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Full Length Research Paper

Assessment of groundwater quality in the rural areas of the North West Province, South Africa

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The aim of this study is to assess the quality of the groundwater currently supplied to the rural communities of the North West Province. Groundwater samples collected from 100 boreholes in these rural areas were subjected to culture-based methods using selective media for the isolation of faecal and total coliform bacteria. Molecular analysis targeting 16S rRNA gene, and the restriction fragment-length polymorphism (RFLP) and sequence analysis of amplified gene for differentiating between species and strains of bacteria, were applied to selected coliform isolates. The physico-chemical parameters such as pH, temperature, total dissolved solids (TDS), turbidity and concentrations of chloride, sulphates, nitrate, fluoride, magnesium, sodium and calcium were also determined. The results revealed that 83% of the boreholes did not comply with the fluoride limit of SANS 241 (0 to 1 mg/l), 40% did not comply with the nitrate limit (0 to 6 mg/l as N), 45% did not comply with the magnesium limit (0 to 30 mg/l as Mg), 43% did not comply with the calcium limit (0 to 32 mg/l as Ca), 31% did not comply with TDS and 47% did not comply with the turbidity limit (<1 NTU). The results also indicated that 23 and 86% of the boreholes did not comply with the limits set by the national guidelines (SANS 241 and DWAF) in terms of faecal (0 cfu/100 ml) and total coliforms (0 to 5 cfu/100 ml), respectively. The results of the molecular study revealed that out of 100 boreholes, 51% tested positive for *Citrobacter freundii,* 28% for *Serratia marcescens,* 12% for *Morganella morganii,* 8% for *Salmonella enterica,* 7% for *Aeromonas veronii,* 5% for *Bacillus cereus*, 2% for *Enterobacter cloacae and* 1% for *Escherichia coli* and *Shigella dysenteriae*. The findings of this study showed convincing evidence that some groundwater supplies in rural areas of North West pose a serious health risk to consumers.

Key words: Groundwater, physical, chemical, microbiological water quality.

INTRODUCTION

Water quality has long been regarded as the primary indicator of health and well-being and water of a good quality is crucial for the economic development of a country. Polluted water not only has the potential to cause human suffering, but also reduces individual productivity. Families experience diminished disposable income due to payments for medical treatment, and valuable time and energy are spent on efforts to secure their water supply. Consequently, thousands of people, especially children, die due to a lack of clean and safe water (World Health Organization (WHO), 2002). According to the WHO (2003a), an estimated 1.1 billion people globally drink unsafe water. Approximately 3.1% of the annual deaths (1.7 million) and 3.7% of the annual health burden (disability-adjusted life years [DALYS]) worldwide) (54.2 million) are attributable to unsafe water and a lack of sanitation and hygiene (WHO, 2003b). Studies have shown that the vast majority of people who lack access to safe water are members of developing nations (UN WWAP, 2003). This implies that many people in the developing world still depend on contaminated water sources for their daily water needs.

As South Africa is classified as a water-stressed country (United Nations, 2006), groundwater sources occupy the third position in terms of the size of their

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contribution (9%) to the total supply of fresh water in South Africa, with 14% sourced from return flows (sewage and effluent purification) and 77% from surface water (dams and rivers) (Van Vuuren, 2009). Pollution of groundwater sources is still the main challenge currently facing rural communities who depend almost exclusively on these water sources. These communities obtain their drinking water directly from uncovered or covered boreholes and wells. In certain areas, natural groundwater complies neither with the limits set for salinity, nitrates, fluorides, iron, manganese and other trace elements, such as arsenic and uranium, nor with potable standards for microbial indicators (Engelbrecht and Tredoux, 2000). The microbiological pollution of groundwater is caused by human and animal activities, which include on-site sanitation, cemeteries, waste disposal, feedlots and unsewered settlements (Engelbrecht and Tredoux, 2000).

Although groundwater has historically been thought to be free of microbial contamination, studies have indicated that contaminated groundwater sources could result in waterborne diseases if consumed without prior treatment (Momba and Mnqumevu, 2000; Momba et al., 2006). Pathogenic bacteria such as *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila, Shigella dysenteria, Salmonella tyhimurium, Pseudomonas* spp. *and Klebsiella* spp. have been reported in groundwater sources (Momba and Mnqumevu, 2000; Momba and Notshe, 2003; Momba et al., 2006). Moreover, outbreaks of cholera, typhoid fever, salmonellosis, shigellosis, gastroenteritis and hepatitis in some parts of South Africa have been linked to contaminated drinking water (Department of National Health and Population Development, 2001). Studies have also established an epidemiologic linkage between bacterial enteropathogens from contaminated water sources and those from stools of patients with diarrhoea in rural South African communities (Obi et al., 2007; Abongo and Momba, 2008; Momba et al., 2008). In these studies, targeted species-specific genes of *Salmonella enteritidis*, *S. typhimurium, E. coli, E. coli* 0157:H7 and *S. dysenteria* showed that the *Salmonella, E. coli, E. coli* 0157:H7 and *Shigella* isolates from stool samples of Human Immunodeficiency Virus-positive (HIV-positive) and HIV-negative individuals with and without diarrhoea were also present in the household drinking water of the same study cohorts. This profile of results indicated that drinking water may have been the source of the organisms in stool samples and gives credence to a possible linkage between enteric bacterial pathogens isolated from water and stool samples. Although the linkage was demonstrated for enteropathogens from HIV-positive and HIV-negative individuals with and without diarrhoea and the household drinking water of the study cohorts, water quality is a more critical factor for HIV-positive cases. HIV-positive individuals are immunocompromised and are thus more susceptible to \ even low-grade pathogens than HIV-negative individuals (Obi et al., 2007). Studies have also indicated that viruses are able to move through several types of soil to contaminate aquifers (Schijven, 2001).

The cause of the increasing incidence of water-related diseases and their complications often remains unknown in rural communities due to the lack of a routine monitoring programme for the quality of their water supplies. This study was therefore aimed at determining the physical and chemical parameters (such as pH, temperature, total dissolved solids (TDS), turbidity, chloride, sulphate, nitrate, fluoride, sodium, potassium, magnesium and calcium concentration) and the bacterial quality of groundwater in the North West Province. The monitoring of groundwater sources has led to increased public health awareness, since it resulted in the detection of specific pathogenic microorganisms and chemical hazards capable of causing infections and diseases in such communities. This might assist local authorities and scientists in developing strategies for addressing groundwater supply problems in these rural communities.

MATERIALS AND METHODS

Study area and sampling points

The North West Province of South Africa shares an international border with Botswana in the north. The climate of this province is characterised by hot summers and cool, sunny winter seasons. The annual rainfall varies from 500 to 700 mm in the eastern part, 400 to 600 mm in the central regions and 100 to 400 mm in the western part of the province (NWPG, 2002).

Groundwater is a vitally important water resource in both the rural and semi-urban areas of the North West Province. The province has a total population of 3.4 million, 60% of whom live in rural areas. More than 80% of the rural population depend on groundwater as their main drinking water source (NWPG, 2002). The aquifers that serve as water sources are composed of limestone, carbonated shale and dolomite. During the study period, none of the boreholes were measured for water level depth. The municipalities distribute water from groundwater sources directly to these communities without any prior purification.

Groundwater samples were collected from 100 boreholes located in the villages of the Bojanala district municipality (which includes the local municipalities of Moretele, Madibeng and Moses Kotane), the central district (Ramotshere Moiloa, Mafikeng and Tswaing) and the Bophirima district municipality (Kagisano and Greater Taung) of the North West Province in South Africa (Table 1 and Figure 1). The communities in these villages obtain underground water from boreholes by using a rotary hand pump that is connected to a standpipe, or directly from standpipes that are connected to the boreholes. Although the boreholes are covered, some are surrounded by animal excreta and are located close to pit latrines. Forty (40) of the boreholes are privately owned, and the remaining 60 are communal boreholes.

Collection of water samples

Water samples from the sources were collected between September and November, 2008. The standpipes or the taps in operation rooms were flushed for approximately 5 min before collecting samples using 2l sterile glass bottles. All the bottles were

Table 1. List and locations of the boreholes surveyed in North West.

Municipality surveyed	Number of boreholes	Locations of boreholes
Moretele	8	Dikebu (1), Makapanstad (2), Noroki (3), Moretele clinic (4), Kgomo-Kgomo (5), Mashilomatsho 1 (6) and 2 (7) and Swartdam (8).
Madibeng	7	Shakung 1 (9) and 2 (10), Dipompong (11), Maboloka (12), Rabokale (13), Jerico (14) and Moiletswane (15).
Moses Kotane	15	Ratau (16), Mabalstad (17), Makgope (18), Khayakhulu (19), Letlhakeng (20), Voordonker (21), Lefaratlhatlha (22), Matau (23), Masekoloane (24), Molorwe (25), Motlhabe (26), Tweelagte 2 (27), Manamela (28), Bapong 2 (29) and Mantsho (30).
Ramotshere Moiloa	14	Luthern Mission (31), Malebelele P.S (32), Braklaagte 1 (33), 2 (34) and 3 (35), Leeufontein 1 (36), 2 (37) and 3 (38), Supingstad 1 (39) and 2 (40), Lekgopung 1 (41) and 2 (42) and Mushane 1 (43) and 2 (44).
Mafikeng	26	Signal Hill 1 (45) and 2 (46), Lonely Park 1 (47) and 2 (48), Moshewane (49), Megokgoane (50), Dimorogwane 1 (51) and 2 (52), Miga 1 (53) and 2 (54), Ikopeleng 1 (55) and 2 (56), six hundred 1 (57) and 2 (58), Dihatshwane 1 (59) and 2 (60), Majemantsho 1 (61) and 2 (62), Setlopo 1 (63), 2 (64) and 3 (65), Motloung (66), Mokgokoe (67), Lekoko 1 (68) and 2 (69) and Morwatshethla (70).
Kagisano	8	Mophohung 1 (71) and 2 (72), Ganyesa 1 (73) and 2 (74), Moswane 1 (75) and 2 (76), Selosesha (77) and Thokoza (78)
Greater Taung	12	Dryharts 1 (79) and 2 (80), Moretele (81), Ntswanahatshe (82), Choseng (83), Pudimoe (84), Matlapeng 1 (85) and 2 (86), Leshobo (87), Myra (88) and Amalia 1 (89) and 2 (90).
Tswaing	10	Groeteland (91), Rapoeli (92), Delareyville (93), Bamberg Farm (94), Eclipse Farm (95), Vriegevagte 1 (96) and 2 (97), Atamelang (98) and Setlagole 1 (99) and 2 (100).

sealed and properly labelled. A mobile laboratory containing all the necessary equipment (a membrane filtration unit, a vacuum pump, kettles, portable incubators, sterile Petri dishes containing selective cultural media, sterile membrane filters, a pH meter, thermometer, turbidity meter and conductivity meter) was used for the on-site analysis of water quality. The plates containing coliform isolates and samples for chemical analysis were then placed in ice bags and transported to the Tshwane University of Technology Water Research Group laboratory for further analysis.

Water quality variables

The water quality tools used to measure the environmental health risk in this study were the SANS 241 (2006) and the South African Water Quality Guidelines for domestic use (DWAF, 1996). Molecular identification of the coliform isolates was also used as a proxy measure that confirmed the possible threat that the microbial quality of groundwater poses to public health in the rural communities of the North West Province.

The pH, turbidity, temperature and TDS were measured on site. The pH was measured using a pH meter (Metrohm Co. Model 713) integrated with a temperature compensation (25°C) device. The pH meter was calibrated with two buffer solutions of pH 4 and 7 before use according to the manufacturer's instructions. Turbidity was measured with a microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100) calibrated by using standard Formazin solutions of 0.02, 20.0, 100 and 800 NTU, according to the manufacturer's operating instructions. The temperature and TDS of the water samples were determined by using a conductivity meter (Hach Co. Sension7) integrated with a temperature compensation (25°C) device. The instrument was calibrated with standard 0, 0100 mol/l KCl solution (to give a value of 14.7 µS/m at 25°C) before it was immersed in the sample. The concentrations of nitrates, fluoride, sulphate and chloride were determined in the laboratory using the Spectroquant Nova 400 manual water analyser (Merck) and photometric test kits (Merck). The manufacturer calibrates the instrument every 6 months. The magnesium, sodium, calcium and potassium concentrations in the water samples were determined by atomic absorption spectrometry (SpectrAA 220FS), according to the standard method (APHA, 1998).

The initial microbiological analysis of water samples performed on site was limited to total and faecal coliforms. The membrane filtration technique, the Chromocult coliform agar (Merck) and the M-FC agar (BioLab) were used for the enumeration of coliforms. Water samples were analyzed for this group of indicator bacteria using internationally accepted techniques and principles. The physicochemical and microbiological water quality parameters were then compared to the standards set by the SANS 241 (2006) and DWAF Water Quality Guidelines for Domestic Use (DWAF, 1996).

Molecular identification of coliform isolates

For the identification of bacterial isolates, individual coliform

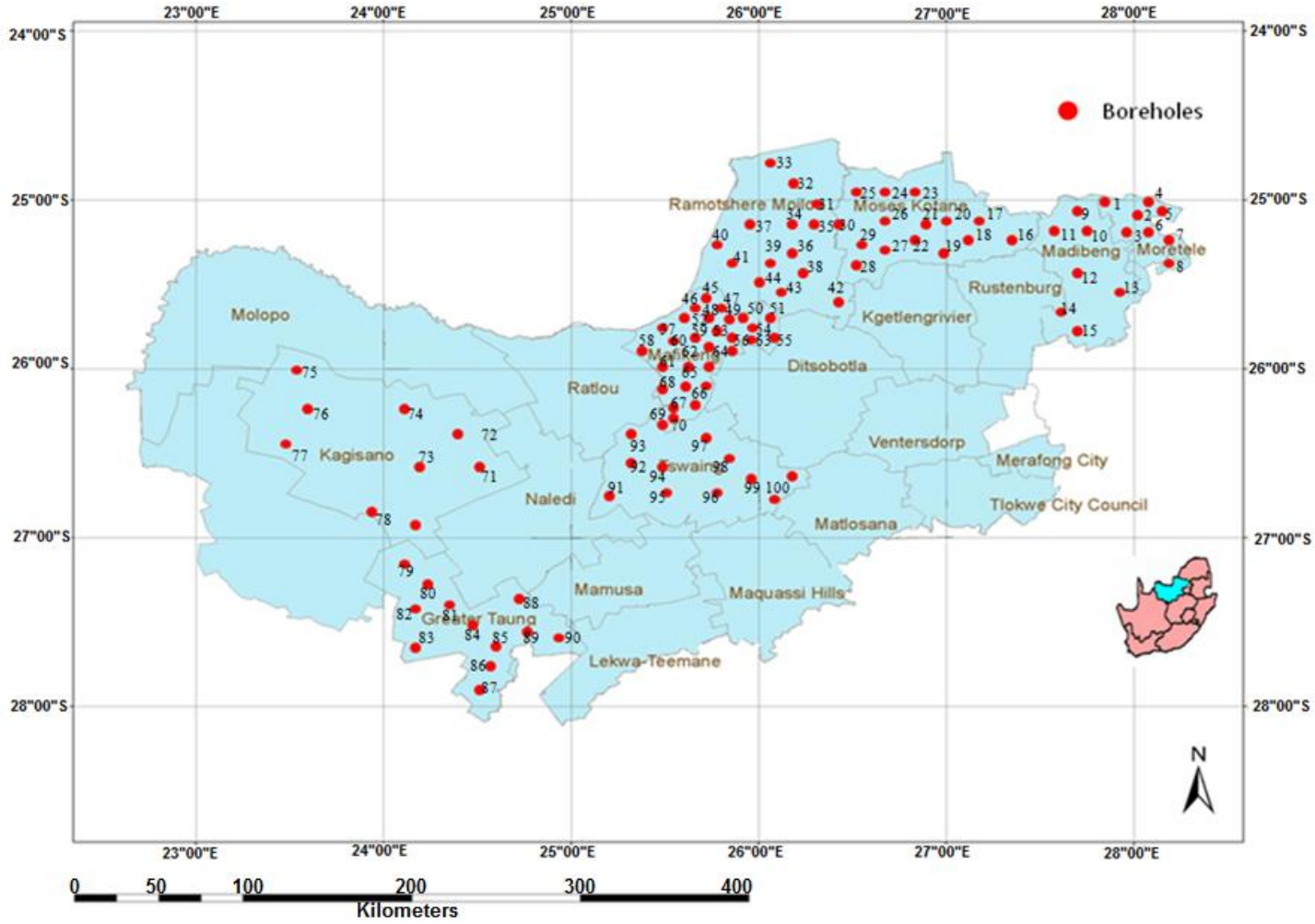

Figure 1. Map of North West Province indicating location of borehole sites in the local municipal areas of Moretele, Madibeng, Moses Kotane, Ramotshere Moiloa, Mafikeng, Kagisano, Tswaing and Greater Taung.

colonies from water samples were randomly selected from different plates based on their size, shape and colour. They were transferred onto chromocult coliform agar (Merck) by the streak plate technique and incubated at 35±2°C for 24 h. The colonies were further purified by the same methods at least three times, using the same medium (Biolab), before Gram staining. Oxidase tests were then done on those colonies that were Gram negative. The oxidase-negative colonies were transferred onto nutrient agar slants, incubated at 35±2°C for 24 h and kept at 4°C until further use.

Extraction of the total genomic DNA

A total of 72 oxidase-negative isolates were used for molecular study. Individual isolates were grown in nutrient broths, followed by incubation at 35±2°C for 24 h. The inoculated broths (1 ml) were centrifuged at 13 300 g for 5 min. The pellets were washed twice with sterile molecular grade water. The total genomic DNA from the bacterial pellet was extracted using the DNeasy DNA purification kit (QIAGEN) according to the manufacturer's instructions. The quality and quantity of the isolated nucleic acids were determined using the NanoDrop™ 2000 spectrophotometer (Thermo Scientific) and agarose electrophoresis (BioRad).

Amplification of the 16S rRNA gene

Eubacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Lane, 1991) and 1507R (5'-TACCTTGTTACGACTTCACCCCA-3') (Heyndrickx et al*.,* 1996) were used in polymerase chain reaction (PCR) for the amplification of the 16S rRNA gene of each of the isolates. The PCR mixtures contained 12.5 µl DreamTaq Master mix (2x) (Fermentas, 140 St. Leon-Rot, Germany), 0.5 µl of each primer (10 pmol), 8.5 µl of nuclease-free water (Fermentas, 140 St. Leon-Rot, Germany) and 5 µl of DNA template. The PCR mixtures were placed in an MJ MINI thermal cycler (BIORAD), and the following thermal cycling conditions were used: pre-denaturation for 10 min, followed by 35 amplification cycles of denaturation at 94°C for 30 s, annealing of primers with template DNA at 55°C for 30 s and primer extension at 72°C for 30 s. This was followed by a final extension at 72°C for 7 min. The PCR amplicons were resolved through electrophoresis of 1% (w/v) agarose gel stained with ethidium bromide, followed by visualisation under ultraviolet light. The low-range Fast Ruler

Table 2. Physicochemical quality of borehole samples analysed in 8 local municipal areas of North West Province during the study period. (n=3 per borehole).

Local municipality	pH value			Temperature (°C)			Turbidity (NTU)			TDS (mg/l)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
Ramotshere Moiloa	N/A	6.5	7.7	24.3 (±0.89)	22.2	25.5	7.63 (±3.78)	0.33	7.89	270.8 (±35.35)	85.51	502.00
Tswaing	N/A	6.9	7.8	24.2 (±0.40)	23.3	24.7	0.93 (±0.46)	0.20	1.60	461.6 (±71.09)	354.20	575.07
Mafikeng	N/A	6.9	8.2	24.1 (±1.06)	21.1	25.8	1.94 (±0.66)	0.33	4.40	486.2 (±84.41)	289.29	630.61
Kagisano	N/A	7.1	7.9	24.4 (±0.29)	23.9	24.8	3.51 (±1.73)	0.19	4.80	403.9 (±44.15)	267.20	635.10
Greater Taung	N/A	7.2	7.9	21.2 (±0.86)	20.3	21.7	1.37 (±0.13)	0.30	2.68	568.7 (±89.49)	376.00	639.03
Moretele	N/A	7.3	8.5	22.5 (±0.47)	22.0	23.1	2.54 (±1.22)	1.15	5.17	567.7 (±79.57)	123.74	578.12
Madibeng	N/A	7.7	8.5	24.1 (±0.60)	23.3	25.1	2.61 (±0.33)	0.37	3.03	508.7 (±74.00)	186.01	530.00
Moses Kotane	N/A	7.7	9.1	23.5 (±0.60)	22.6	24.7	0.73 (±0.72)	0.21	3.21	325.2 (±64.06)	51.53	478.08

N/A, Not applicable; Min, minimum; Max, maximum; Limit for no risk: pH, 5 to 9.5, temperature, 18 to 25°C; TDS, 0 to 500 mg/l; turbidity, 0 to 1 NTU (DWAF, 1996; SANS 241, 2006).

(Fermentas) was included in all gels as size marker. All results were captured using a gel documentation system (Syngene, Cambridge, UK).

Restriction analysis of PCR amplicons

In order to select representative isolates for sequencing, all PCR amplicons were subjected to restriction fragment-length polymorphism (RFLP) analysis. For this purpose, 10 µl of the 16S rRNA amplicons was digested with *Taq1* and *Cs6pI* (Fermentas) according to the manufacturer's instructions. The restriction digests were resolved through electrophoresis of conventional 1.5% (w/v) agarose gel stained with ethidium bromide, followed by visualisation under ultraviolet light. The hyperladder 1 (marker) 100 lines (Bioline) was included in all gels as size marker. All results were captured using a gel documentation system (Syngene, Cambridge, UK). The restriction patterns were determined manually for every five similar profiles. The selected isolate was then ready for sequencing.

Sanger sequencing of the 16S rRNA gene

After grouping the isolates using the PCR-RFLP, the genomic DNA from 32 representative water samples was amplified using the existing 27 F and 1507 R primers as described earlier. The 1500 PCR amplicons were further studied by conventional Sanger (dideoxy) sequencing in both directions using 27 F and 1507 R primers. For this purpose, BigDye for ABI 3130XL was used according to the manufacturer's instructions and the gel was run on a 3130XL sequencer. All the sequences were inspected and manually corrected using Bioedit v.5.0.9 (33) software. For preliminary identification of the bacterial isolates, the corrected sequences were then compared to those in the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) using BLASTn.

RESULTS

Physicochemical characteristics

The physicochemical parameters of the groundwater samples from the North West Province collected during September and October, 2008 are shown in Table 2. The values of pH from water samples of the selected boreholes in local municipalities of North West were observed to be near neutral, ranging from 6.5 to 9.1, with the highest value observed in the Moses Kotane municipal area. In addition, the temperatures of the groundwater samples were observed to range from 20.3 to 25.8°C. The TDS levels of the water samples ranged between 51.53 to 639.03 mg/l, and the highest level was observed in the Greater Taung municipal area. The turbidity levels of all groundwater samples ranged between 0.20 and 7.63 NTU. The highest recorded level of turbidity was 7.63 NTU, in the Ramotshere Moiloa municipal area.

The results of the chemical analysis as shown in Table 3 revealed that the sulphate concentrations of all groundwater samples ranged between 10.42 and 159.38 mg/l, with the highest concentration observed in the Moretele municipal area. The chloride concentrations were highest in the Moretele municipal area (511.89 mg/l) and lowest in the Ramotshere Moiloa municipal area (11.00 mg/l). With regard to nitrates and fluoride concentrations in the water samples, values were observed to range from 0.10 to 14.80 mg/l nitrates and from 0.10 to 41.40 mg/l fluorides, respectively. The highest nitrate concentration of 14.80 mg/l as N was found in the Moses Kotane (Voordonker) local municipal area and the

Table 3. Chemical quality of borehole samples analysed in 8 local municipal areas of North West Province during the study period. (n=3 per borehole).

Local municipality	Chloride (mg/l)			Sulphate (mg/l)			Nitrate (mg/l)			Fluoride (mg/l)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
Ramotshere Moiloa	19.23 (±2.73)	11.00	50.98	14.47 (±1.98)	11.53	18.04	5.00 (±1.68)	2.88	8.00	2.80 (±1.68)	0.64	6.00
Tswaing	59.60 (±11.02)	16.20	66.95	31.31 (±7.21)	12.31	77.54	7.25 (±2.56)	3.61	9.99	3.07 (±0.15)	0.98	8.25
Mafikeng	55.02 (±5.62)	15.98	27.78	31.11 (±8.50)	11.68	128.30	4.62 (±2.89)	0.44	9.16	2.40 (±0.33)	0.10	5.52
Kagisano	89.10 (±11.09)	44.99	73.94	15.65 (±1.58)	12.36	25.81	1.71 (±0.97)	0.48	2.98	1.02 (±0.73)	0.12	2.10
Greater Taung	82.30 (±9.78)	37.99	51.95	120.33 (±9.72)	21.20	152.58	6.52 (±0.51)	3.28	8.59	5.79 (±0.94)	1.00	9.66
Moretele	147.76 (±12.57)	19.43	211.89	98.83 (±15.76)	14.59	159.38	3.16 (±0.88)	0.34	7.91	29.56 (±3.26)	9.89	41.40
Madibeng	148.67 (±12.73)	30.00	186.11	91.24 (±14.08)	25.47	110.33	0.78 (±0.18)	0.10	1.28	12.60 (±1.28)	5.70	16.00
Moses Kotane	28.25 (±3.12)	11.65	35.99	17.23 (±1.60)	10.42	89.97	11.05 (±0.56)	4.73	14.8	28.35 (±1.43)	17.8	33.48

Limit for no risk: Chloride, 0 to 100 mg/l; sulphate, 0 to 200 mg/l; nitrate, 0 to 6 as N mg/l; fluoride, 0 to 1 mg/l (DWAF, 1996; SANS 241, 2006).

Table 4. Chemical quality of borehole samples analysed in eight local municipal areas of North West Province during the study period. (n=3 per borehole).

Local municipality	Sodium (mg/ℓ)			Potassium (mg/ℓ)			Magnesium (mg/ℓ)			Calcium (mg/ℓ)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
Ramotshere Moiloa	4.77 (±1.27)	1.38	16.16	10.61 (±2.60)	3.86	32.73	24.55 (±4.38)	5.31	62.28	14.77 (±2.54)	5.11	38.16
Tswaing	18.48 (±4.05)	6.05	43.38	17.28 (±3.38)	2.74	38.49	22.22 (±3.49)	3.98	39.17	41.12 (±5.87)	12.82	73.94
Mafikeng	17.04 (±1.41)	4.25	29.18	9.74 (±1.59)	0.40	25.56	32.53 (±2.48)	17.33	65.29	26.02 (±3.06)	5.30	57.69
Kagisano	13.96 (±2.12)	7.69	26.23	8.97 (±1.81)	0.11	13.53	21.17 (±4.34)	0.22	40.06	72.57 (±9.11)	41.04	145.8
Greater Taung	17.30 (±4.39)	0.61	55.83	10.59 (±2.83)	1.38	37.42	51.44 (±5.76)	21.23	89.64	34.60 (±5.63)	17.10	82.12
Moretele	125.62 (±6.04)	25.97	346.92	9.21 (±2.43)	2.64	22.05	20 57 (±6.46)	1.63	45.78	61.62 (±8.02)	20.06	136.2
Madibeng	121.01 (±5.97)	33.14	386.87	5.07 (±0.78)	2.89	9.06	15.35 (±3.62)	1.86	26.60	52.22 (±9.91)	15.23	104.2
Moses Kotane	67.18 (±3.14)	23.29	80.12	2.68 (±0.91)	0.09	14.04	48.44 (±5.11)	20.87	82.78	30.46 (±5.00)	9.61	68.93

Limit for no risk: Sodium, 0 to 100 mg/l; potassium, 0 to 50 mg/l; magnesium, 0 to 30 mg/l; calcium, 0 to 32 mg/l (DWAF, 1996; SANS 241, 2006).

highest fluoride concentration, namely 41.40 mg/l, was found in the Moretele (Dikebu) local municipal area.

In addition, sodium, potassium, magnesium and calcium concentrations were also measured and evaluated from borehole samples in different local municipal areas (Table 4). The Madibeng municipal area had the highest recorded sodium concentration (386.87 mg/l), while the lowest was in the Greater Taung municipal area (0.61 mg/l). The potassium concentration was the lowest in the Moses Kotane municipal area (0.09 mg/l) and highest in the Tswaing municipal area (38.49 mg/l). The values recorded for magnesium and calcium concentrations in the water samples ranged from 0.22 to 89.64 mg/l and 5.11 to 145.80 mg/l, respectively. The highest magnesium concentration was observed in the Greater Taung municipal area (89.64 mg/l) and the highest calcium concentration was observed in the Kagisano municipal area (145.80 mg/l).

Table 5. Microbial quality of borehole samples analysed analyzed in eight local municipal areas of North West Province during the study period (n=3 per borehole).

Local municipality	Total coliforms (cfu/100 ml)			Faecal coliforms (cfu/100 ml)		
	Average	Min	Max	Average	Min	Max
Ramotshere Moiloa	73 (±11)	1	240	4 (±2)	0	25
Tswaing	116 (±6)	1	365	12 (±5)	0	45
Mafikeng	105 (±10)	1	440	7 (±1)	0	42
Kagisano	95 (±9)	3	185	5 (±2)	0	12
Greater Taung	126 (±10)	24	328	7 (±3)	0	34
Moretele	76 (±8)	0	320	3 (±1)	0	12
Madibeng	81 (±8)	1	400	8 (±1)	0	50
Moses Kotane	118 (±6)	4	460	11 (±4)	0	58

Limit for no risk: Total coliforms (TC), 0 to 5 cfu/100 ml; Faecal coliforms (FC), 0 cfu/100 ml (DWAF, 1996; SANS 241, 2006).

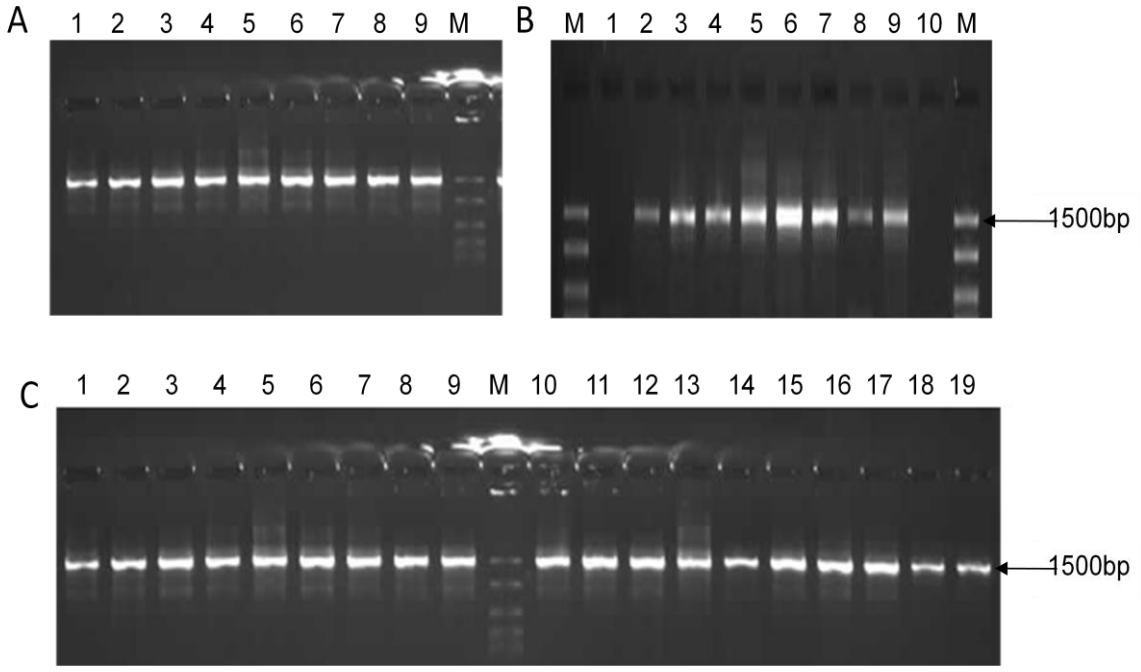

Figure 2. An example of an agarose gel electrophoresis for the amplified PCR product.

Microbiological characteristics

The analyses of the coliform counts obtained from various borehole samples in the different local municipal areas are shown in Table 5. The bacterial counts of all water samples ranged between 1 and 460 cfu/100 ml with regard to total coliforms and between 0 and 58 cfu/100 ml with regard to faecal coliforms. The highest total (460 cfu/100 ml) and faecal (58 cfu/100 ml) coliform counts were recorded in the Moses Kotane municipal area.

Out of 100 borehole samples tested, a total of 72 isolates were oxidase-negative and 64 were processed for genomic DNA extraction. The concentrations of the quantified DNA ranged from 2.0 to 80.0 ng/µl. To identify the possible pathogenic bacteria in groundwater samples, DNA was amplified using universal eubacterial primers of 27F and 1507R. Figure 2 shows the PCR amplicons of the 16S rRNA fragment for water microorganisms. All the samples showed a single band of 1500 bp in agarose gel, indicating the successful amplification of the targeted gene of 16S rRNA from the isolates. The gel showed the

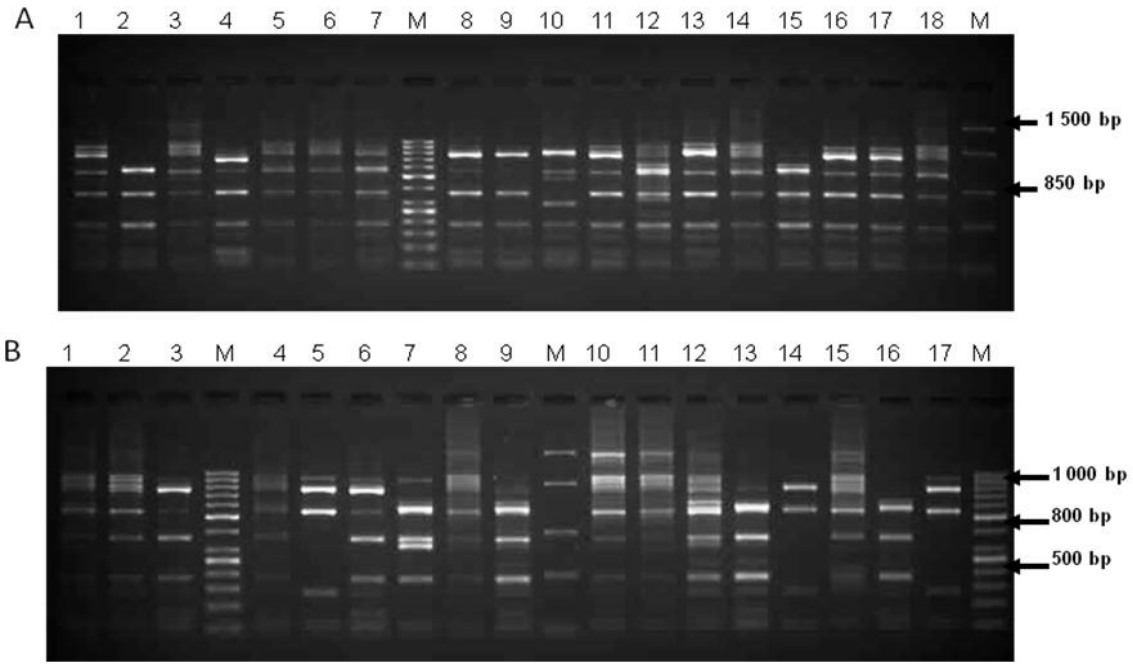

Figure 3. Example of the agarose gel electrophoresis for the restriction fragment profiles of the group-specific PCR products after digestion with (A) *Cs6pI* enzyme and (B) *Taq1* enzyme. Lane M represents molecular weight marker (1500 bp and 1000 bp DNA size ladder).

example of the amplified PCR product of the 36 isolates where Lane 1 and Lane 10 in gel B represent the positive control and Lane M represents the molecular weight marker (1500 bp ladder). These products were further subjected to restriction fragment analysis by using *Cs6pI* enzyme that distinguished two different species, and when using *Taq1* enzyme, 9 different species were identified. These PCR-RFLP types were collapsed into groups based on similarity (Figure 3). The 28 representative PCR amplicons were subjected to DNA sequencing with the original primers 27F and 1507R in both directions. These complete sequences were then probed using the NCBI BLASTn program. The sequences retrieved from the NCBI database giving the closest match in pair-wise BLASTn were identified as *Serratia marcescens, Aeromonas veronii, Citrobacter freundii, Salmonella enterica, Morganella morganii, Bacillus cereus, E. coli, S. dysenteriae and Enterobacter cloacae.*

DISCUSSION

An adequate safe potable water supply in rural communities is essential in order to satisfy basic needs. Consequently, efficient monitoring and management of groundwater is very important to reduce the number of diarrhoeal infections that might pose a public health risk to consumers. This study demonstrated (Table 2) that the pH and temperature values of all the water samples from the different local municipal areas were within the recommended limit of no risk for drinking and domestic purposes of 5 to 9.5 and 18 to 25°C (SANS 241, 2006; DWAF, 1996). The TDS levels of the water samples revealed that 69% of the borehole samples were within the recommended limit of 500 mg/l TDS for no risk (SANS 241, 2006; DWAF, 1996), whereas 10% of water samples collected in Mafikeng and Greater Taung, 6% in Moretele and 5% in Madibeng were above the recommended limit of no risk in terms of TDS. A study conducted by NPWG (2000) also recorded high values of TDS of 580, 850 and 1360 mg/l for Mafikeng and Greater Taung, Madibeng and Moretele. TDS represents the amount of inorganic substances (salts and minerals) in solution. Water with a high TDS usually has an objectionable or offensive taste. A higher concentration of TDS usually poses no health threat to humans until the values exceed 3000 mg/l (DWAF, 1996). In addition, the results also showed that 53% of the borehole samples were within the recommended limits (0 to 1 NTU) for potable water (DWAF, 1996; SANS 241, 2006) with regard to turbidity. However, it was observed that groundwater was turbid in 8 local municipal areas (13% in Mafikeng, 10% in Ramotshere Moiloa, 8% in Moretele, 5% in Madibeng, 4% in Greater Taung, 3% in Tswaing and Kagisano and only one borehole in Moses Kotane) and cannot be considered safe for drinking purposes. The Ramotshere Moiloa local municipality was observed to have a high turbidity level of 7.63 NTU, which might be

due to silt, as the water sample appeared to be brown in colour. Turbidity in water is caused by the presence of suspended matter, which usually consists of a mixture of inorganic matter, such as clay and soil particles, and organic matter (DWAF, 1996). A high level of turbidity in water is often associated with the possibility of microbiological contamination, as high turbidity makes it difficult to disinfect water effectively (DWAF, 1998; WHO, 2006).

The results of the chemical analysis as shown in Table 3, revealed that the sulphate concentrations of all groundwater samples were within the recommended limit of no risk (0 to 200 mg/l) (DWAF, 1996; SANS 241, 2006). The chloride concentrations in the water samples were within the acceptable limit of 0 to 100 mg/l set by DWAF (1996) and SANS 241 (2006), except for 2 local municipalities, namely Moretele (Kgomo-Kgomo and Swartdam) and Madibeng (Shakung 1 and Dipompong). Chloride has no aesthetic or health effects, but the main issue is its ability to increase the corrosion rate of domestic appliances (DWAF, 1996; WHO, 2007). With regard to nitrates, the results revealed that 40% of the borehole samples were above the recommended limits (0 to 6 mg/l as N) for potable water (DWAF, 1996; SANS 241, 2006). These water samples were collected from Moses Kotane (14%), Mafikeng (8%), Greater Taung (8%), Tswaing (5%), Ramotshere Moiloa (4%) and Moretele (1%). Most of the boreholes are privately owned and situated next to onsite unsanitary systems. Consequently, the presence of nitrate in groundwater is usually due to agricultural activity or leaking effluent from on-site sanitation and it is also associated with the simultaneous presence of bacterial contamination (WHO, 2007). However, NPWG (2000) reported that nitrates occur naturally in the Moretele district as inorganic nitrate due to the geological formations such as basalt rocks. High nitrate concentrations can cause methaemoglobinaemia (blue-baby syndrome) in bottle-fed infants and could result in the occurrence of mucous membrane irritation in adults (DWAF, 1996; WHO, 2007). The fluoride concentration results showed that 83% of borehole samples were above the recommended limit of 0 to 1 mg/l (DWAF, 1996; SANS 241, 2006). These water samples were collected from Mafikeng (20%), Moses Kotane (15%), Ramotshere Moiloa (11%), Greater Taung (11%), Moretele (8%), Tswaing (7%), Madibeng (7%) and Kagisano (4%). The present results were in accordance with findings of other researchers (NPWG, 2000; Ncube and Schutte, 2005). Health problems associated with the condition known as fluorosis may occur when fluoride concentrations in groundwater exceed 1.5 mg/l and staining of tooth enamel may become apparent (dental fluorosis). With continued exposure, teeth may become extremely brittle (DWAF, 1996). The incidence and severity of dental fluorosis, and the much more serious skeletal fluorosis, depend on a range of factors including the quantity of water consumed and exposure to fluoride from other sources, such as high-fluoride coal, as was noted in China (WHO, 2006).

The results shown in Table 4 revealed that the all borehole samples were within the recommended limits for no risk (0 to 50 mg/l) with regard to potassium (DWAF, 1996). With regard to sodium, the borehole samples were within the recommended limits for no risk (0 to 100 mg/l) (DWAF, 1996), except for three boreholes in the Moretele (Noroki, Kgomo-Kgomo and Swartdam) and two boreholes in the Madibeng (Shakung 1 and Dipompong) local municipal areas. There is no indication of adverse health effects in the general population associated with high sodium levels in drinking water, although such water may not be suitable for bottle-fed infants because of its faintly salty taste (DWAF, 1996; WHO, 2007). The results also showed that 45% of borehole samples were above the recommended limits of 0 to 30 mg/l and 0 to 32 mg/l (DWAF, 1996) with regard to magnesium, and 43% with regard to calcium. It is important to note that the 45% of the boreholes with high concentrations of magnesium included 13 boreholes located in Moses Kotane, 12 in Greater Taung, 10 in Mafikeng, 4 in Ramotshere Moiloa, 3 in Moretele, 2 in Kagisano and 1 in Tswaing. With regard to calcium, 10 of the boreholes were located in Mafikeng, 8 in Kagisano, 6 in Moretele, 5 in Madibeng and 5 in Moses Kotane, 4 in Tswaing, 4 in Greater Taung and 1 in Ramotshere Moiloa. The presence of high calcium and magnesium in water contributes to water hardness. Groundwater in the dolomitic areas like North West and the northern parts of the country tends to be very hard. This usually has no health implications, except where concentrations of magnesium are extremely high. Magnesium has a bitter taste and may have a laxative effect on people not accustomed to the water (WHO, 2007). Magnesium, together with calcium, is responsible for scaling problems in appliances using heating elements and plumbing (DWAF, 1996; WRC, 1998). High concentrations of calcium impair the lathering of soap (DWAF, 1996; WRC, 1998; WHO, 2007).

Table 5 shows the coliform counts of the water samples analyzed in different local municipalities. In general, both the total and faecal coliform counts in all the local municipalities were above the South African recommended limits for drinking water that is meant for domestic purposes. According to the South African guidelines, the total number of coliforms in drinking water should range between 0 to 5 cfu/100 ml and less than one *E. coli* per 100 ml, while the number of faecal coliforms should be 0 per 100 ml water sample (DWAF, 1996; SANS 241, 2006). The results indicated that, of the 100 boreholes, 86% had more than 5 cfu/100 ml total coliform counts. The highest coliform counts of 460 cfu/100 ml were recorded in the Moses Kotane (Mantsho) local municipality. In Moses Kotane local municipality, most of the boreholes are constructed very close to the pit-latrine toilets. Traditionally, total coliform bacteria were regarded as belonging to the genera *Escherichia*,

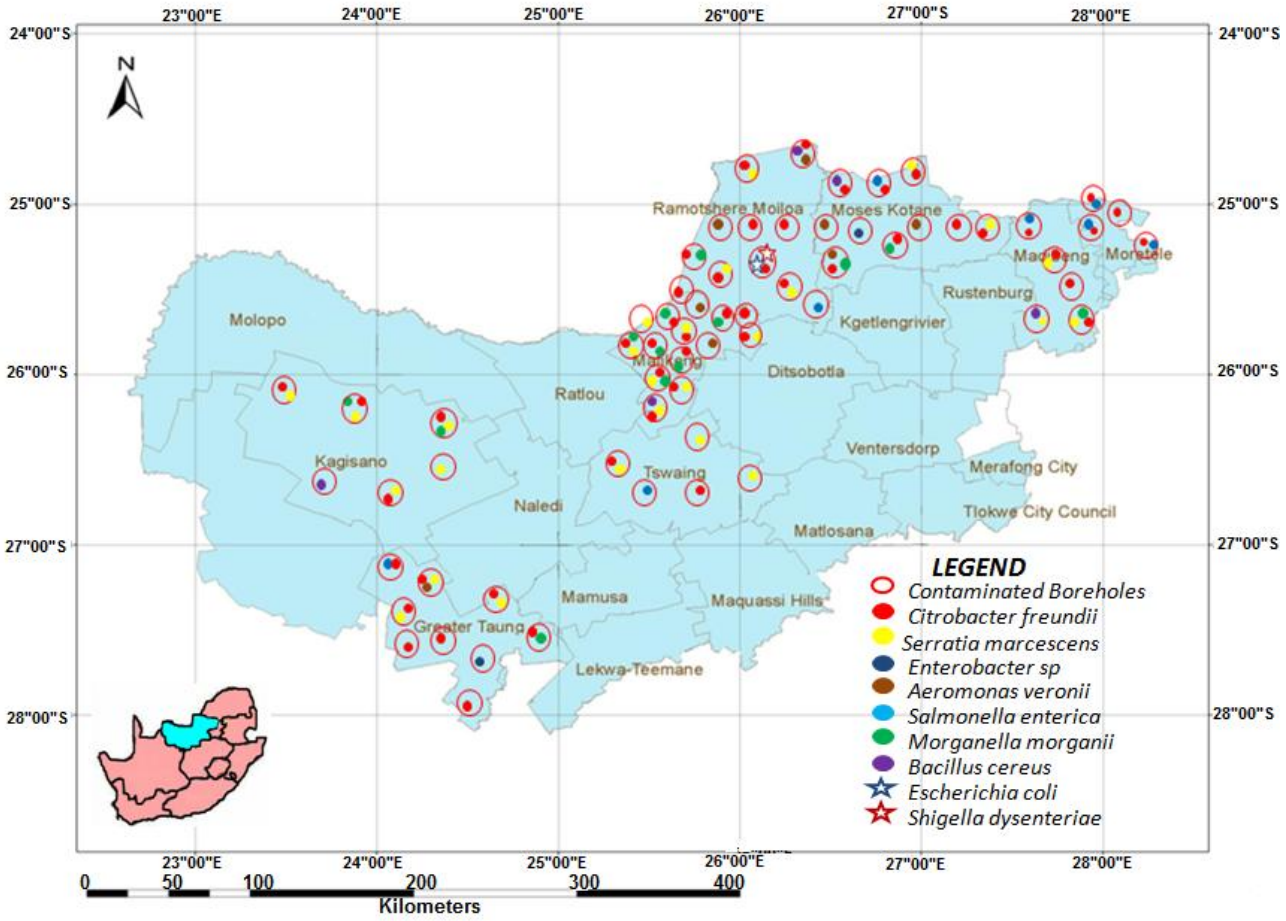

Figure 4. Map of North West Province indicating contaminated boreholes with microorganisms identified in the local municipal areas of Moretele, Madibeng, Moses Kotane, Ramotshere Moiloa, Mafikeng, Kagisano, Tswaing and Greater Taung.

Citrobacter, *Klebsiella* and *Enterobacter*, but the group is actually more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The group includes bacteria of faecal origin and indicates the possible presence of bacterial pathogens such as *Salmonella* spp., *Shigella* spp. and pathogenic *E. coli*, especially when detected in conjunction with other faecal coliforms (DWAF, 1996; WHO, 2006). As far as this group of coliforms is concerned, the results of this study indicated that 77% of the boreholes had less than one faecal coliform per 100 ml of drinking water and that 23% of the boreholes were faecally contaminated and were not within the recommended limits (Figure 3). The highest faecal coliform counts of 150 cfu/100 ml were observed in the Ramotshere Moiloa local municipal area. High concentrations of faecal coliforms in water indicate a risk of contracting waterborne disease, even if small amounts of water are consumed (DWAF, 1996). The culture-based techniques used in this study showed only the general hygienic quality and faecal pollution of groundwater sources based on the detection of both total coliforms and faecal coliforms.

Moreover, the presence of coliform bacteria in water samples might indicate just the possible presence of bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *V. cholerae, Campilobacter jejuni, Campilobacter coli, Yersinia enterocolitica* and pathogenic *E. coli* (DWAF, 1996). Consequently, the range of bacterial pathogens that might result in diseases and sicknesses in the province should be accurately proved. For this purpose, selected coliform isolates were subjected to a subsequent molecular analysis, since molecular tools for microbial diagnosis rely on the *in vitro* amplification of a DNA fragment and offer a higher level of the specificity of strain detection (Rompré et al., 2002; Beneduce et al., 2007). The results of the molecular study revealed that, of 100 boreholes, 51% tested positively for *C. freundii,* 28% for *S. marcescens,* 12% for *M. morganii,* 8% for *S. enterica,* 7% for *A. veronii,* 5% for *B. cereus*, 2% for *E. cloacae, and* 1% for *E. coli* and for *S. dysenteriae* (Figure 4).

The presence of the above opportunistic pathogens in the groundwater samples indicates that communities in the North West Province, especially immuno-

compromised individuals, infants and the elderly, are at a potential risk of contracting infections and diseases such as bacillary dysentery, respiratory infections, urinary tract infections and gastroenteritis during exposure to or consumption of groundwater from these sources (Payment et al., 1991; Bartram et al*.,* 2003). Enteric pathogens such as *E. coli, S. dysenteriae, S. enteric* and *B. cereus* are major causes of diarrhoea and bacillary dysentery everywhere in the world (Gray, 1995; EFSA, 2007; WHO, 2006). In South Africa, diarrhoeal diseases are responsible for about 20% of all deaths of 1 to 5 years old (MacKintosh and Colvin, 2003). In addition, *M. morganii* causes a disease known as "Summer Diarrhoea", which is also often encountered in postoperative patients and is mainly associated with urinary tract infections (Cox, 1985; Senior and Voros, 1990). *C. freundii, S. marcescens, A. veronii and E.r cloacae* are known to cause a wide variety of nosocomial infections of the respiratory tract and urinary tract (Hejazi, 1997; Keller et al., 1998; Whalen et al., 2007). *A. veronii* can cause infections in humans, including septicaemia, particularly in immunocompromised patients, and in patients with wound infections and respiratory tract infections. There have been some claims that *A. veronii* can cause gastrointestinal illness, but epidemiological evidence is not consistent (Song et al., 2004). The outcome of this study revealed that the quality of groundwater supplied in some rural areas of the North West Province poses a health risk for the community.

CONCLUSIONS AND RECOMMENDATIONS

Based on the outcome of the study, it could be ascertained that there is evidence of physicochemical and microbiological pollution of the groundwater supplied to the communities living in rural areas of the North West Province. The high concentrations of total coliforms (>400 times), faecal coliforms (>50 times), fluoride (>41 times), turbidity (>7 times), calcium (>4 times), nitrate (>2 times), magnesium (>2 times) and TDS (>1 times) in these water sources when compared to the exceedance level in the guidelines indicate that the water is not fit for human consumption. The detection of various opportunistic pathogens and pathogenic strains such as *S. marcescens, A. veronii, C. freundii, S. enterica, M. morganii, B. cereus, E. coli, S. dysenteriae and E. cloacae* indicates that communities in rural areas of the North West Province are at a constant risk of contracting waterborne diseases. Consequently, this study calls for urgent involvement by the government to provide protection of groundwater sources and drinking water treatment barriers to ensure the safe distribution of potable water in the province.

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