Comprehensive water testing analyses for improved water management: coliforms, coliphage and cholesterol

Leani Bothma, Lesego Molale-Tom, Chantel Swanepoel, Carlos Bezuidenhout and Rasheed Adeleke

ABSTRACT

The use of faecal coliforms as indicators is the traditional approach of testing water quality. Unfortunately, for a comprehensive water quality analysis, there is an increasing body of evidence that demonstrates coliforms as insufficient indicators for water quality assessment. Therefore, during the last two decades, alternative water testing approaches such as the use of coliphage as well as cholesterol detection have gained popularity. In the present study, we evaluated and compared the reliability of data from three different indicators that included coliforms (*Streptococcus*), coliphage and cholesterol. Four sites were chosen for sample collection and these included one site from Haart river (HR1) and three sites from Barberspan (BP1, 2 and 3) in the North West Province of South Africa. Samples were collected during winter and summer seasons. Collected samples were subjected to different analyses for detection of coliphage, coliforms and cholesterol. Faecal indicator bacteria were detected at all sites and in some cases were relatively high (HR1: 287 cfu/100 mL faecal coliform and 228.6 cfu/100 mL faecal streptococci; BP1: 1,730 cfu/100 mL *Escherichia coli*). The HR1 site consistently had the highest levels of bacterial faecal indicators of the four sampling sites. Most notably, faecal streptococci were detected in higher numbers than any other bacterial indicator. A significant finding was the general higher levels of faecal indicator markers at the BP3. Based on the outcome of this study, a combination of these indicators offers a comprehensive and promising approach for monitoring water quality.

Key words | cholesterol, coliphage, *E coli*, faecal pollution, streptococci

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HIGHLIGHTS

- This manuscript provides historical data for Barberspan, a Ramsar site.
- It shows the strengths of using multiple faecal indicators together at one site.
- It shows the advantages of using Streptococci, coliphages and cholesterol as faecal pollution indicators.
- It informs the water management strategy of Baberspan.
- It informs on the water quality of Barberspan.

INTRODUCTION

Water quality is usually taken for granted despite its associated public health risks that could emanate from faecal pollution (Lemarchand *et al.* 2004). Between 2003 and

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2015, the World Health Organization (WHO) reported that the number of people in the world without access to safe and affordable water for domestic purposes jumped from 1.2 to 1.3 billion (WHO 2003, 2018). A general household survey conducted in South Africa indicated a less than five percent (4.6%) increase in the quality of water supplied to households from 2002 to 2018 (StatsSA 2019). Furthermore, there are still 29.2% of households that have no access to water in their dwellings and have to make use of communal/public taps (StatsSA 2019). According to the WHO (2008), numerous water related health problems are a result of microbial and chemical contaminants in water systems. Water in bird sanctuaries and Ramsar sites that are used by birds as a stop-over during migration are important natural resources and faecal pollution in such entities are of concern (Aburto-Medina *et al.* 2015).

The concept of indicator organisms in water microbiology is vital and well established (George *et al.* 2002). It has provided an efficient framework for assessing the microbial quality and safety of water. The South African (SA) legislature has employed the National Microbial Monitoring Program (NMMP) to assess the faecal contamination of surface waters (DWAF 2002). The 2nd edition NMMP implementation document of 2002 states that faecal coliforms (FC) should be used to assess faecal pollution of surface waters (DWAF 2002). Additionally, the latter document states that the Colilert method for assessing *Escherichia coli (E. coli)* levels should also be employed bi-monthly if a laboratory is capacitated (DWAF 2002). In addition, the Target Water Quality Range (TWQR) established in SA in 1996 (DWAF 1996) incorporates somatic coliphages (SC) in their ranges. However, except for research purposes by independent scientists, no evidence can be found that these are actually being employed by governmental agencies on a regular basis for water quality assessment and management. Despite established dogma that using multiple faecal pollution indicators is more beneficial for risk assessment as opposed to only using *E. coli* or FC (DWS 2019). *E. coli* and FC are still the sole microbial indicators monitored in SA. Bezuidenhout & Adeleke (2011) raised the concern, that even when available, microbiological parameters are not included in Integrated Water Resource Management (IWRM). This is of concern, particularly when considering that organisms such as faecal streptococci (FS), coliphages and faecal sterols are frequently used in water quality assessments in other parts of the world (Cabral *et al.* 2018; Donde *et al.* 2018).

The advantages of FS as bacterial indicators of water quality that makes them preferable to FC are their longer survival rate, and the more closely related persistence patterns of waterborne pathogenic bacteria (Lin & Ganesh 2013). They are more resistant to chlorination and drying, and most of the FS species are not able to multiply in the environment. Most convincingly, more than 91.5% of FS found in the environment are of true faecal origin (Junco *et al.* 2001).

Viruses originating primarily from the gastro-intestinal tract can be monitored using surrogates, bacteriophages (phages) and more specifically coliphages (bacteriophages that infect *E. coli*) Jebri *et al.* 2012). It has also been proven that coliphages more closely resemble the indices of enteric viruses in water than FC (McMinn *et al.* 2017). Regular environmental surveillance of coliphages in water sources can enhance the understanding of the actual burden of disease (community well-being and socio-economic repercussions) caused by those using faecal polluted water without prior treatment (Ganesh *et al.* 2014).

Faecal sterols are a class of compounds that can be correlated quantitatively with major sources of faecal pollution. Sterol biomarkers can be used as chemical tracers to determine sewage transport and distribution in the environment (Kannan *et al.* 2012). Cholesterol, being synthesised by animals and found in their cell membranes, is absent from prokaryotes (Mouritsen & Zuckermann 2004). This molecule thus provides a sterol biomarker for animal faecal pollution.

The aim of this study was to evaluate multiple faecal indicator markers (bacteria, phages and cholesterol) and the impacts of faecal pollution on water quality in a natural water system.

METHODS

The study site Barberspan is a Nature Reserve Inland Lake situated in the North West Province (NWP), South Africa. This Ramsar site is a stop-over for birds migrating between northern Europe and South Africa. The water quality of this lake has been decreasing severely over the years (Matthews & Bernard 2014). As depicted in Figure 1, one

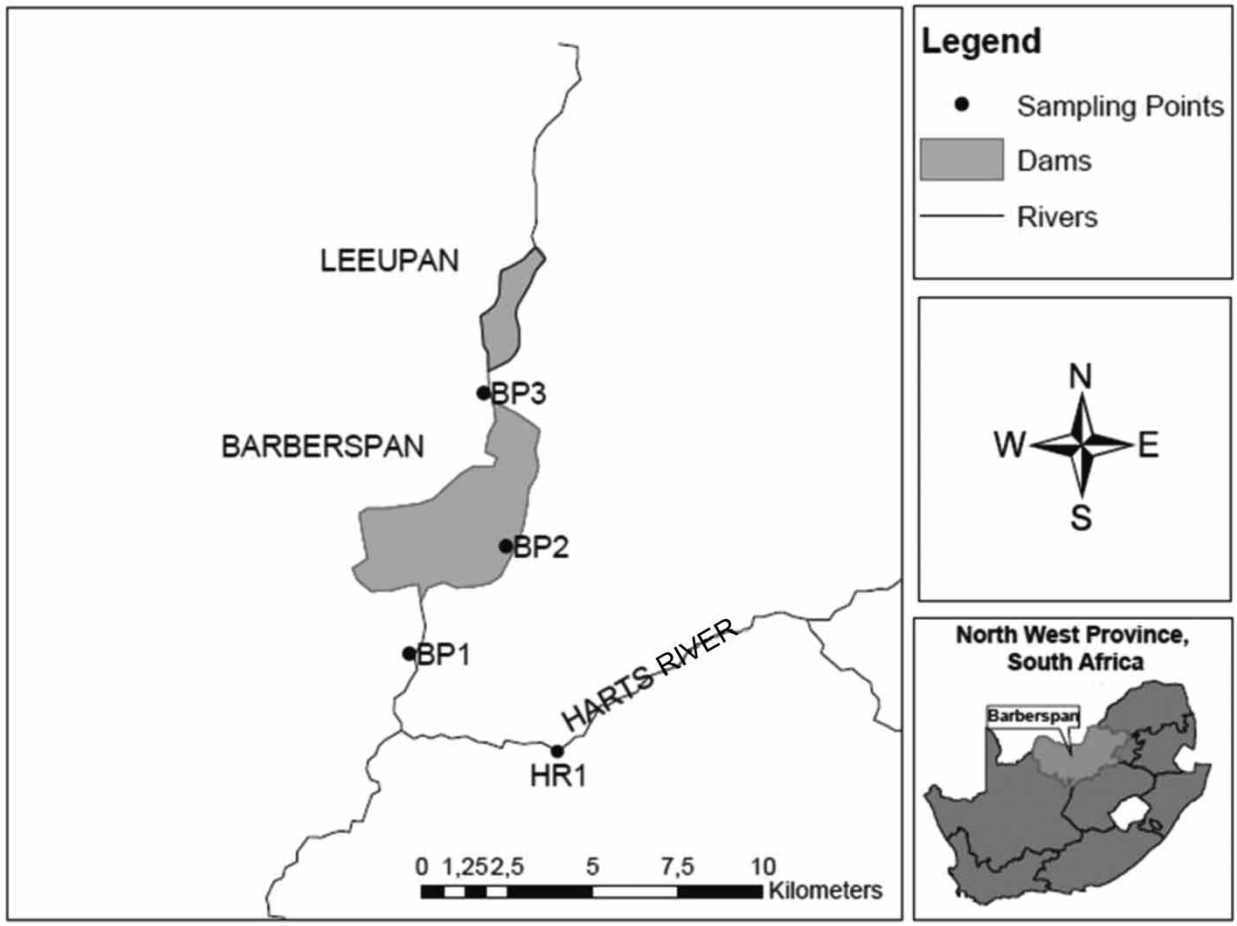

Figure 1 | Map of four sampling sites at Barberspan, North West Province, South Africa. Water is artificially diverted from the west-flowing Harts River into Barberspan that flows from south to north. Barberspan is located in a rural cattle farming area. The only structures close to the pan are two one-storey serving as a small hotel located at BP2 and one permanent residence for the caretaker and his small family at BP3.

sampling site was situated in the Harts River (HR 1) which runs past, as well as into, Barberspan. All the remaining sample sites were in Barberspan and included the Inflow of the Harts River (BP1) into the lake (southern bank), Barberspan Hotel (BP2; eastern bank), and finally at the Outflow into Leeupan (BP3; northern bank; Figure 1).

Initially a pilot study was conducted in 2010 where levels of FC, *E. coli*, FS and cholesterol were determined. However, due to constrains in capacity building, budget and largescale implementation within a developing country; cholesterol screening was halted in 2010. Due to the latter reasons, only FC, *E. coli*, FS were screened in 2011. Thereafter, coliphages were included in the indicators screened in this study due to its affordability and increased notoriety as reliable faecal indicators.

One litre surface water samples were collected in sterile glass Schott bottles using the dip sampling technique (US EPA 1994). These were collected four times periodically during two cold dry winter July 2010 and June 2014 as well as two warm rainy summer August 2011 and October 2014 seasons, representing the temperate climate of the region. Levels of faecal pollution indicators can be found in Table 1 in this manuscript. Physical (other than temperature) and chemical parameter values of the sites can be found in Supplementary Table A.

The temperature of the water samples was measured on site using a multi-meter probe (PCSTestr 35, Eutech Instruments Pte Ltd, Singapore). Bacteria were isolated in triplicates using the standard membrane filtration technique using 0.45 μm Whatman© filters and placed aseptically on

Table 1 | Different average faecal indicator levels for Barberspan during 2010–2014

Sample point	Temperature (°C)				Faecal coliforms (cfu/100 mL)				*E. coli* (cfu/100 mL)				Faecal streptococci (cfu/100 mL)				Somatic phage (pfu/100 mL)		FRNA phage (pfu/100 mL)		Cholesterol (ppm)
Year	2010 (W)	2011 (S)	2014 (W)	2014 (S)	2010 (W)	2011 (S)	2014 (S)		2010 (W)	2011 (S)	2014 (S)		2010 (W)	2011 (S)	2014 (S)		2014 (W)	2014 (S)	2014 (W)	2014 (S)	2010 (W)
HR1	13.70	22.40	18.40	NMT	130.00	287.00	33.60		143.30	NMT			127.30	228.60	NMT		1790.00	NMT	1860.00	NMT	<LOQ
BP1	15.20	22.00	14.50	25.60	14.67	44.00	4.60		14.00	1730.00			36.00	109.30	132.00		470.00	1210.00	650.00	30.00	6.21
BP2	14.70	22.60	14.90	23.40	1.00	132.60	0.30		90.00	80.00			50.00	195.30	17.00		920.00	190.00	110.00	90.00	6.01
BP3	12.00	23.50	12.80	25.40	1.00	76.00	0.00		32.00	180.00			61.30	180.00	103.00		460.00	106.00	4270.00	160.00	7.26
± SD	0	0.60	0	0	0	88.76	0		64.54	57.75			0	55.30	20.51		262.74	59.40	157.68	65.06	0

Sampling was done in winter (W) and summer (S). No measurements were taken (NMT) in the summer of 2014 at HR1 due to the Harts River drying up in the area. Cholesterol levels at HR1 was below the detection level (<LOQ). Standard Deviation (SD) was determined for $n = 3$ unless SD = 0 then $n = 1$.

selective agar (Molale 2012). The selective agars were m-FC agar (Merk, Germany), MLG agar (Oxoid, UK), and KF-*Streptococcus* agar (containing 1 ml of 2,3,5-triphenyltetrazolium chloride (TTC) per 100 ml) (Sigma-Aldrich, South Africa). For isolation and purification of presumptive *E. coli* and FS, representative colonies from the Whatman© filters were aseptically sub-cultured at least three times on nutrient agar. Gram staining was used as the first step for confirming presumptive *E. coli* isolates. The triple sugar iron (TSI) agar (Merck, Germany) test was then used for further confirmation (Fankhauser 2001). Identification of presumptive FS included the Gram reaction, catalase activity, bile solubility, temperature and salt tolerance tests. All presumptive tests were conducted in triplicate. Finally 16S rRNA gene fragments were amplified and sequenced.

E. coli WG5 and *Salmonella enterica* WG49 were used as host strains for somatic coliphage and FRNA phage enumeration. The double-agar layer plaque assay was conducted, in triplicate, for the enumeration of somatic coliphages and FRNA phages using modified versions of ISO 10705-2 (2000) & – 1 (1995), respectively. Modifications were made to the top layer mixture by increasing the semi-solid agar from 3 to 6 mL and the sampled water from 1 to 2.5 mL. Additionally, the incubation period of *Salmonella* WG49 was increased from 3 to 16 hours. This step allowed 1.5 mL *Salmonella* WG49 in 10 mL (top layer mixture) to form a solid bacterial mat when plated on bottom layer of agar.

The Szűcs method (Szűcs *et al.* 2006) was used in triplicate to detect cholesterol. Details of the method are provided and can be found in the institutional repository of North-West University (Boloka) (Swanepoel 2014).

RESULTS

Results contained in this section were recorded from different sampling runs in different years 2010 to 2014. Where appropriate, Microsoft Excel® was used to calculate averages. The bacteriological, general phage data and cholesterol levels were recorded. Only the 2010 and 2014 data are provided for cholesterol and phage levels respectively. For *E. coli*, average data for all three sampling periods are provided. What is evident is that faecal

indicators occurred at all the various sites during all sampling periods albeit that the levels varied. Faecal indicators measured during winter were lower in winter than in the summer sampling period. This could be expected as the higher water temperature normally experienced during summer could support the survival of bacteria (Prest *et al.* 2016). This area is a summer rainfall area and runoff could also have contributed to the higher faecal indicator levels during this period.

Table 1 presents different average faecal indicator levels obtained at Barberspan from 2010 to 2014. In the summer of 2014 average FRNA phage levels increased throughout the system (BP1: 30 pfu/100 mL; BP2: 90 pfu/100 mL; BP3: pfu/100 mL). Average levels of *E. coli*, FS and somatic phage were highest at BP1 compared with the other sites (1,730 cfu/100 mL; 132 cfu/100 mL; 1,210 pfu/100 mL) with *E. coli* and somatic coliphage levels at BP1 (1,730 cfu/100 mL; 1,210 pfp/100 mL) being relatively similar As for, BP3 *E coli* levels were more similar to FRNA phage levels (180 cfu/100 mL and 160 pfu/100 mL) and somatic phage levels to FS (106 pfu/100 mL and 103 cfu/100 mL).

During the winter sampling of 2010, microbiological analysis included FC, *E. coli* and FS. In the case the bacterial levels were highest at HR1 compared to the sites in Barberspan. Once again the levels of bacteria were higher at BP1 compared with BP2 and BP3. During this period cholesterol was detected at all the Barberspan sites (Table 1). The summer of 2011 microbiological analysis shows that the levels of the FC and streptococci at HR1 was higher (287 and 228.6 cfu/100 mL, respectively) than BP1 (44 and 109.4 cfu/100 ml, respectively). The levels for these two parameters were higher at BP2 (132.6 and 195.3 cfu/100 ml, respectively) compared with the BP3 (76 and 180 cfu/100 mL, respectively).

During the summer of 2014 the sampling site HR1, which is situated upstream from the BP1 was dry, due the prolonged drought in the NWP. However, what the previous results have shown is that these sites consistently had the highest level of each indicator organism, except for FRNA phage levels measured during the winter of 2014 when the Outflow site had the highest measurement (427 pfp/100 mL). There was no clear indication of human activity at the HR1 site, thus the origin of the indicators should probably solely be attributed to the observed bird and cattle activity in that immediate area.

DISCUSSION

Construction of records aimed at explaining water quality trends and pollution sources should be an important aspect of IWRM (Fatoki *et al.* 2001; Bezuidenhout & Adeleke 2011). The WHO (2003) has established target water quality ranges for multiple indicators of faecal pollution in water. It is thus advantageous for water management and monitoring practices to rely on a variety of indicators in order to gain a holistic view of a water system. It also validates the claim that no single indicator organism exists that fulfils all the requirements for an indicator organism to the satisfaction of environmental scientists and managers. In SA the National Microbial Monitoring Program (NMMP) is used to assess and report on the faecal contamination of surface waters (DWAF 2002). The 2nd edition NMMP implementation document of 2002 states that FC should be used to assess FC pollution of surface waters and if a laboratory is equipped with the Colilert method *E. coli* levels should also be assessed bi-monthly (DWAF 2002). In practice we observed that *E. coli* is consistently monitored and FC is consistently or sporadically monitored by some of the nine Water Management Areas (WMA). In 2019 two or three WMA have reported their monitoring levels, in 2018 three to six WMA, The Orange WMA last reported its *E. coli* levels in April of 2013 when most sites were classified as high risk of danger for full or partial contact.

It has long been established that the use of multiple faecal pollution indicators is more beneficial for risk assessment than only using *E. coli* or FC (DWS 2019). These standards have been incorporated by various countries for water quality surveys in their guidelines (Tyagi *et al.* 2006). The TWQR established in SA in 1996 (DWAF 1996) incorporates somatic coliphage in their ranges as well. However, except for research purposes by independent scientists, no evidence could be found that these are actually being employed by governmental agencies on a regular basis for water quality management. In SA *E. coli* and FC are still the sole microbial indicators monitored,

and rarely are they both monitored at a single site. It is of vital importance to monitor environmental water systems that run through rural communities, are used for irrigation of crops and watering of livestock. The lack of information on water quality is still great for most water systems in SA (NWDACE-SoER 2008). This study sheds some light on the water quality of one such water system of national and international importance.

Over the years of monitoring bacterial faecal indicators (total coliform, FC, *E. coli*, and FS) arguments had been put forward that FS are more stable of all the bacterial indicators (Wade *et al.* 2010; Byappanahalli *et al.* 2012). Total coliforms and FC are still widely used as indices to measure the quality of surface and groundwater (WHO 2008). However, the applicability of solely using coliform bacteria as indicators of faecal pollution has been extensively argued (Boehm *et al.* 2009). It has also been suggested that not all FC are of faecal origin. Some might multiply in the environment and some are not of true faecal origin, some studies have found them to originate from plants (Scott *et al.* 2002; Simpson *et al.* 2002). It has been suggested that FS rather be used as bacterial indicator for faecal pollution as they have proven to be more reliable than *E. coli* (WHO 2008). Monitoring programmes such as the Microbial Monitoring Programme in South Africa is still only using *E. coli* as the main microbial parameter for microbial quality of water. The survival of the FS group in water is longer than that of FC while they have shown persistence patterns similar to those of waterborne pathogenic bacteria (Curutiu *et al.* 2019). However, if source tracking is to be applied using streptococci the identification of FS using biochemical tests requires a large number of tests, and can prove difficult and time consuming for routine application (Moore *et al.* 2006).

In the summer of 2014 results presented in this study, the trends in phage levels were supported by the *E. coli* and FS levels for the various sites. Faecal streptococci levels in 2010 could also be used to support the observed cholesterol levels. Most data for the parameters indicate that faecal pollution occurs at the various sites and monitoring these will be important for management of this system. The data also demonstrate that a combination of parameters are more informative than just one single microbial parameter.

Coliphages (especially FRNA coliphages) have been recommended as microbial indicators for faecal pollution. They behave more like human enteric viruses, which pose a health risk to water consumers if water has been contaminated by faeces, than any other indicator organism (Jebri *et al.* 2012). In the present study the FRNA phages and 2010 FS and cholesterol showed similar trends and this suggested that these three could be valuable in describing the resource quality of objectives for this water body. (Ogorzaly *et al.* 2009) indicated that in waters where FRNA phage outnumber somatic coliphage human faecal matter is the predominant source of faecal pollution. For BP3, in winter as well as in summer (to a certain extend) this was the case, i.e. FRNA phage levels were higher than the somatic coliphage levels.

Faecal sterols could be useful to provide fingerprints for the various sample points. Faecal sterols are a class of compounds that can be correlated quantitatively with major sources of faecal pollution. Sterol biomarkers can be used as chemical tracers to determine sewage transport and distribution in the environment (Kannan *et al.* 2012). Cholesterol, being synthesised by animals and found in their cell membranes is absent from prokaryotes (Mouritsen & Zuckermann 2004). This molecule thus provides a sterol biomarker for animal faecal pollution. Although the analytical detection method is fast and sterols are overall much more stable and resistant to stressors in the environment (Isobe *et al.* 2002) it is currently expensive to set up the procedure (Szűcs *et al.* 2006). If this method could be developed for easier and cheaper quantification, this could provide a viable method for faecal pollution in developing countries. It has the added benefit of not depending on cultivation of indicators and the levels of detection is therefore less likely to be affected by emerging contaminants in water systems.

Water quality is usually taken for granted in spite of the associated public health risks that could emanate from polluted water, especially faecal pollution (Lemarchand *et al.* 2004). In 2003, the WHO reported that 1.2 billion people in the world lacked access to safe and affordable water for domestic purposes. This situation has not changed much since then. During 2015 this figure was 1.3 billion (WHO 2018) showing that there has been a rise in the number of individuals that still lack access to safe water. This is

contrary to the WHO sustainable development goals (SDG 6) that has as its focus the provision of safe drinking water for all by 2030. Judging from the statistics and the findings of the present and other recent studies it appears that this goal will not be reached.

The main water quality parameters for which large data sets are available are physico-chemical and in some cases *E. coli* levels, representing microbiological parameters. Bezuidenhout & Adeleke (2011) raised the concern, that even when available, microbiological parameters are not included in IWRM. This was echoed in the study by Chidamba *et al.* (2016) where it was demonstrated that for one of the major rivers in SA, the Vaal River, different physical and chemical resource objectives could be specified for specific sections of the river because of the available historic data. The same could not be done for the microbiological parameter(s) and the reason provided was that not sufficient data were available (DWAF 2009). Surface water systems are predominantly polluted by untreated wastewaters, industrial, agricultural and domestic wastes decanting into them (Haller *et al.* 2009). According to the WHO (2008), numerous water-related health problems are a result of microbial and chemical contaminants in water systems.

Additionally, faecal sterols are a class of compounds that can be correlated quantitatively with major sources of faecal pollution. Sterol biomarkers can be used as chemical tracers to determine sewage transport and distribution in the environment (Kannan *et al.* 2012). Cholesterol, being synthesised by animals and found in their cell membranes is absent from prokaryotes (Mouritsen & Zuckermann 2004). This molecule thus provides a sterol biomarker for animal faecal pollution.

In the present study, the discussion supports the present findings and trends regarding the three bacterial indicators in this study and the advantages of including FS as faecal indicator. Listed faecal indicator parameters could be used to determine an index of faecal contamination in water (Rincé *et al.* 2003). The methods described here can be applied to the analysis of other types of environmental water samples. Technical advantages also make it suitable for routine environmental monitoring of faecal pollution and source tracking the pollution. With regard to the data set, it appears that the HR1 and the BP3 sites were, over the sampling period, generally more affected by faecal pollution compared with the other sites. At some stages BP2 site also contributed towards such pollution.

Barberspan is a bird sanctuary, and a multitude of bird species can be found there in great numbers (Ndlovu *et al.* 2017), it seems as though the birds may be responsible for the contamination. Water in bird sanctuaries and Ramsar sites that are used by birds as a stop-over during migration are important natural resources and faecal pollution in such entities is of concern (Aburto-Medina *et al.* 2015). Barberspan is also used for fishing and other recreational activities. These could also be contributing towards the faecal pollution but not to the extent seen with the bacteriophages observed.

CONCLUSION

This study has indicated that the use of a several faecal indicators would provide a better resolution of faecal pollution than when a single indicator is used. The lack of microbiological parameters is still the scenario in many regulatory frameworks and more data are needed to challenge this view. For a strategic water source such a Barberspan, using various parameters to indicate faecal pollution and set up faecal contamination levels that could be correlated with risk, would allow for informed decisions to be taken regarding the use of the water. It would also allow management with a tool to identify hotspots that require interventions. However, constant monitoring is critical to the successful implementation of a risk management strategy.

AUTHOR CONTRIBUTIONS

Leani Bothma: Experimental execution phage, data analysis, writing the first draft, editorial. Lesego Molale-Tom: Experimental execution bacteria, data analysis, co-writing the first draft, editorial. Chantel Swanepoel: Experimental execution cholesterol, data analysis. Carlos Bezuidenhout: Experimental design, data analysis, Supervisor of LB, LM-T and CS, editorial. Rasheed Adeleke: Experimental design, data analysis, co-supervisor of LB, editorial.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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