole production (Mac Faddin, 1980; Rice *et al.*, 1991; Standard Methods, 1995).

Commercially available growth media containing the fluorogenic substrate 4-methyl-umbelliferyl-β-D-glucuronidase (MUG) is used for the isolation and identification of *E. coli* from water samples (Shadix and Rice, 1991; Covert *et al.*, 1992). The *E. coli* bacteria hydrolyse the MUG in the media, which then fluoresces under ultraviolet light (Shadix and Rice, 1991; Covert *et al.*, 1992). However, false negative results on this media have been found due to injured cells, lack of expression of the gene which codes for the enzyme β-glucuronidase by the *E. coli* bacterium isolate, and non-utilization of the MUG reagent in the media by some *E. coli* strains (Chang *et al.*, 1989; Feng *et al.*, 1991; NRC, 2004).

2.3.5 Faecal enterococci bacteria

Faecal enterococci bacteria are found in the genus *Enterococcus* and include species like *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans* and *Enterococcus hirae* (Standard Methods, 1995; WHO, 1996a). The genus *Enterococcus* are differentiated from the genus *Streptococcus* by their ability to grow in 6.5% sodium chloride, pH 9.6, temperatures of 45ºC and their tolerance for adverse growth conditions (Maier *et al.*, 2000). Faecal enterococci are spherical, Gram positive bacteria, which are highly specific for human and animal faecal pollution (Standard Methods, 1995). Most of the species in the *Enterococcus* genus are of faecal origin and is regarded as specific indicators of human faecal pollution, although some species are found in the faeces of animals and plant material (WHO, 1996a).

The recommended test is membrane filtration using mEnterococcus agar and incubation at 35°C to 37°C for 48 h to produce pink colonies (Standard Methods, 1995). Faecal enterococci rarely multiply in polluted water environments and are more resistant to disinfection and treatment processes than the Gram negative faecal coliform bacteria (Standard Methods, 1995). The presence of faecal enterococci in water samples are therefore, an indication of the health risk to waterborne diseases such as meningitis, endocarditis and infections of the eyes, ears and skin (DWAF, 1996; Grabow, 1996).

2.3.6 *Clostridium perfringens* bacteria

Clostridium perfringens is a Gram positive, sulphite reducing anaerobic, rod shaped, spore forming bacteria normally present in faeces of humans and warm blooded animals (Standard Methods, 1995). However, *C. perfringens* are also found in soil and water environments (WHO, 1996a). The spores can survive much longer than coliform bacteria and are highly resistant to water disinfection and treatment processes (Standard Methods, 1995). *Clostridium perfringens* are therefore used as an indicator of faecal pollution to indicate the potential presence of enteric viruses, which may include Enteroviruses, Adenoviruses and Hepatitis viruses as well as the cysts and oocysts of protozoan parasites such as *Giardia*, *Entamoeba* and *Cryptosporidium* in treated drinking water (Payment and Franco, 1993). The enumeration test includes membrane filtration using specific medium (e.g. mCP or *Perfringens* selective OPSP medium with supplements) and incubation 35°C to 37°C for 48 h at in micro-aerophillic conditions to produce black colonies (Standard Methods, 1995).

2.3.7 Bacteriophages

Bacteriophages are viruses, which specifically infect bacteria (Grabow, 2001). Bacteriophages have been suggested as useful indicators to predict the potential occurrence of enteric viruses in water (Grabow *et al*., 1984; Leclerc *et al*., 2000). The survival of bacteriophages is affected by the densities of the host and the bacteriophages in the water sample (Grabow, 2001). In addition, the association of the bacteriophage with solids and the presence of organic matter in the water sample could influence the attachment of the bacteriophages to the host bacterium (Grabow, 2001). Several studies have shown that ultra violet light, temperature, pH of the water, and ion concentrations in the water could

affect the survival of bacteriophages in water (Brion *et al*., 2002; Schaper *et al*., 2002b; Allwood *et al*., 2003). Bacteriophages show higher resistance to environmental stress compared to bacterial indicators such as total coliforms and faecal coliforms and assays for bacteriophages can be conducted quickly, economically and quantitatively (Vaughn and Metcalf, 1975; Havelaar *et al*., 1993). There are several bacteriophages that can be used as indicator organisms which includes the somatic bacteriophages, *Bacteroides fragilis* HSP40 bacteriophages and male specific F-RNA bacteriophages (Grabow, 2001).

2.3.7.1 Somatic bacteriophages

The somatic bacteriophages are a heterogeneous group of organisms that absorbs to bacterial receptors for infection and replication on the cell wall of the laboratory host strain *E. coli* WG5 (Leclerc *et al*., 2000). Somatic bacteriophages are therefore, used as indicators of the potential presence of enteric viruses in water (Grabow, 2001). These bacteriophages can serve as models for the assessment of the behaviour of enteric viruses in water treatment and disinfection processes (Grabow, 2001). The double layer plaque assay is generally used to detect somatic bacteriophages (ISO, 2000; Mooijman *et al*., 2001). However, somatic bacteriophages are not specific to *E. coli*, and may infect and replicate in other species of the Enterobacteriaceae family, which includes the total coliform group (Leclerc *et al*., 2000). Somatic bacteriophages are therefore, not considered a specific indicator for faecal pollution (Leclerc *et al*., 2000).

2.3.7.2 *Bacteroides fragilis* HSP40 bacteriophages

Bacteroides bacteria are present in high numbers in human faeces (Leclerc *et al*., 2000). *Bacteroides* is a strict anaerobic, Gram negative, non-spore forming bacterium which is rapidly inactivated by oxygen levels in water, and needs complex growth media with antibiotics to inhibit the interference from other intestinal microorganisms (Leclerc *et al*., 2000). The *Bacteroides fragilis* HSP40 bacteriophages are a relatively homogeneous group that do not multiply in the environment (Havelaar, 1993; Jagals *et al*., 1995; Puig *et al*., 1999). In some countries, *Bacteroides fragilis* HSP40 bacteriophages is present in relatively low numbers in human faeces (Havelaar, 1993; Jagals *et al*., 1995; Bradley *et al*., 1999; Puig *et al*., 1999). Although this bacteriophage has been shown to be highly

specific for human faeces, tests are complicated and labour intensive (ISO, 2001; Sinton *et al*., 1998).

2.3.7.3 Male specific F-RNA bacteriophages

The male specific F-RNA bacteriophages have small hexagonal capsomers without tails, are approximately 30 nm long with a single RNA genome (Leclerc *et al*., 2000). Male specific F-RNA bacteriophages have been recommended as useful models for monitoring the behaviour of human enteric viruses in water treatment processes because of their size and structure, which are similar to those of the Enteroviruses (Lewis, 1995; Leclerc *et al*., 2000; Grabow, 2001). These bacteriophages are relatively resistant to disinfectants, sunlight, heat- and water treatment processes (Leclerc *et al*., 2000).

Male specific F-RNA bacteriophages specifically attach to the sex pili of the host bacterium [*E. coli* HS(pFamp)R or *Salmonella typhimirium* WG49] in temperatures higher than 30°C (Havelaar and Hogeboom, 1984; Debartolomeis and Cabelli, 1991). The F-pilli are short tube-like protrusions produced by certain bacteria for the transfer of nucleic acid to other bacteria of the same or closely related species and are only produced by the bacteria in the log growth phase which is usually above 30ºC (Havelaar *et al*., 1993; Woody and Cliver, 1995). These bacteriophages are assayed according to an International Standardization Method (ISO, 1995; Mooijman *et al*., 2002). Male specific F-RNA bacteriophages belong to the family *Leviviridae*, which contains two genera, the *Leviviridae* and the *Allolevivirida*e. Both these genera contain distinct subgroups (Watanabe *et al*., 1967; Furuse *et al*., 1979), which is useful in genotyping assays where specific probes are used to distinguish between animal (subgroups I and IV) and human (subgroups II and III) faecal pollution (Osawa *et al*., 1981; Furuse, 1987; Beekwilder *et al*., 1996).

2.4 HUMAN AND ANIMAL FAECAL POLLUTION IN WATER

Water polluted with human and animal faeces may contain potentially pathogenic microorganisms that can cause diseases in consumers (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). The most commonly used faecal indicator microorganisms which include the total coliform

bacteria, thermotolerant coliform bacteria, *E. coli* and faecal enterococci bacteria, are found in both human and animal faeces, but do not differentiate between the origins of faecal pollution (Sinton *et al*., 1998). Human viral pathogens such as Calicivirus, Hepatitis E virus, Reoviruses, Rotaviruses, somatic bacteriophages and male specific F-RNA bacteriophages also infect other animals which can serve as reservoirs (NRC, 2004). Consequently, these animals can be important potential sources of contamination of water sources because the release of microorganisms into aquatic environments by animal hosts could lead to human exposure (NRC, 2004). Poor communities in developing countries share their water sources with cattle and other domestic animals, therefore, the risk of waterborne transmission of zoonotic pathogens to humans, increases (Pournadeali and Tayback, 1980; Meslin, 1997; Sinton *et al*., 1998; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Theron and Cloete, 2002; Hackett and Lappin, 2003). However, water contaminated with human faeces is regarded as a greater risk to human health since it is more likely that it would contain human specific enteric pathogens (Sinton *et al*., 1998). Although various microbial and chemical indicators have been described to identify the origin of faecal pollution in water supplies, different levels of success have been obtained (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

2.4.1 The use of microorganisms to determine the origin of faecal pollution

Several microorganisms have been suggested and tested to distinguish between human and animal faecal pollution in domestic drinking water supplies (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Various factors can have an effect on the specificity of microorganisms that can be used as indicators to determine the origin of faecal pollution, such as: (1) specific bacteria, viruses and protozoan parasites can have multiple hosts (not species specific) (Sinton *et al*., 1998; Gilpen *et al*., 2002); (2) different microorganisms can have similar biochemical reactions in the environment, especially within the same species or genus (Sinton *et al*., 1998; Gilpen *et al*., 2002) and (3) interspecies gene transfer may occur which include small pieces of DNA (eg. plasmids and integrons) and transposons that are carried from one bacteria to another

during sexual and asexual reproduction of bacterial cells (Sinton *et al*., 1998; Gilpen *et al*., 2002).

Microorganisms that have been used in assays to determine the origin of faecal pollution include total coliforms, faecal coliforms, faecal streptococci/enterococci, *Bacteroides* spp, *Bacteroides fragilis* HSP40 bacteriophages, *Pseudonomas aeruginosa*, *Bifidobacterium* spp, *Rhodococcus coprophilus*, male specific F-RNA bacteriophages and specific human enteric viruses (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.1 The ratio of faecal coliform bacteria to faecal streptococci bacteria

The ratio between faecal coliform (FC) and faecal streptococci/enterococci (FS) counts in water is an old method used in several earlier studies to determine the origin of faecal pollution (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). This method is based on the fact that faecal streptococci/enterococci are more abundant in animal faeces than in human faeces while faecal coliforms are more abundant in human faeces than in animal faeces (Sinton *et al*., 1998). The test stipulates that a FC:FS ratio greater than 4 is indicative of human faeces and a FC:FS ration of less than 7 is indicative of animal faecal pollution (Sinton *et al*., 1998).

The limitation of this method is the variable survival rates of some faecal streptococci species, which make this test unreliable (Wheather *et* al., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Sinton and Donnison (1994) have showed that *Enterococcus faecalis* survives longer than *Enterococcus faecium* which survives longer than *Enterococcus durans* which survives longer than *Streptococcus equines* and *Streptococcus bovis* in water environments.

2.4.1.2 The ratio of faecal coliform to total coliform bacteria

Faecal coliforms constitute a subset of total coliforms but grow and ferment lactose with the production of gas and acid at 44.5°C within 24 h (DWAF, 1996). The ratio of faecal coliforms to total coliforms is used to show the percentage of total coliforms that comprises of faecal coliforms which comes from the gut of warm blooded animals (Sinton *et al*., 1998). If the faecal coliforms to total coliforms ration exceeds 0.1 it may suggests the presence of human faecal contamination (Sinton *et al*., 1998). However, this method only shows the possibility of faecal pollution but do not distinguish between human and animal faecal matter (Bartman and Rees, 2000). Another disadvantage of this assay is that some faecal coliforms can multiply in soils in tropical regions and give a false positive result for water pollution (Bartman and Rees, 2000).

2.4.1.3 *Bacteroides* bacteria and *Bacteroides* HSP40 bacteriophages

Bacteroides bacterial species are among the numerous bacteria in human faeces and is also found in low numbers in animal faeces (Maier *et al*., 2000). The bacterium does not survive for long periods outside the human body making the detection of *Bacteroides* difficult (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

However, the *Bacteroidis fragilis* HSP40 bacteriophage strain is a highly specific indicator for human faecal pollution (Grabow, 2001) but is only present in low numbers in human sewage (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). The assays used for the *Bacteroides* bacteria and the *Bacteroides fragilis* HSP40 bacteriophages are expensive, complicated, time consuming and require specialised equipment and skilled labour (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.4 *Pseudomona aeruginosa* bacteria

Pseudonoma aeruginosa bacteria are present in 16% of human adults but occur rarely in lower animals (Sinton *et al*., 1998; Gilpen *et al*., 2002). Unfortunately this bacterium is present in water, soil and sewage samples and can rapidly die-off in aquatic environments and is therefore not a suitable candidate to determine the source of faecal pollution (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.5 *Bifidobacterium* spp

Bifidobacteria spp are strickly anaerobic, Gram positive bacteria present in the gut of humans and animals (Nebra *et al*., 2003). Species such as *Bifidobacteria adolescentis* are specific to humans while species such as *Bifidobacteria thermophilum* are specific to animal faeces (Nebra *et al*., 2003). It is difficult to differentiate between the species based on biochemical and microbiological analysis, which complicates the interpretation of the results (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002).

2.4.1.6 *Rhodococcus coprophilus* bacteria

Rhodococcus coprophilus is a Gram positive, aerobic nocardioform actinomycete which forms a fungus-like mycelium that breaks up into bacteria-like pieces (Sinton *et al*., 1998). The bacteria contaminate grass and when eaten by herbivores these bacteria-like pieces are found in the herbivore dung (Jagals *et al*., 1995; Sinton *et al*., 1998). *Rhodococcus coprophilus* has never been found in human faeces and is therefore used as an indicator of animal faecal pollution (Jaggals *et al*., 1995). The disadvantage of this bacterium is the long growth time of 21 days (Wheather *et al*., 1980; Mara and Oragui, 1985; Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002). Saville and co-workers (2001) have designed a PCR protocol to detect this organism in faecal specimens of animals, which showed potential to be used as a routine laboratory test, but more studies are needed to evaluate this detection technique.

2.4.1.7 Male specific F-RNA bacteriophages

Male specific F-RNA bacteriophages are a homogeneous group of microorganisms belonging to the Family *Leviviridae* (Leclerc *et al*., 2000). This family comprise of four subgroups, those predominating in humans (groups II and III), and those predominating in animals (groups I and IV) (Leclerc *et al*., 2000). Genotyping with specific probes or serotyping with specific antisera can be used to classify male specific F-RNA bacteriophages into one of the four distinct subgroups (Beekwilder *et al*., 1996). The application of these assays makes it possible to distinguish between environmental contaminations from human or animal faecal origin (Beekwilder *et al*., 1996). Grouping is based on serological and physico-chemical properties of each subgroup (Leclerc *et al*., 2000). However, antisera necessary for serotyping are expensive, not readily available and some isolates are difficult to serotype (Furuse *et al*., 1978; Havelaar *et al.*, 1986).

Genotyping of F-RNA bacteriophages are based on molecular techniques, which include specific oligonucleotide probes and nucleic acid hybridisation (Hsu *et al*., 1995; Beekwilder *et al*., 1996). Hsu and co-workers (1995) investigated genotyping with non-radioactive oligonucleotide probes as an alternative to serotyping for the grouping of male specific F-RNA bacteriophages. Beekwilder and co-workers (1996) also described a method which identifies male specific F-RNA bacteriophages quantitatively by a plaque hybridisation assay. Comparison of genotype and serotype results showed that genotyping is a more effective and technically feasible method for the grouping of male specific F-RNA bacteriophages (Hsu *et al*., 1995; Beekwilder *et al*., 1996).

Several studies have suggested that male specific F-RNA bacteriophage subgroup classification, especially subgroups II and III that predominates in human faeces, will not always distinguish between human and pig faecal contamination due to similar dietary and living conditions of pigs as well as exposure of the pigs to human faecal wastes (Osawa *et al*., 1981; Havelaar *et al.*, 1990; Hsu *et al.*, 1995). Consequently, a small percentage of overlapping between the serotypes and their expected animal sources were found with studies showing that animal samples might contain all 4 serotypes (NRC, 2004). In addition, Schaper and co-workers (2002) have showed that human samples contained serotypes I and IV that is mainly associated with animal hosts. Despite these results, various studies have used genotype and serotype analysis

successfully to distinguish between faecal pollution of either human or animal origin (Osawa *et al*., 1981; Havelaar *et al.*, 1990; Hsu *et al.*, 1995; Beekwilder *et al*., 1996; Schaper *et al*., 2002a). Rose and co-workers (1997) have used reverse transcriptase polymerase chain reaction (RT-PCR) to isolate male specific F-RNA bacteriophages from polluted marine waters. However, a study conducted by Schaper and Jofre (2000) comparing RT-PCR followed by southern blotting with plaque hybridisations on male specific F-RNA bacteriophages in sewage samples, indicated that RT-PCR was less sensitive than plaque hybridisation analysis to identify the various F-RNA bacteriophages present in the sewage water samples. Therefore, genotyping of male specific F-RNA bacteriophages using nucleic acid hybridisation seems to be the microbial method of choice to distinguish between human and animal origin of faecal pollution (Schaper and Jofre, 2000).

2.4.1.8 Human enteric viruses

Human enteric viruses associated with waterborne diseases include Adenoviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses (Grabow, 2001). Although excreted in high numbers in faeces by infected individuals, these viruses may be present in low numbers in environmental samples due to dilution (Grabow, 2001). The detection of specific human enteric viruses can be used to confirm the presence of human faecal pollution (Grabow, 2001). Since the detection of viruses is mostly based on molecular techniques, it is not a cost-effective method to include in routine monitoring of water (Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002; NRC, 2004).

Viability of viruses can also not be indicated by molecular techniques and additional cell culture techniques should be included, thereby further increasing the cost and labour (Grabow, 2001; Gilpen *et al*., 2002). However, all viruses are not able to grow in cell cultures (Grabow, 2001). In addition these techniques are labour intensive and skilled personnel are required (Tartera and Jofre, 1987; Gavini *et al*., 1991; Arango and Long, 1998; Sinton *et al*., 1998; Gilpen *et al*., 2002; NRC, 2004).

2.4.1.9 Multiple antibiotic resistant analyses

Resistant bacteria have the ability to survive exposure to antibiotics or disinfectants and through rapid multiplication pass their resistant genes on to other pathogenic as well as to non-pathogenic bacteria (Sergeant, 1999). These antibiotic resistant genes are often associated with transposons (genes that can easily move from one bacterium to another bacterium or by bacteriophages) (Sergeant, 1999). Many bacteria also possess integrons and plasmids, which are small pieces of DNA that accumulate new genes (Sergeant, 1999). Over a period of time, a bacterium can build up a whole range of resistant genes, which is referred to as multiple resistances, which may be passed on within a genus or species to other strains or species (Sergeant, 1999).

The multiple antibiotic analysis (MAR) includes the use of antibiotic resistance patterns of specific microorganisms to differentiate between phenotypes within a specific genus (Krumperman, 1983; Sergeant, 1999). In *E. coli*, *Salmonella* spp and *Shigella* spp, a chromosomal locus is used to determine the intrinsic levels of these organisms for their susceptibility to structurally different antibiotics and disinfectants (Krumperman, 1983). Over expression of this chromosomal locus due to mutations or chemical induction, produces a range of new bacterial phenotypes within a bacterial species (Krumperman, 1983). Bacteria isolated from humans have different MAR profiles than isolates from domestic animals (Krumperman, 1983; Hair *et al*., 1998; Sergeant, 1999). Individual bacterial isolates can be classified into phenotypic groups when the MAR profiles are combined with discriminant statistical analyses (eg. a variation of multivariant analysis of variance) (Krumperman, 1983; Hair *et al*., 1998; Sergeant, 1999). However, MAR studies are time consuming, complicated and expensive. In addition, antibiotic resistance encoded on plasmids can be lost during isolation and there are constant population shifts in antibiotic resistance (Sergeant, 1999).

2.4.1.10 Deoxy Ribonucleic Acid based profiles of microorganisms

The microbial Deoxy Ribonucleic Acid (DNA) based profile approach provide genomic profiles of microbial communities and are used to identify the genus, species, subspecies and strains of microorganisms (Turner *et al*., 1996; Nebra *et al*., 2003). The DNA based profile techniques used to distinguish between microbial genus and species

include ribotyping, Internal Transcribed Spacer-Polymerase Chain Reaction (ITS-PCR), tRNA-PCR and 16S rRNA sequencing (Nebra *et al*., 2003). The DNA based profile techniques used to distinguish between microbial subspecies and strains include Amplified Ribosomal DNA Restriction Analysis (ARDRA), Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR), plasmid or chromosomal restriction-fragment-length-polymorphism (RFLP), Internal Transcribed Spacer-sequencing (ITS-sequencing) and Pulsed Field Gel Electrophoresis (PFGE) methods (Nebra *et al*., 2003). These DNA profiling methods are expensive, labour intensive, require skilled personnel, need specialised equipment and are therefore not used routinely (Turner *et al*., 1996; Nebra *et al*., 2003; Wei *et al*., 2004).

Although several microbiological methods have been proposed and tested to determine the origin of faecal contamination, many of these microorganisms have proved to be difficult to use in routine laboratory procedures because of the type of equipment required, the cost and the skill necessary to perform the assay (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). Genotyping of male specific F-RNA bacteriophages seems to be the most promising microbiological method presently available to distinguish between human and animal faecal pollution of water supplies in rural communities based on results obtained by various studies on animal and human faeces (Osawa *et al*., 1981; Havelaar *et al*., 1990; Hsu *et al*., 1995; Beekwilder *et al*., 1996; Schaper *et al*., 2002a).

2.4.2 The use of chemicals to determine the origin of faecal pollution

Several chemical indicators have been used to identify the source of faecal pollution in various water supplies (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). However, expensive equipment and high concentrations of the chemical in the water sample is needed for accurate identification of the origin of faecal pollution (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

2.4.2.1 Direct chemical indicators

Direct chemical indicators include chemicals present in the faeces, e.g. faecal sterols, uric acid and urobilin (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). The

breakdown products of sterols are stanols (Leeming *et al*., 1996). Leeming and co-workers (1996) have conducted tests on human and animal faeces and especially on sterols and stanols and found that stanols produced in animals were distinctively different than the stanols formed in humans.

Faecal sterol cholesterol is reduced in the gut of humans to coprostanol and in the gut of animals to epicoprostanol (Leeming *et al*., 1996). These compounds can be found in the environment as cholestanol (Leeming *et al*., 1996). Coprostanol is used exclusively as a marker of human faecal pollution (Leeming *et al*., 1996). Plant derived 24-ethylcholestrol is reduced to 24-ethylpicoprostanol in the intestinal tract of herbivores and found in the environment as 24-ethylcholestanol (Leeming *et al*., 1996). The 24-ethylcoprostanol is used as an exclusive marker of animal faecal pollution (Leeming *et al*., 1996).

2.4.2.2 Indirect chemical indicators

Indirect chemical indicators are specific for human faecal contamination (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). These chemicals are associated with faecal discharge in wastewater and septic tank discharges (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003). Fluorescent whitening agents (FWA) and sodium tripolyphosphate (STP) present in washing powders, long chain alkylbenzenes (LAB) present in commercial detergents and polycyclic aromatic hydrocarbons have been used as indirect indicators of human faecal pollution (Sinton *et al*., 1998; Gilpen *et al*., 2002; Gilpen *et al*., 2003).

Although different studies have described the use of these microbiological and chemical indicators, it is apparent that no single chemical determinant could reliably distinguish human from animal faecal contamination (Jagals *et al*., 1995; Sinton *et al*., 1998). It seems that the use of a combination of these determinants may provide the best solution for identifying the origin of faecal pollution in water environments (Jagals *et al*., 1995; Sinton *et al*., 1998).

2.5 SOURCE WATER SUPPLIES

The World Health Organization (WHO) classifies source water supplies as either improved or unimproved (WHO, 2000; Gundry *et al*., 2004). Improved water sources include public standpipes, household connections, boreholes, protected dug wells, protected springs, boreholes and springs connected via a pipe system to a tap, as well as rainwater collection (WHO, 2000; Gundry *et al*., 2004). Unimproved water sources include unprotected wells, unprotected springs, vendor-provided water, rivers as well as tanker truck provision of water (WHO, 2000; Gundry *et al*., 2004).

Several studies carried out in developing countries have determined the microbiological quality of these improved and unimproved water sources and depending on the water source, different results were obtained (Pournadeali and Tayback, 1980; Obi *et al*., 2002; Sobsey *et al*., 2003; Gundry *et al*., 2004; Obi *et al*., 2004). Studies conducted in Iran (Pournadeali and Tayback, 1980) and in northern Sudan (Musa *et al*., 1999) have both showed that water at communal taps were microbiologically of a better quality than untreated irrigation canal water. Contrary to these findings, a study in Burma (Han *et al*., 1989) has showed that tube well and shallow well water supplies were microbiologically of a better quality than municipal tap water and pond water source supplies.

In South Africa, studies in the Limpopo Province (Verweij *et al*., 1991) have showed that communal standpipes were microbiologically less contaminated than borehole and unprotected spring water sources. Another study in the rural Kibi area of the Limpopo Province of South Africa (Davids and Maremane, 1998), have indicated that spring and borehole water sources were microbiologically less contaminated than river water sources.

In addition three recent studies conducted in the Vhembe region of the Limpopo Province in South Africa indicated that rivers and fountains used by rural communities for domestic water were all contaminated by enteric pathogens including *E. coli*, *Plesiomonas shigelloides*, *V. cholera*, *Enterobacter cloacae*, *Shigella* spp, *Salmonella* spp, *Aeromonas hydrophila*, *Aeromonas caviae* and *Campylobacter* spp (Obi *et al*., 2002; Obi *et al*., 2003; Obi *et al*., 2004). *Escherichia coli* isolates obtained from the

different rivers during this study were typed using molecular techniques to determine the presence of virulent genes (Orden *et al*., 1999; Kuhnert *et al*., 2000; Obi *et al*., 2004). Enterotoxigenic *E. coli* isolates (11.8%) contained heat stable and heat labile genes; Shigatoxin producing *E. coli* (4.4%) isolates contained *stx1* and *stx2* genes; Necrotoxigenic *E. coli* (35.6%) contained *cnf1* and *cnf2* genes and Enteropathogenic *E. coli* (34.1%) isolates contained *BfpA* and *EaeA* genes (Obi *et al*., 2004). Necrotoxigenic *E. coli* may play a role in possible zoonotic transmission since it has been shown that human and animal strains share similar serogroups and carry the same genes coding for fimbrial and afimbrial adhesion (Mainil *et al*., 1999). All of these studies indicated that the water sources used by communities in developing countries are microbiologically contaminated and pose a health risk to the consumers (Pournadeali and Tayback, 1980; Obi *et al*., 2002; Sobsey *et al*., 2003; Gundry *et al*., 2004; Obi *et al*., 2004).

2.5.1 Water collection from the source water supply

In most developing countries, women are responsible for the collection of water (Sobsey, 2002). The work involved in fetching the water may differ in each region, it may vary according to the specific season, it depends on the time spent queuing at the source, the distance of the household from the source and the number of household members for which the water must be collected (WHO, 1996b; WHO, 1996c). Water for domestic use is collected either by dipping the container inside the water supply Fig 2.1), collecting rainwater from a roof catchment system (Fig 2.2) or by using different types of pumps connected to the water supply system (Fig 2.3) (Sobsey, 2002). The transportation of the water from the source water supply could be either by a wheelbarrow (Fig 2.4), a donkey cart (Fig 2.5), a motor vehicle (Fig 2.6), using a rolling system (Fig 2.7) or by carrying the container by hand or on the head (Fig 2.8) (CDC, 2001). A common practice often seen in rural areas was the use of leaves or branches with leaves to stop water slopping out during transit in wide-neck storage and transport containers (Fig 2.9) (Sutton and Mubiana, 1989). Consequently, a study by Sutton and Mubiana (1989) has showed that these leaves can be an additional source of coliform bacteria to the drinking water.

Figure 2.1: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Dipping containers inside theprimary water source**

Figure 2.2: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Collecting rain water from the roof of the household**

Figure 2.3: **Water collection by rural people in the Vhembe region of the Limpopo Province of South Africa: Ground water pumped to a communal tap**

Figure 2.4: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a wheelbarrow**

Figure 2.5: Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a donkey cart

Figure 2.6: Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a motor vehicle

Figure 2.7: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of a rolling drum**

Figure 2.8: **Water transportation by rural people in the Vhembe region of the Limpopo Province, South Africa: Use of hands and head**

Figure 2.9: Methods used by rural people in the Vhembe region of the Limpopo Province, South Africa to stop water from spilling while in transport: Use of leaves/branches

Water sources could be some distance away from the households, particularly in rural areas (WHO, 1996b; WHO, 1996c). In studies conducted in Malawi, Kenya, Uganda and Tanzania (Lindskog and Lundqvist, 1989; White *et al*., 2002), it was found that if the water taps were situated closer to the dwelling, the amount of water collected/person/day increases from 9.7 to 15.5 litres. Studies in Mosambique (Cairncross and Cliff, 1987) showed that households collect on average 11.1 litres of water/person/day if the source is less than 300 m from the dwelling, while the households who have to walk more than 4 km collected on average 4.1 litres of water/person/day. In Lesotho, Esrey and co-workers (1992) made a rough estimate of 10 litres of water/person/day based on direct observations of households in rural communities. Studies in rural communities in the Limpopo Province of South Africa (Verweij *et al*., 1991) showed that on average 11.4 litres of water/person/day was collected if the source was close to the household, compared to an average of 8.6 litre of water/person/day if the sources were more than 1 km from the household. The Department of Water Affairs and Forestry in South Africa recommends 25

litre/person/day from a source within a distance of 200 m from the dwelling (DWAF, 1994) and the WHO estimates a minimum of 20 litres of water/person/day is sufficient (WHO, 1996b), while Gleick (1998) recommends 50 litres of water/person/day is efficient. These studies indicated that more water was collected per person per day if the source was closer to the dwelling (White *et al*., 2002; Lindskog and Lundqvist, 1989; Verweij *et al*., 1991).

Very few studies have investigated the microbiological quality of water during collection and transportation. In a study in Rangoon, Burma (Han *et al*., 1989) the water at the source and during collection were analysed and indicated that the faecal coliform counts in the collection samples were higher than the counts in the source water samples (Han *et al*., 1989). The increase in faecal contamination of the water in the collection containers after collection from the source could have been due to unhygienic handling of the water and posed a potential health risk of diseases to the consumers (Sobsey, 2002). In a study in Sri Lanka (Mertens *et al*., 1990) it was found that only 5% of tube well water samples were contaminated if the pump was sterilised prior to collection of the sample compared to 50% if the pump was not sterilised. This implied that the taps were contaminated by hands or animals during collection (Mertens *et al*., 1990).

In another study in rural communities in South Africa (Verweij *et al*., 1991), water samples were taken immediately after collection from communal taps and unprotected borehole and springs. Special precautions were taken to prevent contamination during collection, which included rinsing of the container before filling, using a calabash to scoop water from the source and demarcation of a special area for water collection (Verweij *et al.*, 1991). The results from this study indicated no significant difference between faecal coliform counts at the source and immediately after collection of the water (Verweij *et al.*, 1991). The drawbacks of this study however included the sample size (only 8 households were studied), and inadequate information given regarding who collected the water samples e.g. a technician or a woman from the study households (Verweij *et al*., 1991). A study carried out in a Malawi refugee camp has found that hands are primarily responsible for contamination of collected water because the women rinses the container with small amounts of water using their hands to rub around the container opening in an effort to clean it (Roberts *et al*., 2001). A study by Dunker

(2001) has concluded that rural communities in South Africa spent little time on proper cleaning of the collection containers, especially if water has to be collected more that once a day.

These studies have shown that although the microbiological quality of the source water could be classified as safe for domestic purposes, the water collected by the households from these sources, become contaminated after collection (Sobsey, 2002). The origin of the contamination includes: transport and unhygienic collection and handling practices such as dirty utensils, dirty hands and unclean storage containers (Dunker, 2001; Sobsey, 2002).

2.5.2 Interventions to improve source water supplies

Various intervention strategies to improve the water at the source have been described in the literature (Sobsey, 2002). These improvements can include the building of reservoirs, building protective structures around boreholes and fountains, providing communities with communal taps closer to the dwelling and the treatment of the water source with a disinfectant (Sobsey, 2002). A study in Shangai (Xian-Yu and Hui-Gang, 1982) have showed that continuous chlorination rather than periodic chlorination of wells is more reliable, safes time and labour and showed a reduction in the mortality rates due to enteric diseases from 13.7 per 100 000 people to 1.1 per 100 000 people. However, Jensen and co-workers (2002) have found that in rural areas of Parkistan, where public water supply systems was chlorinated, no reduction in diarrhoea incidence in children from these villages were found compared to diarrhoea incidence in children from villages where the people used untreated ground water supplies.

Different interventions can be implemented to improve the microbiological quality of the source water supply. A study in rural Malawi (Lindskog and Lindskog, 1988) has showed that communal piped water supplies situated within a distance of 400 m from a specific household, improved the microbiological water quality used for drinking because people collected water more often and did not store water which could have become contaminated during storage. A 3 year study by Ghannoum and co-workers (1981) in Libya have showed that the installation of water treatment plants did reduce the incidence of bacillary and amoebic dysentery between 10% and 50%, but not

Giardia infections. However, studies carried out in peri-urban communities in South Africa (Genthe *et al*., 1997; Jagals *et al*., 1999) have showed that although the households were supplied with good quality water complying with South African drinking water specifications (DWAF, 1996), the water in the household storage containers had increased levels of indicator microorganisms. This implied that secondary contamination was introduced after the water collection. Consequently, many of these studies have indicated that improvements at the water source are useless as water is contaminated during collection and storage in households due to poor sanitation practices.

2.6 POINT-OF-USE WATER SUPPLIES IN THE HOUSEHOLD

Source water contamination is likely to have a wide effect on the community because it can introduce new pathogens in the home environment (Sobsey, 2002). However, several studies have reported that the microbiological quality of the water deteriorate after collection, during transport and during storage at the point-of-use due to secondary contamination factors (Rajasekaran *et al*., 1977; El Attar *et al*., 1982; Han *et al*., 1989; Lindskog and Lindskog, 1989; Sandiford *et al*., 1989; Blum *et al*., 1990; Henry and Rahim, 1990; Mertens *et al*., 1990; Pinfold, 1990; Verweij *et al*., 1991; Simango *et al*., 1992; Swerdlow *et al.*, 1992; Shears *et al*., 1995; Kaltenhaler and Drasar, 1996; Genthe *et al*., 1997; Jensen *et al*., 2002; Wright *et al*., 2004). Due to the distances and unavailability of piped water supplies on the dwelling or inside the households in many developing regions of the world, people are forced to store their drinking water (Sobsey, 2002).

Transmission of microorganisms inside the household can occur through several routes (Briscoe, 1984; Roberts *et al*., 2001). The most important transmission routes include water, food, person-to-person contact, unhygienic behaviour (eg. intra-household transmission of faeces), the storage conditions of the water storage containers at the point-of-use and the abstraction conditions of water from the storage container (Briscoe, 1984; Roberts *et al*., 2001). In addition, a number of studies (as shown in Table 2.3) suggested that inadequate storage conditions increased the risk of contamination, which can lead to infectious diseases.

Table 2.3 Summary of studies indicating increased microbiological contamination of stored water and the associated infectious disease risk due to inadequate storage conditions (Sobsey, 2002)

Study Area	Storage container	Storage time	Impact on Microbial quality	Disease Impact	Reference
Bangladesh	Water jars	1-2 days	Increased *Vibrio cholera* presence	Increased cholera rates	Spira *et al.*, 1980
Bahrain	Capped plastic vessels, jars, pitchers	Not reported	*Vibrio cholera* present in stored water and not in source water	Uncertain	Gunn *et al.*, 1981
Sudan	Clay jars (zeers)	2 days – 1 month	Increased faecal indicator bacteria over time, in summer and during dust events	Not measured	Hammad and Dirar, 1982
Egypt	Clay jars (zir)	<1- 3 days	Algae growth and accumulated sediment	Not detected	Miller, 1984
India	Wide mouth vs narrow neck	Not reported	Not measured	Cholera infections fourfold higher in wide mouth storage vessels	Deb *et al.*, 1986
Burma	Buckets	Up to 2 days	Higher levels of faecal coliform bacteria than sources	Not measured	Han *et al.*, 1989
Liberia	Large containers, open or closed	Long time	High levels of enterobacteria in stored samples compared to sources	Not measured	Molbak *et al.*, 1989
Sri Lanka	Earthen pots and others	Not reported	High levels of faecal coliforms in unboiled stored water	Not measured	Mertens *et al.*, 1990
South Africa	Plastic container	4 hours	Higher coliform levels over time	Measured; no effect	Verweij *et al.*, 1991
Africa	Traditional and metal jars	24 hours and more	High total and faecal coliform levels	Not measured	Empereur-Bisonette *et al.*, 1992
Malaysia	Various containers	Not reported	Higher levels of faecal coliforms in unboiled than boiled water	Higher diarrhoea risks from water unboiled or stored in wide neck than narrow neck containers	Knight *et al.*, 1992
Zimbabwe	Covered and uncovered containers	12 hours or more	Higher *E. coli* and *Aeromonas* levels with storage and use	Not measured	Simango *et al.*, 1992
Peru	Wide mouth containers	Not reported	Higher faecal coliform levels in stored waters than source waters	Increased cholera risks	Swerdlow *et al.*, 1992
Bangladesh	Traditional pots	Not reported	Increased faecal coliform levels and antibiotic resistance	Increased faecal coliforms and multiple antibiotic resistant flora	Shears *et al.*, 1995
Trinidad	Open drum, barrel, bucket vs tank or none	Not reported	Increased faecal bacteria levels in open storage vessels than tank	Not measured	Welch *et al.*, 2000

Some studies showed an increase in the number of *V. cholera* in stored water (Spira *et al*., 1980; Gunn *et al*., 1981), while other studies indicated an increase in faecal coliform bacteria and enterobacteriaceae (*E. coli* and *Aeromona* spp) in the stored water (Deb *et al*., 1986; Hammad and Dirar, 1982; Han *et al*., 1989; Molbak *et al*., 1989; Mertens *et al*., 1990; Verweij *et al*., 1991; Empereur-Bisonette *et al*., 1992; Knight *et al*., 1992; Simango *et al*., 1992; Swerdlow *et al*., 1992; Shears *et al*., 1995; Welch *et al*., 2000).

The geometric design of household water storage containers could play an important role in ensuring that the stored drinking water does not become contaminated during storage (Sobsey, 2002). Many different types and sizes of traditional storage containers (Fig 2.10 and 2.11) are commonly used in developing countries such as the nomadic people of Sudan which uses a container made from animal hide called a girba (Musa *et al*., 1999) and communities in Africa which use traditional African clay pots or urns (Patel and Isaacson, 1989; Sutton and Mubiana, 1989; Sobsey, 2002).

Figure 2.10: Typical 25 litre water storage containers and buckets used for point-of-use water storage by rural people in the Vhembe region of the Limpopo Province, South Africa

Figure 2.11: Typical 200 litre water storage container used for point-of-use water storage by rural people in the Vhembe region of the Limpopo Province, South Africa

The material of the container is also important because the chemical material of the storage container could be conducive to bacterial growth and survival of potentially pathogenic microorganisms if contamination of the water occurs. This was shown in a study conducted by Patel and Isaacson (1989), which showed that *Vibrio cholera* 01 survived longer in corroded iron drums than in new iron drums.

The studies in Table 2.3 have showed that water can be stored between 4 h and 1 month at the point-of-use. Faechem and co-workers (1983) indicated that the time of storage was important, with the highest increase in faecal contamination occurring if the storage time was longer than 10 h. Similar observations were reported by other studies, especially if the storage periods were longer than 12 h (Han *et al*., 1989; Mertens *et al*., 1990; Verweij *et al*., 1991; Simango *et al*., 1992 Ahmed and Mahmud, 1998; Momba

and Kaleni, 2002). These studies have showed that the microbiological quality of water deteriorates during long storage times and increased the risk of the transmission of waterborne diseases.

Other factors, which could contribute to the contamination of the water during storage at the point-of-use, included unsanitary and inadequately protected (open, uncovered, poorly covered) containers (Dunker, 2001). Many of the studies listed in Table 2.3 had either uncovered containers, containers with wide openings or buckets, which were used as storage containers. Storage containers need to be covered at all times to prevent flies, animals (Fig 2.12) and small children from touching the water (Fig 2.13) (Sobsey, 2002). It was noted by Jensen *et al*., (2002), that containers with openings of less than 10 cm were less contaminated with coliform bacteria than those with wider openings. Water was poured from these containers, while water was dipped out with hands and utensils where containers with wider openings were used. However, a study by El Attar and co-workers (1982) showed no differences in water quality between containers that were covered versus those that were uncovered.

Figure 2.12: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: animals licking containers while containers are filled with water

Figure 2.13: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: small children touching water storage containers which are not closed

Human faecal pollution from children and adults who do not wash their hands after being to the toilet can contribute to secondary contamination of household stored drinking water (DeWolf Miller, 1984; Dunker, 2001). Several studies have indicated that *E. coli* can survive for 10 min, *Klebsiella* spp for up to 2.5 h (Casewell and Phillips, 1977) and *Shigella sonnei* and faecal enterococci for up to 3 h (Knittle, 1975; Pinfold, 1990) on unwashed hands, which could contaminate food and water in the household.

Finally, inadequate cleaning measures of the storage containers could lead to the formation of biofilms (Fig 2.14) which could harbour potentially pathogenic and opportunistic microorganisms such as total coliforms, faecal coliforms, *E. coli*, somatic and F-RNA bacteriophages, *C. perfringens*, *Salmonella* spp and *Helicobacter pylori* (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002; Sobsey, 2002). These

indicator and pathogenic microorganisms could survive longer than 48 h in biofilms inside household drinking water storage containers and pose a potential risk factor for humans consuming this water (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002; Sobsey, 2002).

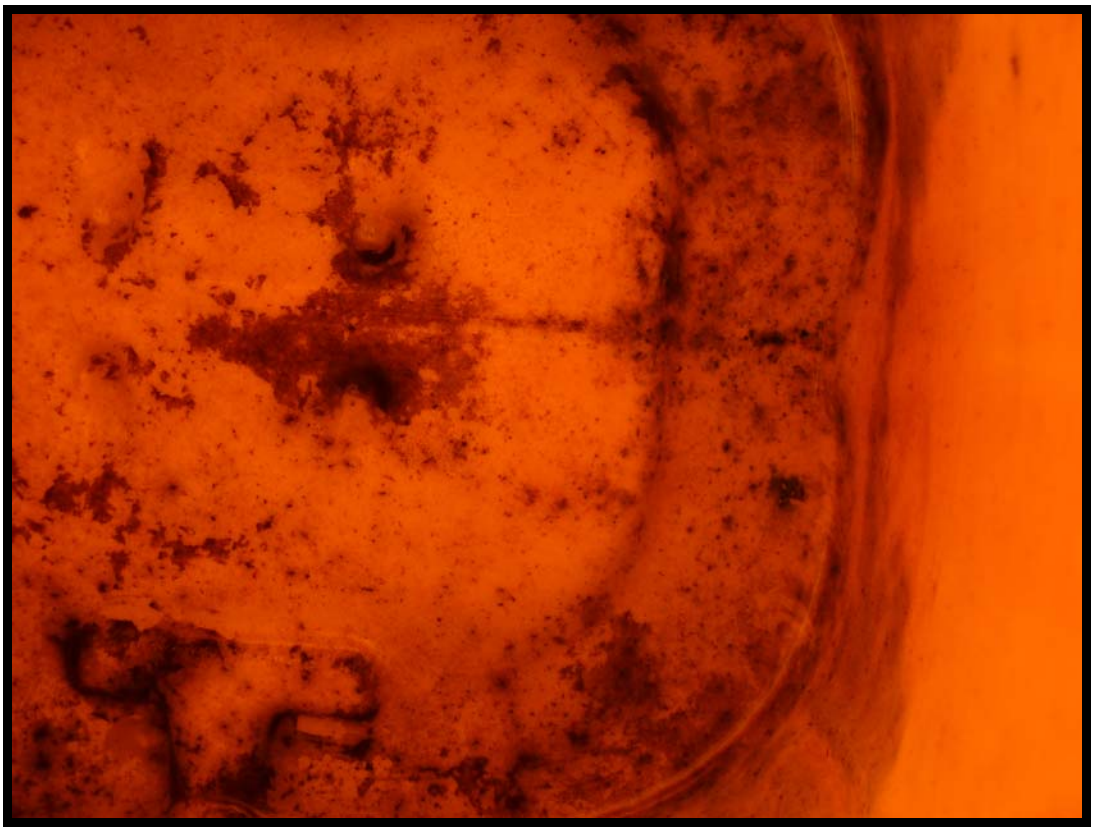

Figure 2.14: Possible contamination route of stored drinking water in rural households in the Vhembe region of the Limpopo Province, South Africa: biofilm formation inside a 25 litre water storage container

The studies mentioned in this section clearly showed that contamination of water occurred during collection and storage at the point-of-use and does contribute to the risk of disease transmission and possibly the spread of anti-microbial resistant genes (Shears *et al*., 1995; Sobsey, 2002). Therefore, the focus must be on point-of-use interventions rather than water source interventions because point-of-use interventions will be more effective in the removal and inactivation of potential disease causing microorganisms introduced during collection and storage inside a family cohort.

2.6.1 Interventions to improve point-of-use water supplies in the household

Point-of-use interventions must improve the water used for drinking at the household level (Sobsey, 2002). This can be achieved by educating household members to improve their hygienic behaviour, by improving the water storage container and by appropriate treatment of the stored water (Dunker, 2001). All of these interventions will be discussed in the following sections.

2.6.1.1 Improving the point-of-use water supply by improving hygienic practices in the household

Basic hygiene practices such as hand washing was shown to be an effective intervention in the reduction of diarrhoea in developing countries (Curtis *et al*., 2000; Trevett *et al*., 2005). A study in Burma (Han and Hlaing, 1989) showed a 30% reduction in diarrhoeal incidence if people washed their hands after defecation, prior to food preparation. Studies in Indonesia (Wilson *et* al., 1991) and Bangladesh (Shahid *et al*., 1996) have showed an 89% and 66% reduction of diarrhoea respectively after hand washing was introduced.

However, factors like the distance from the washing area and the frequency of hand washing do affect the influence of the intervention on the disease outcome (Faechem, 1984; Hoque *et al*., 1995). Faechem (1984) has showed that soap and water together removes 100% of inoculated bacteria while water alone removed less bacteria. Hoque and co-workers (1995) has showed that soap, ash and soil were equally effective hand-washing reagents, however, drying wet hands on clothing, resulted in recontamination of the hands. Proper education should therefore be given to people from rural communities to promote the correct hygiene practices and these communities should be informed on the transmission risk and the causes of waterborne diseases (Dunker, 2001).

2.6.1.2 Improving the point-of-use water supply by using an improved storage container

The United States Centres for Disease Control and Prevention (CDC) and the Pan American Health Organization (PAHO) have studied and reviewed the advantages and disadvantages of different types of water collection and storage containers from studies carried out in various regions of the world. These two organisations have written guidelines for the most desirable container to be used by households for drinking water storage. The guidelines include the following (Mintz *et al*., 1995; Reiff *et al*., 1996; CDC, 2001):

- The container must have a capacity of 15 to 25 litres, rectangular or cylindrical with one or more handles and flat bottoms for portability and ease of storage;
- Should be made of lightweight, oxidation-resistant plastic, such as high-density polyethylene or polypropylene, for durability and shock resistance;
- Should be fitted with a 6 to 9 cm screw-cap opening to facilitate cleaning, but small enough to discourage or prevent the introduction of hands or dipping utensils;
- Should have a durable, protected and preferably easily closed spigot or spout for dispensing water;
- Should have an affixed certificate of approval or authenticity;
- Should be affordable to the user.

Based on these guidelines, the CDC and PAHO designed a 20 litre container to decrease the risk of contamination during storage (Fig 2.15) (Mintz *et al*., 1995; Reiff *et al*., 1996; CDC, 2001; Sobsey, 2002). Together with the use of a sodium hypochlorite solution, this container has proved effective in several studies carried out in different developing countries in Africa, Europe and South America as indicated in Table 2.4 (CDC, 2001; Sobsey, 2002).

Figure 2.15: The CDC safe storage container designed by the CDC and PAHO in the USA for point-of-use treatment

Several of the studies mentioned in Table 2.4, have investigated the reduction of disease, especially the reduction of diarrhoea during the intervention phase (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). The results from all of these studies showed that the diarrhoea incidences were reduced between 20% and 85%, while cholera incidence were reduced by 90% during a cholera outbreak in Madagascar (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). Unfortunately most of these studies have only used *E. coli* and thermotolerant indicator bacteria to assess the microbiological quality of the stored household water (Semenza *et al*., 1998; Quick *et al*., 1999; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). However, none of these studies investigated the survival of pathogenic microorganisms in the CDC safe storage container nor have any study investigated the origin of the faecal contamination in the CDC safe storage container. Although, the incidence of diarrhoea decreased during the intervention studies, little information is available on the origin or the causative microorganism of the diarrhoeal diseases (Sobsey, 2002).

Table 2.4 Efficacy of chlorination and water storage in the CDC safe storage container to disinfect household water, reduce waterborne diseases and improve the microbiological quality of water (Sobsey, 2002)

Location	Water and service level	Treatment	Storage vessel	Disease reduction (%)	Significant microbe decrease?	Intervention	Reference
Uzbekistan	Household On site and off plot Mixed sources	Free chlorine	CDC safe storage container	85% diarrhoea	No But based on small number of samples	Water intervention only	Semenza *et al*., 1998
Guatamala	Street vendor water Off plot Mixed sources	Free chlorine	CDC safe storage container	No data	Yes *E. coli* positive counts decrease from >40 to <10%	Water intervention and Santation and Health intervention	Sobel *et al*., 1998
Guinea-Bisseau	Oral rehydration solution Off plot Ground water or Surface water	Free chlorine	CDC safe storage container	No data	Yes Mean *E. coli* positive counts decrease from 6200 to 0 counts.100 ml^{-1}	Water intervention and Santation and Health intervention	Daniels *et al*., 1999
Bolivia	Household On site Ground water	Electrochemical oxidant (mostly free chlorine)	CDC safe storage container	44% diarrhoea	Yes *E. coli* positive counts decrease from 94 to 22%; median E. coli counts from >20 000 to 0	Water intervention and Sanitation and Health intervention	Quick *et al.*, 1999
Parkistan	Household On site and off plot Municipal	Free chlorine	CDC safe storage container	No data	Yes Thermotolerant coliforms counts decrease by 99.8%	Water intervention and Santation and Health intervention	Luby *et al*., 2001
Madagascar	Household	Free chlorine (traditional vessel)	CDC safe storage container or traditional vessel	90% cholera (during outbreak)	Yes Median *E. coli* positive counts decrease from 13 to 0 counts.100 ml^{-1}	Water intervention and Santation and Health intervention	Mong *et al*., 2001
Zambia	Household Off plot or on site Not reported Ground water	Free chlorine	CDC safe storage container or traditional vessel	48% diarrhoea	Yes *E. coli* positive counts decrease from 95 to 31%	Water intervention and Santation and Health intervention	Quick *et al*., 2002
Bolivia and Bangladesh	Household Onsite Shallow groundwater and municipal water	Free chlorine	CDC safe storage container or traditional vessel	20.8% diarrhoea	Yes *E. coli* counts decreased in intervention households	Water intervention and Health intervention	Sobsey *et al*., 2003

The studies in Table 2.4 have also included additional interventions together with the CDC safe storage container and sodium hypochlorite solution interventions. The additional interventions included sanitation and health interventions where people were informed and educated on hygiene and handling practices (Sobel *et al*., 1998; Daniels *et al*., 1999; Quick *et al*., 1999; Luby *et al*., 2001; Mong *et al*., 2001; Quick *et al*., 2002; Sobsey *et al*., 2003). Generally all of these studies have showed that proper education will influence the compliance with point-of-use interventions (Sobsey, 2002). People should be made aware and educated on the benefit of using interventions to improve the microbiological quality of the household drinking water.

2.6.1.3 Improving the point-of-use water supply by chemical or physical treatment

Several physical and chemical treatments have been developed and tested under various field conditions in several countries as interventions to improve the water at the point-of-use (Sobsey, 2002; Nath *et al*., 2006). However, many of these treatments are not suitable for conditions in rural communities. The various advantages and disadvantages with regards to the use of some of these treatment interventions in rural regions will be discussed in the following sections.

2.6.1.3.1 Physical treatment methods

Physical treatment methods include boiling, heating, settling, filtration and exposure to ultraviolet radiation from sunlight (Gilman and Skillikorn, 1985; Mintz *et al*., 1995; Conroy *et al.,* 1996; CDC, 2001; Sobsey, 2002). Boiling is widely used since it is easy to use and effective in destroying bacteria, viruses and protozoa in all types of water (Sobsey, 2002). However, the collection of firewood is time consuming, could lead to deforestation and is an expensive method for general use (Gilman and Skillicorn, 1985; Barau and Merson, 1992). A further concern is that water is often transferred to storage containers for cooling and thus can become re-contaminated (Sobsey, 2002).

Solar disinfection such as the SOLAIR and SODIS systems, which makes use of plastic water collection bottles which is left in the sun, have been widely tested in rural African communities (Conroy *et al*., 1996; Conroy *et al*., 1999; Meyer and Reed, 2000; Conroy *et al*., 2001). Both these systems inactivates pathogens by disinfecting small quantities

of water for consumption, requires relative clear water (turbidity< 30 NTU) and the effectiveness of the inactivation is dependant on exposure times (Conroy *et al*., 1996; Conroy *et al*., 1999; McGuigan *et al*., 1999; Meyer and Reed, 2000; Conroy *et al*., 2001; Rijal and Fujioka, 2001; Sobsey, 2002; Mascher *et al*., 2003; Oates *et al*., 2003).

Sedimentation and settling is used for very turbid water (Sobsey, 2002). The turbidity is usually due to the presence of sand particles (mud) (Sobsey, 2002). After the water is collected, the container is left undisturbed for a few hours (Sobsey, 2002). The large dense particles (sands and silts) together with large microorganisms will settle out (sediment) due to the effect of gravity (Sobsey, 2002). The upper cleaner water is carefully removed without disturbing the sedimented particles (Sobsey, 2002). Unfortunately sedimentation is not very effective in reducing microbial pathogens in stored household water (Sobsey, 2002).

Filtration is a widely used method to remove particles and some microorganisms from water samples (Potgieter, 1997; Sobsey, 2002). Several types of filter media and filtration processes are available for household treatment of water (Sobsey, 2002). However, the effective removal of microorganisms, the cost and the availability of the filter media in developing countries varies from easy to moderate to difficult (Sobsey, 2002). Granular type of filters include bucket filters, barrel or drum filters and roughing filters and filter cisterns which can rapidly reduce turbidities and enteric bacteria by >90% and larger parasites by >99% efficiency, and enteric viruses by 50% to 90% (Sobsey, 2002; Clasen and Bastable, 2003). Slow sand filters, fibre, fabric and membrane filters, porous ceramic filters and diatomaceous earth filters are alternative filters that have been tested and used for household water treatment in developing countries (Sobsey, 2002; Clasen and Bastable, 2003). Many of these studies have showed to reduce turbidity by 90% and bacteria by 60%, although the cost of the filters is high (Sobsey, 2002; Clasen and Bastable, 2003). A study by Clasen and co-workers (2004) in Bolivia, indicated a reduction of diarrhoea of 70% and a 100% reduction of thermotolerant coliforms in households using ceramic filters compared to control households not using ceramic filters. Unfortunately, little information is available on the effectiveness of these filter systems in the reduction of viruses from household water (Sobsey, 2002).

2.6.1.3.2 Chemical treatment methods

Various chemical methods are available for the treatment of drinking water at the household level and include methods such as coagulation-flocculation, precipitation, adsorption, ion exchange and chemical disinfection with agents such as sodium hypochlorite (Gilman and Skillicorn, 1985; Mintz *et al*., 1995; Conroy *et al.,* 1996; CDC, 2001; Sobsey, 2002). Unfortunately most of these methods are expensive, requires technical skilled persons, regular monitoring, specific materials and the efficacy varies (Sobsey, 2002). Chemical disinfectant agents have proved to be the most successful types of treatment and include free chlorine (which will be discussed in more detail), chloramines, ozone and chlorine dioxide (Sobsey, 2002).

Several factors might play a role in the effectiveness of a chemical disinfectant. These factors include pH, turbidity, temperature, degree of microbial contamination and the contact time of the disinfectant to the water and microorganisms (LeChevallier *et al*., 1981; Reiff *et al*., 1996). According to Reiff and co-workers (1996), an ideal chemical disinfectant should have the following qualities:

- The disinfectant must be reliable and effective in the inactivation of pathogens under a range of conditions likely to be encountered;
- The disinfectant must provide an adequate residual concentration in the water as to assure safe microbial quality throughout the storage period;
- The disinfectant must not introduce nor produce substances in concentrations that may be harmful to health, nor otherwise change the characteristics of the water so as to make it unsuitable for human consumption;
- The disinfectant must be reasonable safe for household storage and use;
- The disinfectant must have an accurate, simple and rapid test for measurement of the disinfectant residual in the water, which can be performed, when required;
- The disinfectant must have an adequate shelf life without significant loss of potency;
- The disinfectant must have a cost that is affordable for the household.

A chemical disinfectant that has been used effectively since 1850, is chlorine (sodium hypochlorite) (White, 1999). During a cholera outbreak in London, chlorine was used

to disinfect water supplies (White, 1999). During the 1890's, Europe used hypochlorites against epidemics of typhoid (White, 1999). Only in the early 20th Century Great Britain and New Jersey City began treatment of potable water supplies on a continuous basis. Since then chlorine has become the most widely used water treatment disinfectant because of its potency, ease of use and cost effectiveness (White, 1999).

Chlorine reacts with water to form hypochlorous acid (HOCl) and hydrochloric acid (HCl) (Carlsson, 2003). The HOCl dissociates further into a hypochlorite ion (OCl^-) and a hydrogen atom (H^+) which are commonly referred to as the free chlorine residual (Carlsson, 2003). The main problem to overcome when chemical treatment is used is the differences in resistance of bacteria, viruses and parasites to these chemical disinfectants (Sobsey, 1989; Sobsey, 2002). The resistance of waterborne microbes to be inactivated by chemical disinfectants is influenced by several factors: (1) their physical status; (2) their physiological status; (3) the presence of microorganisms within microbial aggregates (clumps); and (4) microorganisms embedded within other matrices such as a membrane, a biofilm, another cell, or fecal matter (Sobsey, 1989; Sobsey, 2002). The microorganisms could be protected against chemical disinfectants and by the oxidant demand of the material in which they are located (Sobsey, 1989; Sobsey, 2002). Consequently it has been showed that bacteria are more susceptible to chlorine than viruses or enteric parasites (Sobsey, 1989; Sobsey, 2002).

In bacterial cells the free residual chlorine reacts with various structures on the bacterial cell (Carlsson, 2003). The free residual chlorine can also kill the microorganism by disrupting the metabolism and protein synthesis, to decrease respiration, glucose transport and adenosine triphosphate levels and to cause genetic effects by modification of the purine and pyirimidine basis (LeChevallier and Au, 2004). In viruses the free residual chlorine targets mainly the nucleic acid and do not have a noticeable effect on the protein coat (Carlsson, 2003). This means that viruses containing a protein coat are more resistant to the effect of free residual chlorine (Carlsson, 2003). Free chlorine residual is not very effective against parasites because of the tough outer coat, which makes them very resistant to the action of hypochlorous acid (Carlsson, 2003). Therefore, parasites need to be exposed for longer times to the free chlorine to be inactivated (Venczel, 1997; Carlsson, 2003). Studies have showed that *Giardia lamblia*

cysts are inactivated at 1 $mg.l^{-1}$ free chlorine in water with a pH of 6 to 7 and at temperatures of 5°C only after 1 to 2.5 h (USEPA, 1989) and *Giardia muris* cysts under the same conditions are only inactivated after exposure of 10 h (USEPA, 1989).

Studies have showed that the use of free chlorine residual together with the CDC safe storage container (Table 2.4) has improved the microbiological quality of the water and reduced the prevalence of diarrhoea (Quick *et al*., 1996; Luby *et al*., 1998; Macy and Quick, 1998; Semenza *et al*., 1998; Quick *et al*., 1999). The CDC recommends the addition of either a 0.5% or a 1.0% stabilized concentration of sodium hypochlorite solution to obtain a free chlorine residual between 0.5 and 1.5 $mg.l^{-1}$ after 60 min (WHO, 1996a; CDC, 2001; Dr R Quick, CDC, Atlanta, USA, personal communication). In South Africa, the DOH's recommendations do not specify the free chlorine residual concentration. However, the DOH do recommend the addition of 5 ml of a 3.5% stabilized concentration of sodium hypochlorite solution to a 20 or 25 litre storage container (Appendix C) (Mr H Chabalala, Department of Health, Pretoria, personal communication).

In addition, several studies have showed that the use of some chemical disinfectants resulted in the formation of chemical by-products such as trihalomethanes, haloacetonitriles, chlorinated aldehydes, chlorinated acetones, chlorinated phenols and chlorinated acetic acids (WHO, 1996a; Carlsson, 2003). Some of these by-products are potentially hazardous (carcinogenic and mutagenic) (WHO, 1996a; Carlsson, 2003). However, the health risk posed by these by-products is small in comparison to the health risk caused by waterborne pathogenic and opportunistic microorganisms (WHO, 1996a; Carlsson, 2003).

Although various point-of-use interventions have been proposed, the interventions selected for a particular community must be tailored for the needs of the community and consider the resources available to the community (Nath *et al*., 2006). The ideal solution will be to provide these communities with treated municipal tap water in the dwelling to eliminate storage of the water. However, this is not possible in many developing countries due to economical constraints. In the meantime, interventions at the point-of-use should focus on point-of-use treatments that are cost effective, easy to obtain and easy to use (Sobsey, 2002). The rural communities of the Vhembe region in

South Africa could benefit from point-of-use interventions such as the use of the CDC safe storage container together with a sodium hypochlorite solution to improve the quality of household drinking water (Sobsey, 2002).

2.6.2 Sustainability of point-of-use interventions

The microbiological effectiveness of household interventions at the point-of-use has been indicated by several studies (Sobsey, 2002; Fewtrell *et al*., 2005). However, questions on acceptability, affordability, long term utilization and sustainability of household treatments must still be answered (Nath *et al*., 2006). Only one published study on the sustainability of a point-of-use water treatment system could be obtained from the literature: Conroy and co-workers (1999) found that one year after the completion of a solar disinfection intervention in Masaai communities, almost all households were still using the intervention. The lack of adequate follow up studies on the long term utilization and sustainability of household treatments therefore, needs to be addressed in order to determine the success of point-of-use treatment systems.

2.7 SUMMARY

In South Africa almost 80% of the population are living in rural communities without adequate water and sanitation infrastructures (Statistics South Africa, 2003). Many of the communities have to share water sources with cattle and domestic animals (Dunker, 2001). Communal standpipes provide water on infrequent time schedules and the majority of communal standpipe water is untreated. The Vhembe region is situated in the Limpopo Province of South Africa. The Vhembe region was a former homeland for the Venda people in South Africa before the 1994 elections and known as the Venda homeland. In the Vhembe region, the majority of rural communities are poverty-stricken, lack access to potable water supplies and rely mainly on water sources such as rivers, streams, ponds, springs and boreholes for their daily water needs (Davids and Maremane, 1998; Obi *et al*., 2002; Obi *et al*., 2004). Water from these sources is used directly by the inhabitants and the water sources are faecally contaminated and devoid of treatment (Nevondo and Cloete, 1991; Davids and Maremane, 1998; Obi *et al*., 2002; Obi *et al*., 2004). Consequently, a significant proportion of residents are exposed to potential waterborne diseases (Central Statistics, 1995).

A pilot study, which consisted of a questionnaire survey, was conducted initially to serve as a background study before the initiation of this study. The purpose of the pilot study was to obtain information concerning the baseline microbiological quality of the source water and the storage containers as well as to observe sanitation and hygiene practices of rural people in the Vhembe region. Many of the households in rural areas of South Africa do not have individual connections to treated, piped water supplies. These households typically store water in the household. The stored water is vulnerable to contamination from handling during collection, transport and storage. Results from the pilot study indicated the need for education aimed at diseases associated with polluted water supplies and the improvement in the sanitation and hygienic behaviours of the household members during water collection and storage at the point-of-use. Based on the results obtained from the pilot study it was evident that intervention strategies at the point-of-use in the rural communities were needed as interim solutions to prevent waterborne diseases and improve the microbiological quality of domestic stored drinking water.

The literature study has showed that depending on water collection and storage practices, deterioration of the microbiological quality of the water may occur before the water is actually consumed, mostly due to secondary contamination at the point-of-use. Reviews by Sobsey (2002) and Gundry and co-workers (2004) suggested that more point-of-use intervention field studies must be conducted. The bacteriological evidence in their studies showed that improved storage containers may be effective at reducing microorganisms in stored water if the sources were of good microbiological quality or uncontaminated. However, many of the point-of-use interventions mentioned in the literature review, especially the physical and chemical treatment interventions, are impractical because of costs and sustainability and therefore not suitable for impoverished rural households in developing countries such as South Africa (Sobsey, 2002; Gundry *et al*., 2004). In addition, the literature study has also showed that improving the microbiological quality of water before consumption would reduce diarrhoeal disease together with sanitation and hygiene education (Mertens *et al*., 1990; Hoque *et al*., 1995). However, many of the studies have used indicator microorganisms to assess the effectiveness of interventions. The literature review has indicated that most of the currently used indicator microorganisms used to evaluate the microbiological quality of water have shortcomings and will only give an indication of

the potential risk associated with the transmission of waterborne diseases (Moe *et al*., 1991; Payment and Franco, 1993; Sobsey *et* al., 1993; Sobsey *et al*., 1995).

Several potentially pathogenic microorganisms in water polluted by human and animal faeces could cause diarrhoeal diseases in consumers (Sobsey *et al*., 1993; Gerba *et al*., 1996; Grabow, 1996; Leclerc *et al*., 2002; Theron and Cloete, 2002). Little information on the origin of faecal contamination in the traditional and CDC safe storage containers are presently available. Literature has showed that microbiological and chemical indicators can be used to distinguish between human and animal faecal pollution in water (Jagals *et al*., 1995; Sinton *et al*., 1998). However, no single microorganism or chemical determinant could reliably distinguish human from animal faecal contamination and therefore, the use of a combination of chemical and microbial determinants together may provide the best solution for identifying the origin of faecal pollution at the point-of-use (Jagals *et al*., 1995; Sinton *et al*., 1998).

Consequently, the literature study has indicated that the best interventions available that will be applicable to conditions in rural communities in South Africa included the use of the CDC safe storage container together with a chemical treatment such as sodium hypochlorite solution. The aim of this study was therefore to improve the microbiological quality of drinking water in rural households at the point-of-use by the implementation of intervention strategies which included the use of traditional storage containers as well as the CDC water storage container, with or without the addition of a sodium hypochlorite solution. The results obtained from this study would be used to provide information to the DOH and DWAF, which can be used in future water and health policy formulations to prevent waterborne outbreaks in these rural communities.