Borehole water: a potential health risk to rural communities in South Africa

S. Taonameso, L. S. Mudau, A. N. Traoré and N. Potgieter

ABSTRACT

Sporadic outbreaks of diarrhoea in children in the Vhembe rural areas could be an indication of contamination in drinking water sources. In areas where improved water sources are used, not all rural households experience the benefits of these improved water sources. Water samples were collected from boreholes in three wards in the Vhembe District to determine microbiological risks over a 5-month period. A Water Point Mapping tool was used to indicate the borehole distribution. Water samples were taken from each functional borehole and analysed for total coliform and *Escherichia coli* counts, electrical conductivity, pH and temperature. A multiplex PCR protocol was used for identification of pathogenic *E. coli*. A total of 125 boreholes were identified of which only 12 were functional. Seven boreholes tested positive for total coliforms and *E. coli* counts. Four boreholes (33.3%) tested positive for diarrhoeagenic *E. coli*. Fifty-eight percent (58%) of water samples were without health risks, 17% were low risk and 25% could cause infection according to the South African water quality standards. This study indicated the importance of the role of the Municipalities and the maintenance plans that need to ensure that all boreholes are functional and provide safe drinking water to the rural communities.

Key words | borehole water, diarrhoeagenic *Escherichia coli*, drinking water quality, health risk assessment, PCR, rural communities

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INTRODUCTION

Poor water quality is known to be a major cause of disease outbreaks and impact on health (Hunter *et al.* 2010; Galadima *et al.* 2011). The Joint Monitoring Programme (JMP) estimated that 1.8 million people globally use a source of drinking water that is faecally contaminated (WHO/UNICEF 2014). It further indicated that 1.1 billion people drink water that is at least of 'moderate' risk (>10 cfu/100 ml *Escherichia coli*). Data from national randomized studies suggest that 10% of improved drinking water sources may be 'high' risk containing at least 100 CFU/100 ml sample (WHO/UNICEF 2014). In South Africa, contamination of improved water sources is usually a result of poor operation and maintenance that cause frequent failures and contamination (Rietveldt *et al.* 2009). Consequently, this causes concerns about the safety of improved sources used in communities especially in rural areas where Municipalities are failing to maintain good water quality performance (NEPAD 2015). The emergence of communicable disease outbreaks related to water in most parts of South Africa's rural areas remains a major challenge, even though there is a vast improvement of infrastructure dedicated to accessing safe drinking water to these communities (UNICEF 2009). The recent outbreak of cholera (December

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2008–March 2009) in the Mpumalanga Province of South Africa was due to unsafe water sources (Sigudu *et al.* 2015). Bezuidenhout (2013) indicated the risks of drinking water from boreholes that do not always provide safe water and have bacteria and chemical contaminants in the North West Province of South Africa. Studies conducted in the Tshitale-Hlanganani area of the Vhembe district in the Limpopo Province of South Africa also detected contamination and risks of *E. coli* in borehole water used by communities for drinking purposes (Potgieter *et al.* 2006). Therefore, it is recommended to use available water guidelines to assess the compliance for both physical and microbiological parameters to avert outbreaks of waterborne diseases. Despite risk assessment of drinking water that are performed every year, rural Municipalities including the Vhembe district are still lagging behind in terms of providing rural communities with safe water (DWS 2016). This study aimed to determine the safety of drinking water from boreholes in rural areas by assessing the microbiological and physical quality of the functional borehole water sources as an indispensable step to assess the health risks faced by the communities.

METHOD

Study site

The study was conducted in the Thulamela local Municipality [Wards 15, 18 and 19] in the Vhembe District, Limpopo Province of South Africa between 2014 and 2015. These three wards cover a total surface area of 75.48 km^2 comprising 21 villages that have approximately 48,448 inhabitants, as shown in Figure 1 (Statistics South Africa 2012). A total of 125 boreholes were available in the area during the study period (Figure 1). The borehole water that is used in the three study wards in the Thulamela Local Municipality is pumped by electrical-, diesel-, hand- and wind-powered mono pumps.

Questionnaire survey

A questionnaire (Figure 2) was completed for each of the 125 borehole water points that were visited in the whole study area and documented a range of characteristics which included: type of water point and pump; whether

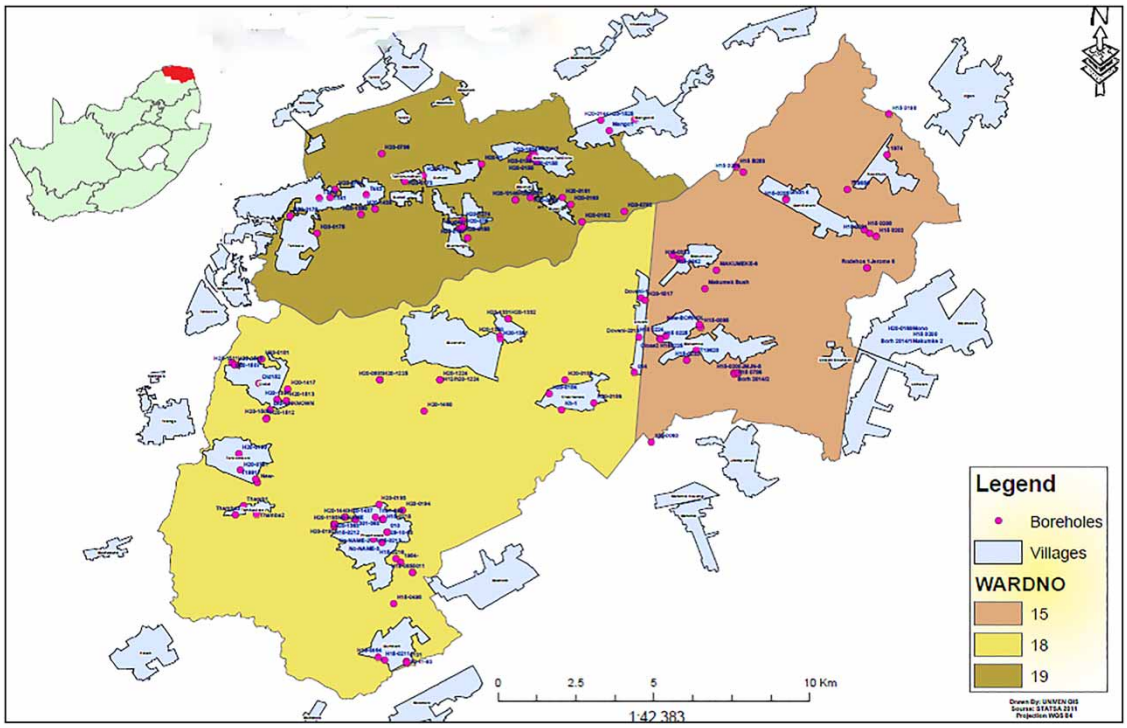

Figure 1 | The ward boundaries of the Thulamela Local Municipality's wards 15, 18 and 19 indicating Municipal boreholes surveyed.

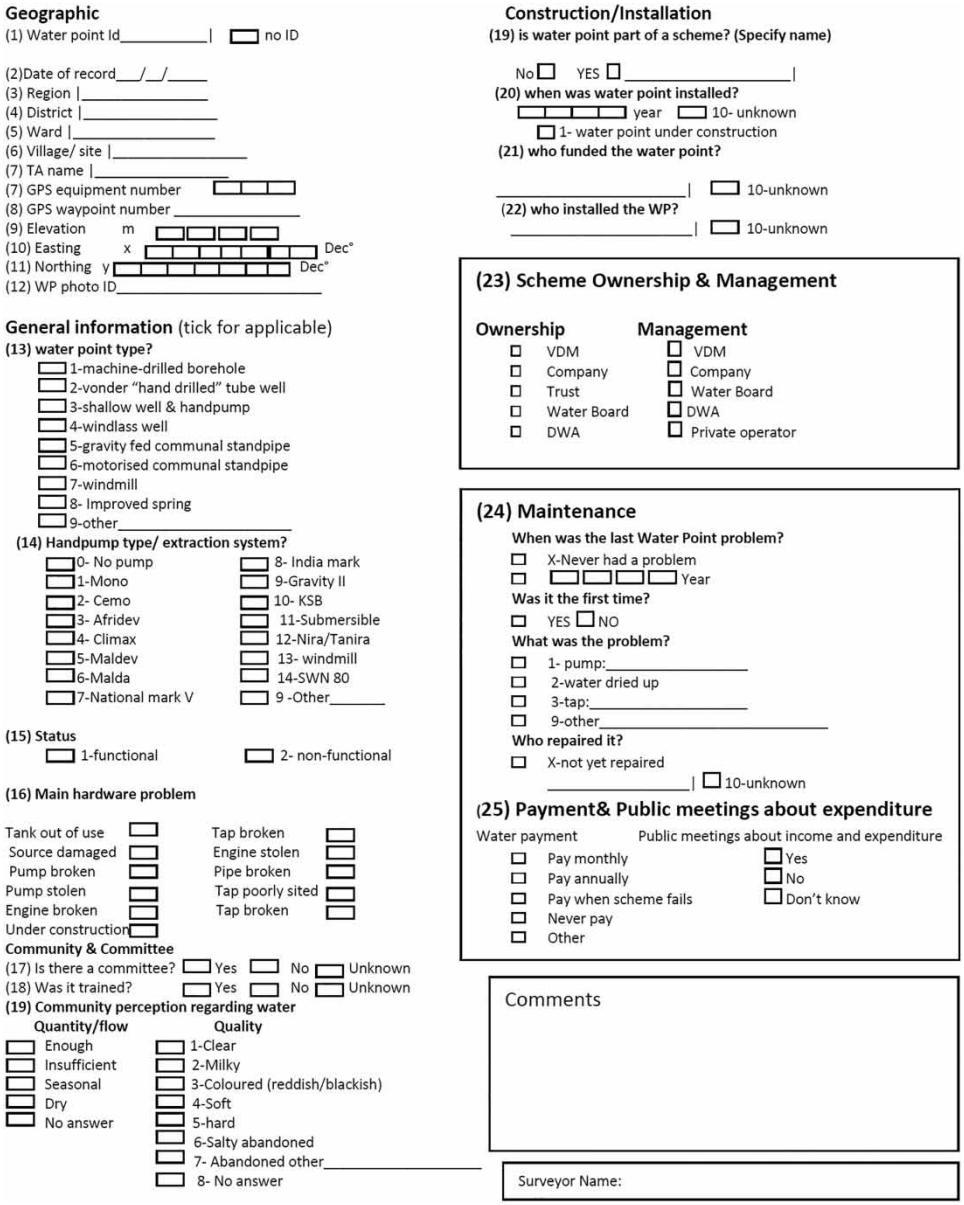

Figure 2 | The survey questionnaire used in the study.

the water point is functional or not, and reasons why it does not work; year of construction; funding and implementing agent (ownership); perception of water quality and quantity; and community training. The questionnaire was completed at the water point and questions were directed to the Headman or his assistant who was knowledgeable about borehole operations and services. The Surveyor filled in the responses from the Tribal Authority's designated assistant/aid. Field verification was undertaken and, where additional information was needed, the other village elders with knowledge regarding the questions were consulted. The questionnaire survey was used in conjunction with a Water Point Mapping tool to indicate borehole distribution and functionality. This was a descriptive survey and it used qualitative methods to assess health risk factors relating to the borehole water used in the three study wards. This study used primary data from the respondents and direct observation of the boreholes and the environment.

Physical quality assessment

A HM-digital meter, COM-100 (HM Digital, Culver City, CA, USA) was used to measure pH, electrical conductivity (EC) and temperature on-site.

Water quality assessment

Water samples were collected from all the functional boreholes using 500 ml sterile sampling bottles. The samples were stored in cooler boxes with ice packs and transported to the laboratory within 6 hours. Total coliforms and *E. coli* were analyzed using the Colilert-18 method (IDEXX 2001). Samples were analyzed according to the manufacturer's instructions (IDEXX Laboratories, Westbrook, Maine, USA). The trays were incubated at 37 °C for 18 to 24 hours. The yellow wells were identified as positive for total coliforms and all fluorescent yellow wells were identified as positive for the presence of *E. coli*. The Most Probable Number (MPN) tables provided by the manufacturer were used to determine the count per 100 ml for each sample. The SANS-241 (SABS 2011) guidelines were used to assess microbiological and physical water quality and health risk.

Molecular identification of *E. coli* genes

A total of 2 ml of media was taken from 10 positive *E. coli* wells of each positive Colilert® Quanti-Tray/2000 with a Neomedic disposal syringe with mounted needle (Kendon Medical Supplies) and aliquoted into 2 ml sterile Eppendorf tubes (Hamburg, Germany). The tubes were centrifuged for 2 minutes at 13,000 × g (Thermo-scientific, Carlsbad, California, United States) to pellet the cells, and the supernatant was discarded. DNA was extracted from the collected bacterial cells using the adapted version guanidinium thiocyanate/silica method reported by Boom *et al.* (1990) and an adaptation of spin methodology as reported by Borodina *et al.* (2003). The DNA extraction method included the addition of 250 µl 100% ethanol (Merck; Kenilworth, NJ, USA) as a lysis buffer to enhance the binding of DNA to the celite. The celite containing the bound DNA were loaded into a DNA binding membrane (Borodina *et al.* 2003) in the spin columns. DNA was eluted from the celite with 100 µl Qiagen elution buffer (Qiagen®; Helden, Germany) (Omar *et al.* 2010). The extracted DNA was used as a template in all PCR reactions (Omar *et al.* 2010).

In order to assess the health implications of borehole water used, molecular identification of *E. coli* pathogenic strains was undertaken on all *E. coli* positive samples using the multiplex PCR method published by Omar & Barnard (2014). All m-PCR reactions were performed in a Biorad MycyclerTM Thermal cycler in a total volume of 20 µl. The primers used are shown in Table 1. Each reaction consisted of 1X Qiagen® PCR multiplex mix (containing HotstartTaq® DNA polymerase, m-PCR buffer, and dNTP mix) (Qiagen, Helden, Germany); 2 µl of the primer mixture [0.1 lM of *mdh* and *lt* primers, 0.2 lM of *ial*, *eagg*, *astA*, *bfp* and *gapdh* primers (F and R), 0.3 lM of *eaeA* and stx2 primers (F and R), 0.5 lM of *stx1* and *stx2* primers], 2 µl of DNA sample, 1 µl of *gapdh* cDNA, hotstart *Taq* polymerase and 5ul of PCR grade water. The reactions were subjected to a denaturing step at 95 °C for 15 minutes, 35 cycles that consisted of denaturation at 95 °C for 45 seconds, annealing at 55 °C for 45 seconds, extension at 68 °C for 2 minutes and final elongation at 72 °C for 5 minutes (Omar & Barnard 2014). Positive and negative controls were also included in all PCR reactions The positive control contained a mixture of the five pathogenic *E. coli* and commensal *E. coli* DNA. The negative control contained PCR grade water.

Bacterial DNA was analysed using a 2.5% (w/v) agarose horizontal agarose slab gel in TAE buffer (40 $mmol^{-1}$ Tris-acetate; 2 $mmol^{-1}$ EDTA, pH 8.3) with 0.5 μgml^{-1} Ethidium Bromide. DNA was electrophoresed for 1–2 hours in the electric field strength of the 8 Vcm^{-1} gel. DNA was visualized using UV light (Gene Genius Bio Imaging system, Vacutec®, USA) and the relative sizes of the DNA fragments were estimated by comparing their electrophoretic mobility with that of the standards run with the samples on each gel, either 1 kB or 100 bp markers (Fermentas®, Baden Wurttemberg, Germany).

RESULTS

From 125 boreholes identified in the area, only 12 boreholes were found to be functional (10%). Seven (58%) of the 12

Table 1 | Primers used for the identification of *E. coli* pathogenic strains (Omar & Barnard 2014)

Pathogen	Primer	Sequence	Size (bp)	Reference
E. coli	*Mdh (F)*	GGT ATG GAT CGT TCC GAC CT	300	Tarr *et al.* (2002)
	Mdh (R)	GGCAGA ATG GTA ACA CCA GAG T		
EIEC	*ial (F)*	GGT ATG ATG ATG ATG AGT GGC	630	López Saucedo *et al.* (2003)
	ial (R)	GGA GGC CAA CAA TTA TTT CC		
EHEC/Atypical EPEC	*eaeA (F)*	CTG AAC GGC GAT TAC GCG AA	917	Aranda *et al.* (2004)
	eaeA (R)	GAC GAT ACG ATC CAG		
Typical EPEC	*bfpA (F)*	AAT GGT GCT TGC GCT TGC TGC	410	Aranda *et al.* (2004)
	bfpM (R)	TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT		
EAEC	*stxl (F)*	ACA CTG GAT GAT CTC AGT GG	614	Moses *et al.* (2006)
	stxl (R)	CTG AAT CCC CCT CCA TTA TG		
	stx2 (F)	CCA TGA CAA CGG ACA GCA GTT	779	
	stx2 (R)	CCT GTC AAC TGA GCA CTT TG		
ETEC	*It (F)*	GGC GAC AGA TTA TAC CGT GC	360	Pass *et al.* (2000)
	It (R)	CGG TCT CTA TAT TCC CTG TT		
	ST (F)	TTT CCC CTC TTT TAG TCA GTC AAC TG	160	
	ST (R)	GGC AGG ATT ACA ACA AAG TTC ACA		

F = forward.
R = reverse.

boreholes were positive for total coliform counts and four (33%) were positive for *E. coli* counts. Table 2 indicates that 58% of water samples were without health risks (0 count/100 ml for both total coliforms and *E. coli*), 17% were of low risk (<10 count/100 ml for total coliforms and 1 count/10–0 ml for *E.* coli) and 25% could cause risk of infection (>100 counts/100 ml for total coliforms and >10 counts/100 ml for *E. coli*). Measurements of pH and

Table 2 | Risk assessment of borehole water quality

			Microbiological water quality		Physical water quality		
Ward	Village	Borehole	Total coliform MPN/100 ml (*colour coding)	*E. coli* MPN/100 ml (*colour coding)	Temp (°C)	Electrical conductivity (μS/m)	pH
15	Mavambe	Borehole 1	<1 (Blue)	<1 (Blue)	21	87.2	8.76
		Borehole 2	<1 (Blue)	<1 (Blue)	21	87.2	8.76
		Borehole 3	<1 (Blue)	<1 (Blue)	23	86.0	8.65
18	Mulenzhe	Borehole 4	<1 (Blue)	<1 (Blue)	20	136.4	7.64
		Borehole 5	>2419.6 (Purple)	<1 (Blue)	20	720	7.64
	Dididi	Borehole 6	5.2 (Green)	2.0 (Red)	16	1092	8.09
	Doveni	Borehole 7	>2419.6 (Purple)	1.0 (Red)	20	865	7.89
		Borehole 8	>2419.6 (Purple)	2.0 (Red)	21	865	7.85
	Phaphazela	Borehole 9	21.6 (Yellow)	<1 (Blue)	20	941	7.92
19	Makhuvha A	Borehole 10	9.7 (Green)	<1 (Blue)	21	793	8.89
		Borehole 11	14.8 (Yellow)	6.3 (Red)	20	798	8.03
	Budeli	Borehole 12	<1 (Blue)	<1 (Blue)	20	92.8	8.70

*Colour coding:
Blue (Class 0) - ideal water.
Green (Class I) – good water.
Yellow (Class II) – marginal water.
Red (Class III) – poor water.
Purple (Class IV) – completely unacceptable/unsafe.

EC for all functional boreholes were within the safe risk guidelines (Table 2). Ward 18 was found to be the ward with the highest risk of infection as compared to the other two wards. The safest ward was Ward 15 with no detectable health risks.

Table 3 indicates the pathogenic *E. coli* strains that were identified from the four *E. coli* positive samples found in Wards 18 and 19. Three *E. coli* strains were identified as harbouring genes of Shiga-like toxins, enterotoxins (heat-stable and heat-labile toxins) and an enterocyte effacing factor (also known as intimin).

DISCUSSION

Rural and low-economic communities are known to use water of poor water quality and this is recognized internationally as an issue of critical concern to public health (Bain *et al.* 2014). The operation and management of these water supplies may be inadequate due to the limited resources and lack of awareness of factors affecting water quality (Rietveldt *et al.* 2009). In Dididi, the community leaders claimed that they raised the problem of the broken distribution pipe with the Municipality early in 2013 (own communication with community leaders 2013/2014). There was no progress of any maintenance at the time of this survey during 2014. The principal public health concern is the use of vulnerable groundwater aquifers without water purification or disinfection measures for drinking purposes. The deficiencies in a multi-barrier approach in vulnerable drinking water supplies increase the risk of drinking contaminated water (Cool *et al.* 2003).

The presence of Atypical Enteropathogenic *E. coli* (aEPEC) and Enterotoxigenic *E. coli* (ETEC) in borehole water used in Dididi is a major concern because of their ability to cause acute infections (Kaper 1996). In a study conducted in Libya using molecular identification, *E. coli* isolates cultured from 243 diarrhoeal stool samples obtained from children and 50 water samples showed 1.2% of the diarrhoeal cases were identified as EPEC, 3.3% were ETEC but EHEC was not detected (Ali *et al.* 2012). In a similar study, Varga and co-workers in Tanzania reported that diarrheagenic *E. coli* (35.7%) was the most isolated enteropathogens and was predominated by Enteroaggregative *E. coli* (EAEC), ETEC (9.3%) and EPEC (5.3%) (Varga *et al.* 2004). The contamination of the borehole water in Dididi is most probably a result of a swampy area observed around the borehole. A broken distribution water pipe conveyed water to the reservoir could be the main cause of contamination. Stagnant water accumulated next to the borehole was a drinking source for community livestock (Figure 3). Animal faeces were also observed and might be the source of pathogenic *E. coli* strains (Table 3). According to Todar (2008) dairy and beef cattle are primary reservoirs of *E. coli* (EHEC) serotype 0157:H7 which causes bloody diarrhoea and they carry it asymptomatically and shed it in faeces. The review by Onda *et al.* (2012) indicated that unprotected wells without sanitary protection are capable of causing groundwater contamination. It is reported that groundwater resources are at less risk of being contaminated by animal faeces when livestock densities are low or when livestock are spread out over a wide area of land (DWAF 2004).

The water point status for the borehole in Doveni is similar to that observed in Dididi where stagnant water accumulated around the borehole enabling underground contamination through seepage. Stagnant water, (Figure 4) found next to or upslope of boreholes, wells and springs has the potential to contaminate the water source (Nguyen *et al.* 2006). The penetration of surface water carrying animal waste or sewage to groundwater abstraction wells could lead to gastrointestinal illnesses as faecal material could carry various pathogenic microbes.

The one borehole in Doveni was positive for Enterohaemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli (STEC)* and ETEC. EHEC harbours the *stx1* gene and is found in humans, cattle and goats where it causes bloody diarrhoea (Nguyen *et al.* 2006). Entero-pathogenic *E. coli* and Enterotoxigenic *E. coli* cause diarrhoea in humans (Nguyen *et al.* 2006). Enterotoxigenic *E. coli* (ETEC) is the

Table 3 | *E. coli* strains isolated from borehole water samples

Ward	Village	Borehole	*E. coli* pathotype
18	Dididi	Borehole 6	aEPEC, ETEC
	Doveni	Borehole 7	Commensal *E. coli*
	Doveni	Borehole 8	EHEC, ETEC
19	Makhuvha A	Borehole 11	Commensal *E. coli*

Figure 3 | Animals close to borehole.

Figure 4 | Stagnant water around borehole.

leading bacterial cause of diarrhoea in the developing world as well as the common cause of traveller's diarrhoea (Nguyen *et al.* 2006). Each year, ETEC reportedly causes more than 200 million cases of diarrhoea and 380 000 deaths monthly in children in developing countries (Nguyen *et al.* 2006). Two boreholes (Doveni and Makhuvha A) shown in Table 3 harboured genes for the non-pathogenic strain of the commensal *E. coli*. Apparently, the presence of these strains still raises a concern of acute infection that could affect the water consumers.

Abandoned wells (Figure 5) are directly linked to aquifers and can channel harmful materials such as sewage, pesticides, fertilizer, toxic chemicals, and bacteria from the land surface into aquifers as they provide a good preferential

Figure 5 | Abandoned borehole; main hole destroyed.

flow pathway to these contaminants (Nguyen *et al.* 2006). A preferential flow pathway is a short cut that a contaminant can take from the surface to the groundwater, or to an abstraction point. This means that the contaminants take much less time to reach the groundwater or abstraction point, than they would if the contaminants were to travel through undisturbed soils, sub soils and underlying bedrock (WHO 2004). Illegal dumping of waste material in these open main holes is a source of contamination of the aquifers. During the survey, many open abandoned boreholes were identified. Abandoned wells are not difficult to seal properly, but many remained open. Because of their large number and wide distribution, abandoned wells pose a significant threat to local ground water supplies (Mawdsley *et al.* 1995; ADEM 2001). When a well is no longer useful, it should not simply be left as an open hole because it is a threat to the environment (Cool *et al.* 2003; Onda *et al.* 2012).

The results of this study indicate that even though boreholes are viewed as improved drinking water sources and expected to provide safe drinking water, some of the boreholes had very poor microbiological quality. Pathogenic bacteria, viruses and other substances from excreta in pit latrines can move through the sub-surface soils and contaminate groundwater (Onda *et al.* 2012). Borehole water in areas with high rainfall and shallow water tables is more vulnerable to contamination from pit latrines (Nguyen *et al.* 2006). Groundwater vulnerability is also high in fractured rock and other high permeability environments, such as

sandy or gravel soils (Babiker *et al.* 2004). This may account for the contamination of borehole water in Doveni, in a borehole that is situated on a down slope and closest to households where the distance does not give enough time for the geological sub-surface material to remove potential contaminants before they reach the water table or the water source (Nguyen *et al.* 2006).

CONCLUSION

Vulnerability of borehole water to contamination is a serious concern in public health risks. This study shows that safety of borehole water in rural areas cannot be guaranteed and is subject to the condition of the infrastructure (pump and distribution system) provided and the site of the borehole. Sustainability and regular monitoring of borehole water is essential to prevent potential public health problems that could result in communicable disease outbreaks. There is a need to provide safe drinking water to rural communities that rely on borehole water for drinking purposes. It is therefore recommended that appropriate measures should be taken to save communities from the burden of outbreaks of diarrhoea. In addition, a maintenance plan to make sure that all boreholes remain functional is essential in good service delivery and should be part of the contingency plans of the Municipalities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

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