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Identification and enumeration of microbial organisms in grey water in the Khayelitsha informal settlement, Western Cape, South Africa

Qenehelo A. Leuta, James P. Odendaal and Arnelia N. Paulse

ABSTRACT

Grey water, as opposed to blackwater, is generally assumed to be a safer and more acceptable wastewater source that could be considered for reuse. This is mainly due to a common misconception that its microbial load is lower compared to that of blackwater (domestic sewage). This study aimed to determine the presence of microbial contaminants, specifically pathogens, in stagnant grey water in the RR Section in Khayelitsha, Western Cape. The most probable number (MPN) technique was conducted to determine faecal coliform (FC) and *Escherichia coli* (EC) counts in the samples. The API 20E and the RapID ONE systems were used to identify possible pathogenic Gram-negative microorganisms, while the isolated Gram-positive microorganisms were identified using the BBL Crystal Gram-Positive (GP) Identification (ID) system. The highest respective FC and EC counts observed during this period were recorded as 2.8×10^7 microorganisms/100 mL (Site F, week 5). The RapID ONE and the API 20 identification systems identified mostly *Escherichia coli, Klebsiella* species and *Enterobacter cloacae*, amongst others, while the BBL Crystal-GP ID system identified mostly *Corynebacterium* and *Bacillus* species. The presence of these organisms raises health concerns for the community of RR Section.

Key words | API 20E, BBL Crystal Gram-Positive Identification, grey water, informal settlements, RapID ONE

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INTRODUCTION

The South African Government has a standard rule for providing basic water and sanitation in informal settlements with a minimum of one tap for every 25 households within a distance of 200 meters and one toilet for every five households (City of Cape Town 2011). However, although these basic services are provided, the maintenance of sanitary facilities and water collection points, i.e. communal standpipes, are not well managed. This study was conducted in collaboration with the Social Justice Coalition who campaigns for the constitutional rights of all people, especially those living in informal settlements. The microbial quality of grey water is primarily measured by the presence of indicator microorganisms (Rodda *et al.* 2010). These stagnant grey water pools, containing various solid material varying between food and different waste particles, provide a breeding ground for potential pathogens such as *Escherichia coli*, *Vibrio cholerae* and *Pseudomonas aeruginosa* (Granfone *et al.* 2008) to accumulate. However, previous literature have implicated the presence of pathogenic microorganisms such as *P. aeruginosa* (Khalaphallah & Andres 2012), *Salmonella*, *Shigella* and *V. cholerae* (Nganga *et al.* 2012) in the grey water.

Previously, communities such as those in Khayelitsha had a system of public toilets connected to the township sewer and running water that were provided by communal taps scattered throughout the settlement. However, most of the flush toilets and water taps broke down and some were often in a very bad state with poor hygienic conditions (South African Slum Dwellers International Alliance 2018).

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Lali (2018a, 2018b) conducted interviews in various Khayelitsha communities, such as the K2 community where municipal workers claimed that K2 had the worst kind of toilet facilities because they are not cleaned on a regular basis. Some toilets were not only blocked and dirty, but some had no doors or cisterns. In certain toilets, taps were lacking or sinks were blocked. Workers claimed that the blockages were due to illegal connections to the sewer system, which caused sewage to back up and thus clog the facilities. In another article, residents in the RR Section were also struggling with poor living conditions, i.e. lack of proper sanitary facilities and the availability of water and electricity (Lali 2018a, 2018b).

In the current study, similar conditions were observed in the RR Section in Khayelitsha. Figure 1 illustrates the RR Section in the informal settlement of Khayelitsha near Cape Town in the Western Cape. In 2009, the director of Strategy, Support and Coordination for the City of Cape Town reported a total of 569 communal toilets and 61 standpipes in RR Section (City of Cape Town 2009). To date, the conditions in the RR Section has seemingly not changed significantly. In 2011, various surveys conducted in the RR Section identified the presence of approximately 4,000 households, with an estimated 25 households per communal tap and one toilet per five households. At the time, it was established that the ratio of allocated communal taps and available toilets to the number of households in the RR Section, was not sufficient. The poor condition and management of certain sanitary facilities and standpipes led to a heightened burden being placed on those facilities that were in working order (Silber 2011; Department of Monitoring and Evaluation (DPME) & Department of Human Settlement (DHS) 2012).

The broken toilets and standpipes could also be a result of the poor quality of the construction or building materials used (DPME & DHS 2012). At the same time, the inadequate provision of toilets and standpipes led to an overload of the existing sanitation infrastructure. Coupled with infrequent or inadequate maintenance, this led to many toilets and standpipes breaking down (SJC 2011).

According to the City of Cape Town municipality, the area where most informal settlements, like Khayelitsha, are located makes it difficult to construct proper and adequate sanitary facilities that could accommodate all the inhabitants. The City of Cape Town municipality stated that full flush toilets cannot be installed on privately owned property, in areas of extremely high density, under power lines or on landfill sites. In addition, full flush toilets may not be constructed in flood plains, outside an urban edge and in or close to water bodies or retention ponds.

With the broken down sanitary facilities, inhabitants have no choice but to make use of plastic bags or night pots in which they urinate and defecate at night (Tokota 2012), which are emptied in the morning and cleaned at communal standpipes (Tokota 2012). This results in the water at the base of the standpipes becoming contaminated with human faecal waste.

The raw human waste from the night pots, i.e. diluted faecal matter and urine, has found its way from broken sanitary facilities to these stagnant pools at the base of the standpipes (Figure 2), forming a mixture of unidentified and possibly pathogenic organisms (SJC 2011), and increasing the possibility of disease outbreak in the already confined and overpopulated area (SJC 2011). The problem is exacerbated by the inhabitants' inadequate knowledge of pathogens and its possible presence at standpipes, which are also sometimes used as a wash-up area for dishes, laundry and food (Granfone *et al.* 2008).

It can therefore be deduced that the grey water accumulating at the base of the standpipes, with the addition of microbial pollutants, cannot be regarded as grey water anymore, but rather as blackwater instead. The following techniques were employed to determine the microbial contamination level as well as the identification of possible microbial pathogens in the sampled water.

The Most Probable Number (MPN) enumeration technique determines the planktonic microbial levels associated with faecal contamination. This method provides a statistical estimate of the bacterial density within a sample (UNICEF 2008). It is easy to conduct with little requirement for specialised equipment and is best for enumerating cells in highly turbid or contaminated samples (UNICEF 2008). In addition, the technique distinguishes between the faecal coliforms (FC) and *E. coli* (EC) counts in water samples (Rompre *et al.* 2002).

Flow cytometry (FCM) is an optically based method for analysing individual cells in complex matrices (Castaño-Boldú & Comas-Riu 2012). The technique identifies different cell types within a heterogenous population (Macey 2007). On its own, the FCM technique lacks the ability to

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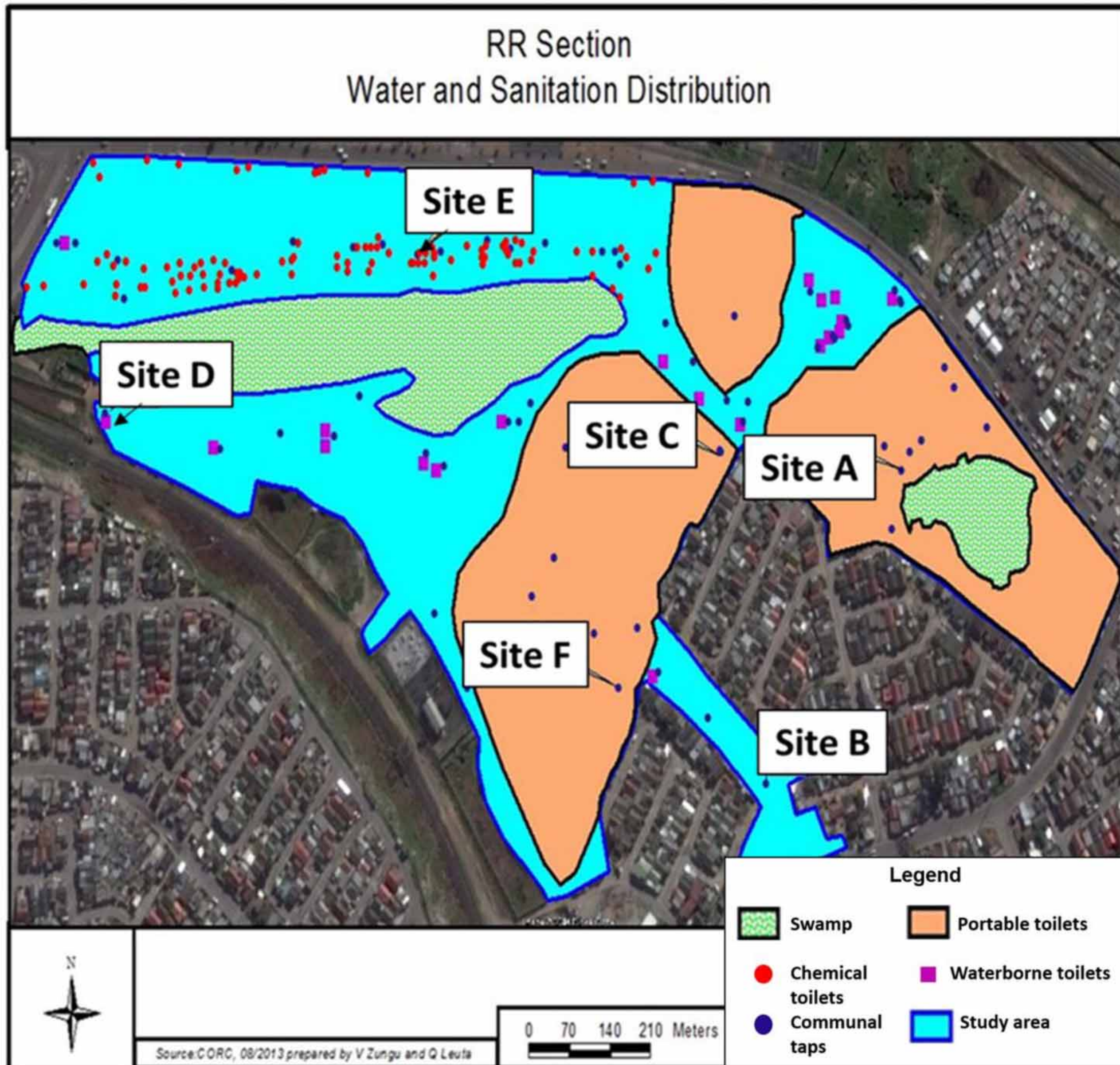

Figure 1 | A map of RR Section showing all the six sampling sites adapted from CORC (2013).

differentiate between dead and viable bacterial cells and therefore should be used in conjunction with the LIVE/DEAD *Bac*Light Viability Probe (Berney *et al.* 2007). With the use of LIVE/DEAD *Bac*Light Viability Probe, the total amount of microorganisms, irrespective of the conditions of the microbial cells, provides a complete indication of the amount of microbial cells in a sample.

The API 20E system is a standardised, miniaturised version of conventional procedures for RapID identification of *Enterobacteriaceae* and other Gram-negative bacteria through the use of 20 miniaturised biochemical tests (or cupules) (Hill *et al.* 2005). When comparing the API 20E system with *invA* polymerase chain reaction (PCA) and 16S rRNA sequencing, Nucera *et al.* (2006) found the API 20E system to be more cost-effective if it is used in small laboratories with limited equipment and low sample number. The system was found to be 100% sensitive and also had 96% specificity compared to the 16S rRNA sequencing in the

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Figure 2 | Example of the stagnant water that was used as sampling points for microbial analyses.

identification of *Salmonella enterica* (Nucera *et al.* 2006). Ndlovu (2013) was able to identify 87% and 85% of colonies isolated from the Berg and Plankenburg Rivers, respectively, using the API 20E system.

The RapID ONE system uses both the conventional and chromogenic substrates for the identification of *Enterobacteriaceae* and other oxidase-negative and Gram-negative bacteria (O'Hara 2005). The system consists of a test strip with 18 wells, which will give 19 test results (O'Hara 2005). This is due to the fact that the last well is bifunctional after addition of the Innova spot indole reagent (O'Hara 2005). Ndlovu (2013), however, found the interpretation of results from the RapID ONE system more challenging compared to the API 20E system. The system can identify *Enterobacteriaceae* without additional tests and results can accurately be acquired on the day of incubation. Ndlovu (2013), when comparing the RapID ONE system to the API 20E system, found the API 20E system to be more sensitive than the RapID ONE system. Ndlovu (2013) was also able to identify 82% and 75% of colonies isolated from the Berg and Plankenburg Rivers, respectively.

The BBL Crystal Gram-Positive (GP) Identification (ID) system is an 18-h miniaturised identification method that uses modified conventional, fluorogenic and chromogenic substrates. It is intended for identification of both Gram-positive cocci and bacilli (Bullock & Aslanzadeh 2013). The system panels consist of 29 dehydrated substrates and a fluorescence control on tips of plastic prongs (also known as spikes) (Becton Dickinson, BD 2012). Venter (2010) found the BBL Crystal GP ID system to be reliable and successfully identified 80% of isolates subjected for identification, which included *Bacillus* spp., such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. megaterium*, and also *Kocuria rosea.* Stovcik *et al.* (2008) successfully used the BBL Crystal GP ID system to identify *Enterococcus faecium*.

This study therefore not only reflects on the possible presence of pathogens, but also provides evidence of the microbial contamination levels and how it compared to acceptable allowable levels in stagnant grey water at the bases of communal standpipes located in the RR Section of the Khayelitsha informal settlement.

METHODS

Sampling sites

Sampling started in January and continued until May. Samples were collected as early in the morning as possible (between approximately 8 am and 11 am). Samples of stagnant grey water were collected twice a month from the base of six communal standpipes for a period of 5 months. The six sampling points (Figure 1) were identified as possible pathogen-containing sites. Figure 2 shows an example of the stagnant water that was used as sampling points for microbial analyses. Where possible, 250 mL water samples were collected in sterile Schott bottles as per the South African Bureau of Standards (SABS) water quality sampling standards (SABS 2008). Upon collection, samples were stored and transported in an ice-filled cooling container for further analyses.

In addition, temperature and pH levels of the water samples at each sampling point were measured with a hand-held mercury thermometer and portable pH meter, respectively (Table 1).

MPN enumeration technique

The MPN technique or Multiple Tube Fermentation technique was used according to the method described by Paulse *et al.* (2007, 2009). The water samples were diluted from 10^{-1} to 10^{-5} and inoculated into Lauryl Tryptose Broth (LTB) tubes containing Durham tubes (presumptive test). Subsequently, positive LTB tubes were re-inoculated

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Table 1 | Physical parameters recorded during the sampling period at all sites

	Site A		Site B		Site C		Site D		Site E		Site F	
Sampling week	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH
1	24	6.2	17.5	6.4	18	6.2	20	6.4	19.5	6.4	21	6.7
2	22	6.2	19	5.4	18	5.8	22	6.7	19	6.4	19	6.7
3	25	6	21	6	20	6	22	5	22	6	25	6.5
4	24	5.8	21	5.8	20	6.2	23	6.7	22	6.2	25	6.4
5	20	6.4	18	6.7	19	6.2	19	4.8	19	5.8	18	6.2
6	17	7.2	16	7.5	15	7.2	16	7.4	16	7.7	11	7.2
7	12	6.3	13	7.2	13	7.4	13	7.4	15	7.2	17	6.9
8	11	6.4	10	6.7	8	6.5	9	6.4	10	6.6	12	6.6
9	12	7.1	12	7.1	11	7	12	6.4	11	6.7	13	6.2

into Brilliant Green Bile Broth and Tryptone Water tubes and incubated (confirmation test). Results for both the presumptive and confirmation tests were compared to the corresponding De Mans codes (FDA/CFSAN – BAM Appendix 2 1998) to obtain the respective FC and EC counts.

FCM analysis

FCM analysis was conducted based on the method described by Paulse *et al.* (2007, 2009), in conjunction with the BD Cell Viability Kit, BD Liquid Counting Beads and the LIVE/DEAD *Bac*Light Viability Probe. For this analysis, however, the SYTO 9 in *Bac*Light was replaced with Thiazole Orange (TO9) for green emittance fluorescence.

The sample was analysed using FACSCalibur and the concentrations of total cell populations were determined using the following equation:

$$\frac{\text{Number of events in cell region}}{\text{Number of events in bead region}} \times \frac{\text{Number of beads/test}}{\text{test volume}} \times \text{dilution factor} \quad (1)$$

Note the bead concentration recorded at 1,043 μL for BD Liquid Containing Beads obtained from BD.

Statistical analysis

Repeated measures analysis of variance (RMA) was performed on all data as outlined by Dunn & Clark (1987) using Statistica. In each RMA, the residuals were analysed to determine their normal distribution. In all hypotheses tests, a significant level of 5% was used as standard.

The MPN results are expressed in microorganisms per 100 mL, whereas the FCM results are expressed in microorganisms per mL.

API 20E system

A serial dilution of 10^{-1} to 10^{-3} was performed on the obtained samples. The diluted samples were directly spread-plated (in duplicate) onto selective media, which included MacConkey, MacConkey Without Salt and Eosin Methylene Blue agars and incubated at 37 °C for 18 to 24 h as per manufacturers' instructions. The isolated colonies were then re-streaked onto Nutrient Agar (NA) plates, incubated for 18 to 24 h and the follow-up procedure completed as per manufacturer's instructions (BioMérieux, South Africa). The ApiWeb identification software (BioMérieux, South Africa) was used to identify the organisms. An *E. coli* laboratory strain was used as a positive control.

RapID ONE system

Bacterial colonies were prepared in the same manner as for the API 20E test kits. As with the API 20E system, only Gram-negative and oxidase-negative colonies were considered for further analysis. The RapID ONE system (Innovative Diagnostic Systems) procedure was followed according to the manufacturer's instructions. Specific isolated organisms were identified by the Electronic RapID

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Compendium on the manufacturer's website. An *E. coli* laboratory strain was used as a positive control.

BBL crystal™ GP ID system

A serial dilution of 10^{-1} to 10^{-3} was performed on the obtained samples. The diluted sample was directly spread-plated (in duplicate) onto selective media, which included Sabouraud Dextrose (SD) and Baird Parker (BP) agars. The isolated colonies were then re-streaked onto NA plates and incubated at 37 °C for 18 to 24 h as per manufacturers' instructions. Prior to identification, a Gram stain was performed on each isolated and selected colony and the results recorded. Only Gram-positive colonies were considered for further analysis. The BBL Crystal GP ID system (BD Microbiology System) procedure was used. This was followed by reading all panels using the BBL Crystal Panel Viewer to interpret the reactions. The reaction results were recorded on the results pad to get a possible isolate profile number. This number, as well as the cell morphology, was then entered into BBL Crystal MIND.

RESULTS AND DISCUSSION

Specific South African guidelines pertaining to the exposure to and reuse of raw grey water regarding acceptable microbial levels could not be found (Rodda *et al.* 2011). For the purpose of this study the obtained enumeration results were compared to the acceptable limits for recreational water sources (Department of Water Affairs and Forestry, DWAF 1996) as children were seen playing close to or with the water from the stagnant pools.

MPN

The results obtained for the total coliforms (total MPN count), FC and EC counts within the water samples are depicted in Figure 3. Only FC and EC results will be discussed, however.

Table 2 indicates the recommended maximum limits for indicator organisms in South African water (DWAF 1996). According to Table 2 the maximum acceptable limit of FC in recreational waters is ≤2,000 microorganisms/100 mL. Most of the FC and EC counts observed and recorded exceeded the acceptable limits as indicated by DWAF (1996).

On average, the MPN counts ranged between 1.7×10^3 microorganisms/100 mL (lowest) recorded at Site E (week 4) and 1.6×10^8 microorganisms/100 mL (highest) observed at Sites A (weeks 3 and 5), B (week 5), D (week 5) and F (week 5). The lowest FC counts observed were 1.3×10^3 microorganisms/100 mL (Site E, week 4), while the highest FC counts were 1.3×10^7 microorganisms/100 mL and 2.8×10^7 microorganisms/100 mL recorded at Site A (week 3) and Site F (week 5), respectively. The EC counts recorded during the sampling period ranged from 0 recorded at Site F (week 9) to 2.8×10^7 microorganisms/100 mL recorded at Site F (week 5). The lowest acceptable EC counts of 0, 70 microorganisms/100 mL and 200 microorganisms/100 mL were all observed at Site F in weeks 7, 8 and 9, respectively. No significant trend influencing the microbial counts could be observed at the same site during the different weeks of sampling.

The infrequent and inadequate maintenance of communal toilets and taps, as well as security concerns, causes inhabitants to either resort to urinating and defecating in plastic bags or in night pots (Bregman 2011; Tokota 2012) throughout the course of the night. As the night pots are emptied in the morning and cleaned at the standpipes (Granfone *et al.* 2008; SJC 2011), it introduces human waste carrying possible pathogenic microorganisms into the stagnant water pools. The result is the high FC and EC counts as illustrated in Figure 3. As previously mentioned, the poor drainage and raw sewage from broken toilets may combine with rainwater where it finds its way to the stagnant pools at the base of the taps (SJC 2011).

FCM analysis

The total FCM counts (all microorganisms, i.e. alive, injured or dead), recorded throughout the sampling period is represented in Figure 4. The total FCM counts ranged from the lowest, 4×10^6 microorganisms/mL at Site E in week 1 to the highest, 3.4×10^9 microorganisms/mL at Site A in week 5.

The highest total FCM counts recorded at each of the sampling sites throughout the entire sampling period were 3.4×10^9 microorganisms/mL (Site A, week 5), 8.9×10^7 microorganisms/mL (Site B, week 5),

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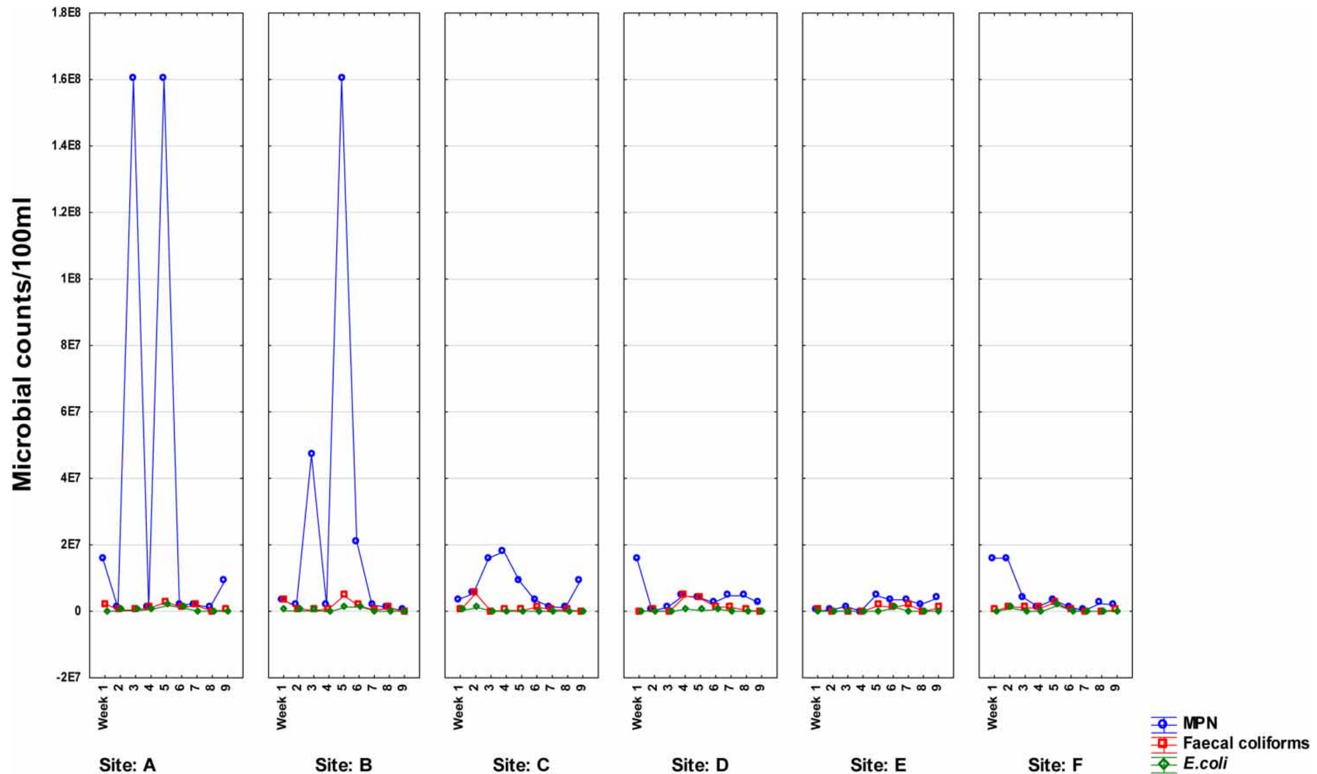

Figure 3 | Comparison of the total MPN, demonstrating all possible gas-producing organisms, FC, and *E. coli* per 100 mL of stagnant water pools sample for all sites.

Table 2 | Recommended guidelines for indicator organisms in South African waters (for recreational purposes) (DWAF 1996)

Organism	CFU per 100 ml[a]
Faecal coliforms	≤2,000 (r);
Enterococci (faecal streptococci)	≤30 [full contact (r)]; ≤230 [interm. contact (r)]
Clostridium perfringens	–
Staphylococcus (coagulase positive)	–
Pseudomonas aeruginosa	–
Acid-fast bacteria	–
Coliphages	≤20 [full contact (r)]
Escherichia coli	≤130 (r);
Bacteroides	–

[a]r, water used for recreational purposes (swimming, bathing etc.).

2.2×10^8 microorganisms/mL (Site C, week 6), 9.6×10^8 microorganisms/mL (Site D, week 6), 2×10^8 microorganisms/mL (Site E, week 3) and 1.1×10^9 microorganisms/mL (Site F, week 5).

Even after some of the microbial load had died off, the viable FCM counts remained significantly ($p < 0.05$) higher compared to the acceptable guidelines proposed by DWAF (1996) for recreational water. The highest viable FCM counts observed at various sites were 3.1×10^9 microorganisms/mL (Site A, week 5), 8.2×10^7 microorganisms/mL (Site B, week 5), 1.9×10^8 microorganisms/mL (Site C week 6), 9.2×10^8 microorganisms/mL (Site D, week 6), 1.8×10^8 microorganisms/mL (Site E, week 3) and 1×10^9 microorganisms/mL (Site F, week 5). As mentioned before, the constant high counts in week 5 proved to be unclear as similar activities were observed at all the sites throughout the entire sampling period. Week 5 did not record the highest environmental temperature or highest rainfall pattern throughout the sampling period. One scenario could be the lower rainfall pattern observed during this week, which could allow for the higher microbial concentrations measured.

When comparing the MPN results to the highest total FCM counts (Table 3(a) and 3(b)), the MPN results

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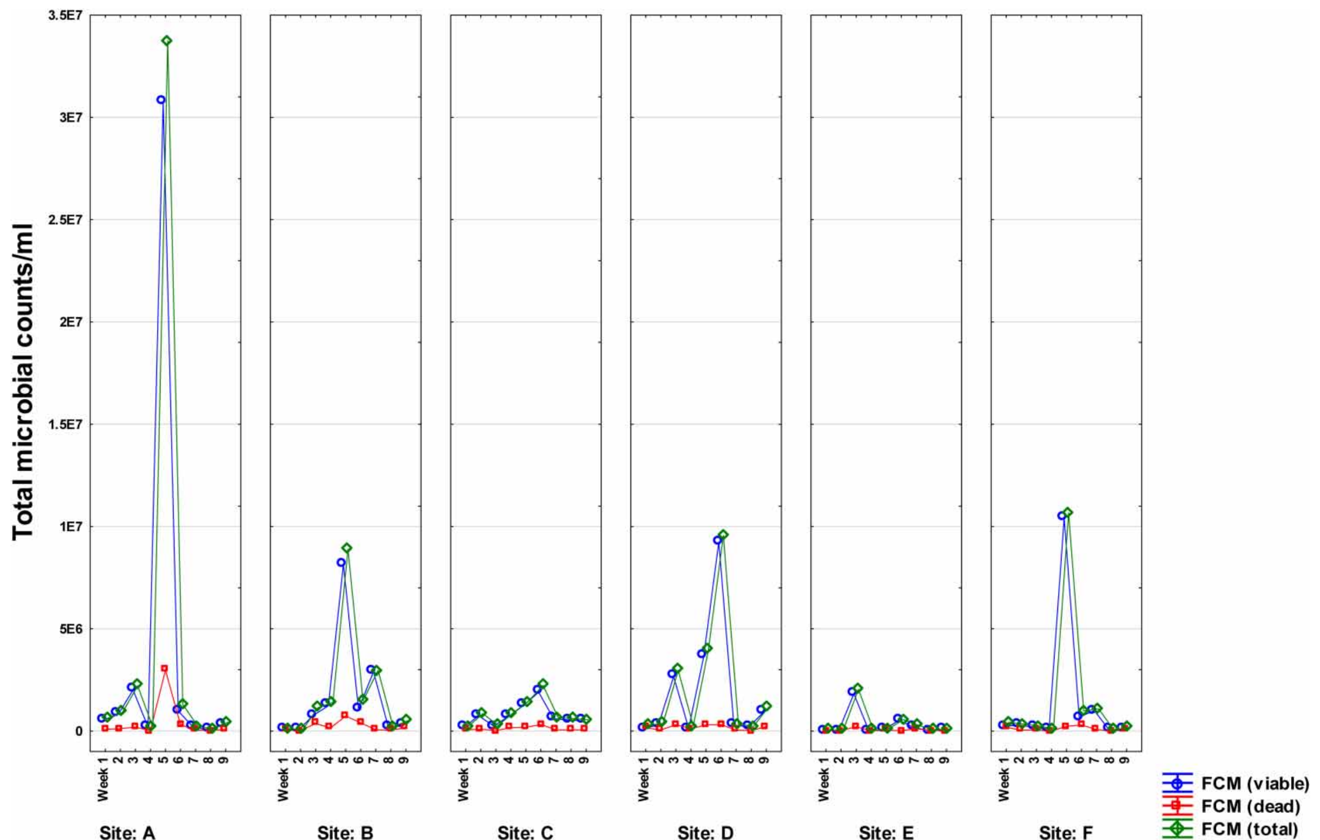

Figure 4 | FCM results of total, viable and non-viable bacterial cells within stagnant water samples at RR Section.

accounted for 4.74% (Site A, week 5), 17.99% (Site B, week 5), 1.57% (Site C, week 6), 0.27% (Site D, week 6), 0.74% (Site E, week 3) and 0.33% (Site F, week 5). These results indicate that even though faecal contamination is a significant contributor to the microbial pollution of the water in the stagnant pools at some of the sampling points, various other non-gas-producing microorganisms are also major contributors to the microbial load. In comparison, the total FCM counts were significantly ($p < 0.05$) higher than the total MPN counts. Figure 5 illustrates the comparison of the MPN to the total FCM results.

When compared to the highest total FCM counts, as mentioned above, the total MPN counts were 1.6×10^6 microorganisms/mL (Site A, weeks 3 and 5), 1.6×10^6 microorganisms/mL (Site B, week 5), 9.2×10^5 microorganisms/mL (Site C, week 5), 1.6×10^6 microorganisms/mL (Site D, week 5), 3.5×10^5 microorganisms/mL (Site E, week 6) and 1.6×10^6 microorganisms/mL (Site F, week 5). This proves to be an obvious expectation as the MPN technique only accounts for the gas-producing microorganisms in the water samples, thus illustrating a fraction of the total microbial count as indicated by the FCM technique. The high total FCM counts observed at these sites during the sampling period is clearly a result of microorganisms other than the gas-producers.

The reliability of the FCM technique has been employed by various researchers and was found to yield more accurate results than other enumeration techniques (Paulse *et al.* 2007; Hammes *et al.* 2008, 2010; Van der Wienlen & Van der Kooij 2010; Wang *et al.* 2010; De Roy *et al.* 2012; Liu *et al.* 2013). This could be due to the FCM technique having the ability to detect organisms that have entered a viable but non-culturable state (Berney *et al.* 2007; Wang *et al.* 2010).

API 20E and RapID ONE systems

Fifty-one morphologically different colonies grown on the three selective media were randomly isolated.

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Table 3 | (a) Average percentage ratio of MPN to total FCM counts based on FCM analysis for all samples analysed over sampling period and (b) average percentage ratio of viable FCM count to total FCM based on FCM analysis for all samples analysed over the sampling period

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
(a)									
A	25.51	0.99	72.33	5.11	4.74	1.69	8.34	12.70	21.78
B	27.02	22.83	40.99	1.43	17.99	14.07	0.67	5.67	0.46
C	16.34	6.32	57.58	20.43	6.36	1.57	2.11	1.81	16.99
D	56.90	0.42	0.40	18.03	1.09	0.27	13.51	19.85	2.15
E	3.50	5.33	0.74	1.11	41.16	6.22	12.27	33.22	38.35
F	37.65	46.29	17.56	11.86	0.33	1.42	0.18	43.41	10.50
(b)									
A	85.67	86.98	90.92	89.92	91.17	75.65	81.64	64.46	80.14
B	68.60	55.46	67.66	88.71	91.68	72.99	97.70	75.47	67.97
C	71.67	88.86	90.09	80.58	87.07	85.56	90.38	89.49	88.47
D	40.21	83.20	89.53	55.19	92.42	96.66	85.13	88.37	80.86
E	65.63	51.43	89.43	58.76	57.25	95.51	59.87	48.18	68.63
F	56.60	83.10	78.96	91.87	97.81	69.19	92.61	91.34	60.24

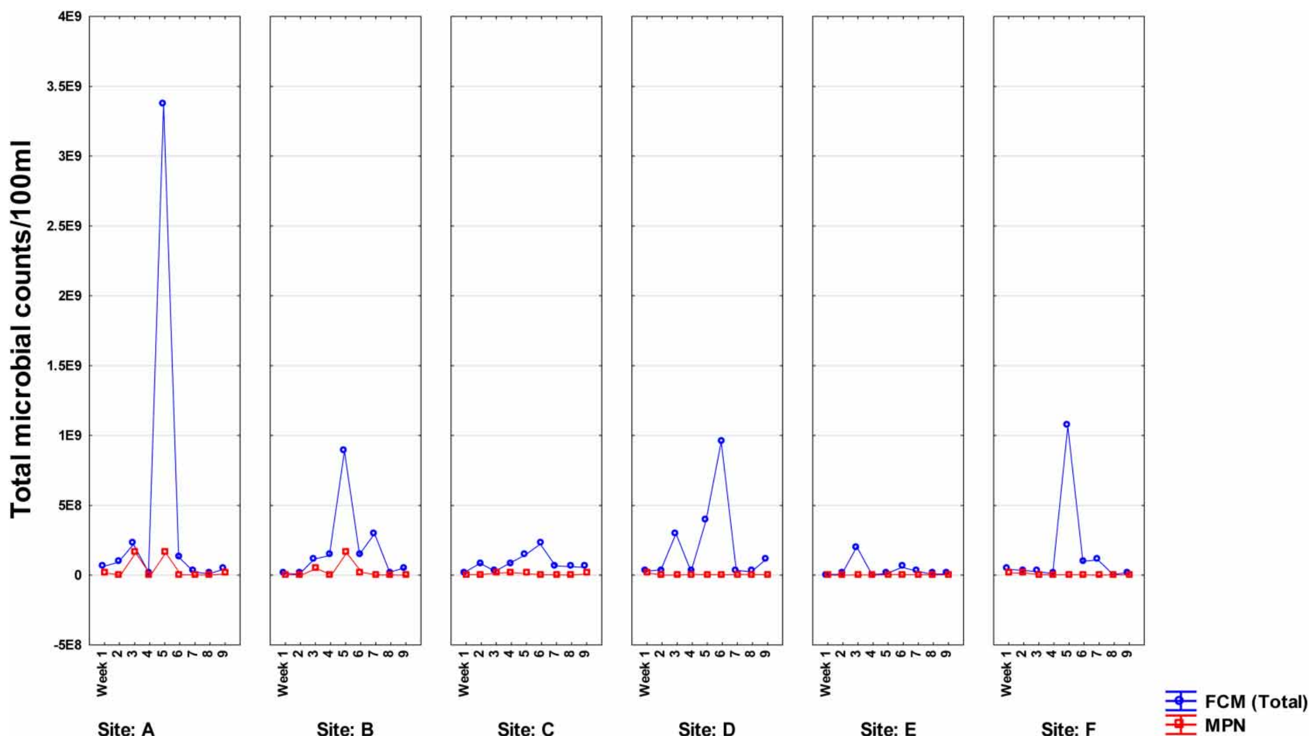

Figure 5 | Comparison of MPN (representing all possible gas-producing organisms) results to total FCM results.

However, only 49 and 36 could be subjected for identification by the API 20E and RapID ONE systems because they met the manufacturers' requirements, respectively. Most organisms identified by the two systems belong to the *Enterobacteriaceae*, which include the coliform bacteria.

Of the 49 isolates subjected to identification by the API 20 E system, only 80% (39 isolates) were successfully

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identified while the remaining 10 could not be identified as they required further biochemical analysis. The API 20E system was able to identify 14 species of *Enterobacteriaceae*. The *Enterobacteriaceae* identified most often by this system included *E. coli* (21%), *Klebsiella pneumonia* (13%), *Klebsiella oxytoca* (13%) and *Acinetobacter baumannii/calcoaceticus* (10%). Even though the API 20E system identified *Enterobacter asburiae* and *Pseudomonas oryzihabitans,* 100% identification was inconclusive and thus required further biochemical analysis. For this reason, these isolates were not considered as a positive identification.

Of the 36 isolates subjected for identification by the RapID ONE system, only 72% (26 isolates) were positively identified, while the remaining 28% required further biochemical analysis. The RapID ONE system identified 13 species of *Enterobacteriaceae*. The *Enterobacteriaceae* mostly identified by the RapID ONE system included the coliform bacteria *K. pneumonia* (19%), *Acinetobacter calcoaceticus* (12%), *E. coli* (12%) and *Enterobacter cloacae* (12%).

Most of the isolated microorganisms belong to the coliform bacteria, which are commonly used as bacterial indicators of the sanitary quality of food and water. The significance of the coliform bacteria present therefore suggests the possible presence of pathogenic microorganisms in the obtained water samples in the RR Section.

It is assumed that these coliform bacteria were introduced into the stagnant water pools as a result of inhabitants washing night pots (Granfone *et al.* 2008; SJC 2011) and the intestines of slaughtered animals at the base of the communal standpipes. The presence of the *Enterobacteriaceae* group throughout the sampling period is a cause for concern as it is an indication of the possible presence of highly pathogenic bacteria.

Both the API 20E and RapID ONE systems identified organisms belonging to the *Enterobacteriaceae* group. Both identification systems were able to identify different species of *Enterobacter*, which included *E. aerogenes*, *E. asburiae, E. cloacae* and *E. sakazakii*. In addition, *A. calcoaceticus, Citrobacter freundii, Providencia alcalifaciens* and *Salmonella gallinarum* were also identified by the RapID ONE system. Similarly, the API 20E system could identify *K. oxytoca, Pantoea* and *Raoultella terrigena,* whereas the RapID ONE system could not. *Leclercia adecarboxylata* and *K. oxytoca* could only be identified with the API 20E system. The RapID ONE system was able to identify more of the *Salmonella* isolates to the species level than the API 20E.

Although both systems have been successfully used before in the identification of *Enterobacteriaceae* (Nucera *et al.* 2006; Ndlovu 2013), Ndlovu (2013) further compared both systems with the polymerase chain reaction (PCR) technique and found PCR to be more sensitive, cheaper and requires less time for identifying organisms than the API 20E and RapID ONE systems. Thus, both of these systems should be used for preliminary identification and final identification should be used with a more sensitive system like PCR and 16S rRNA gene sequencing.

BBL crystal GP ID system

Sixty-eight morphologically different isolates were isolated; however, only 38 isolates could be subjected to identification using BBL Crystal GP ID based on their Gram-positive reaction. Thirty-eight isolates were subjected to the BBL Crystal GP ID system, but only 71% (27 isolates) were successfully identified.

According to the manufacturer's instructions, only isolates with a confidence level of 0.9 (and above) could be considered as a positive identification. Although BP agar is usually used for the isolation of *Staphylococcus aureus,* this organism was not identified during this study.

Furthermore, organisms with typical colonial morphology of *S. aureus* were identified as *Bacillus* spp. and other species of the *Staphylococcus* genus. This is in agreement with a previous study where BP agar was used to isolate *S. aureus*.

Aerococcus, Bacillus, Brevibacillus, Corynebacterium, Gemella, Kocuria, Micrococcus, Staphylococcus and *Streptococcus* were the nine genera identified by this system. *Corynebacterium* species (16%), which included *C. bovis, C. diphtheria* and *C. pseudodiphtheriticum*, were the most identified isolates. *Aerococcus urinae* and *B. cereus* accounted for 15%, respectively, of the organisms identified, while *Micrococcus luteus, Staphylococcus equorum* and *Streptococcus intermedius* accounted for 7% each of the identified species.

Site A recorded the highest genus diversity where *Aerococcus urinae*, *Bacillus* spp., *Corynebacterium* spp., *Kocuria kristinae*, *Micrococcus* spp. and *Staphylococcus* spp. were identified. The system also identified various organisms at

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Site B (*Brevibacillus brevis*, *Corynebacterium* spp., *Gemella morbillorum* and *Streptococcus* spp.), Site C (*A. urinae*, *Bacillus* spp., *Micrococcus luteus*, *Staphylococcus* spp. and *Streptococcus* spp.), Site D (*Bacillus* spp., *Corynebacterium* spp. and *Staphylococcus* spp.), Site E (*Bacillus* spp., *Corynebacterium* spp. and *Streptococcus* spp.) and Site F (*A. urinae*, *Corynebacterium* spp. and *Streptococcus* spp.).

Even though a wide variety of organisms were identified at the different sites throughout the sampling period, various strains (such as *B. megaterium*, *Corynebacterium pseudogenitalium* and *Corynebacterium genitalium*) had a confidence level below 0.9, which according to the manufacturer's instructions could not be considered as a positive identification.

The effects of inadequate, unmaintained and broken sanitary facilities in the RR Section

Many studies that assess grey water quality in informal settlements in South Africa concentrate on the chemical analysis (Mofokeng 2008). For microbial analysis, the focus is usually on indicator organisms such as total coliforms, faecal coliforms and *E. coli*. This study, however, has given a much-needed basis for understanding the pathogenic microbial organisms that could be present in grey water in informal settlements, and can therefore be utilised in informal settlement wastewater management. In certain informal settlements, community gardens are a high priority that provides essential food crops to inhabitants, and thus grey water might be considered for irrigational purposes. However, the fact that human excreta is introduced into the grey water after the washing of night pots in the RR Section clearly indicates that this area's grey water cannot be considered for irrigational reuse.

According to the two enumeration techniques employed, microbial counts proved to exceed the maximum allowed microbial limit for recreational water, which changes the grey water concept to that of blackwater. This causes major health concerns as the stagnant wastewater serves as a playground to children in the area.

Waterborne pathogens are of a great public concern because they can cause diseases through drinking contaminated water, contact with contaminated water or poor hygiene. The isolated microorganisms *Salmonella* Paratyphi A, a serovar of *S. enterica*, is responsible for enteric fever, and *Acinetobacter baumannii*, *E. aerogenes* and *E. cloacae*, opportunistic nosocomial pathogens, have previously been linked to multidrug-resistant infections in hospitals (Davin-Regli & Pagès 2015). According to Bottone (2010), *B. cereus* has been associated with severe local and systemic infections, such as pneumonia and meningitis, which may pose public health problems. The fact that this organism may have a beneficial role as a probiotic in humans, despite also containing lethal toxic disease strains, makes this microorganism highly complex, especially in terms of economic and sanitary significance (Hong *et al.* 2005).

Of the other microbial strains also isolated from the water samples, *Bacillus licheniformis* and *Corynebacterium bovis* are commonly recognised as opportunistic pathogens. Even though human infections are rare, *B. licheniformis* has been linked to infections in immunocompromised individuals, whereas *C. bovis* has shown to cause eye infections (Chow *et al.* 2013) and has also been isolated from abscesses in humans (Cheleuitte-Nieves *et al.* 2018).

Although only a few possible diseases are highlighted, it is a cause for concern because many of the microorganisms isolated, in addition to the above-mentioned diseases, may pose major health risks in the RR Section where children and the immunocompromised may come into contact with the highly polluted stagnant water sources. The inadequate drainage at the bottom of the standpipes also creates a health hazard to the community of RR Section.

CONCLUSION

The extremely high microbial load and presence of pathogenic microorganisms such as *K. pneumonia*, various *Salmonella* spp., *B. cereus* and *M. luteus* in the stagnant grey water is a possible health risk to the RR Section community. The children playing in close proximity to the grey water at the base of the communal standpipes water and the elderly using this water for hand washing are immunocompromised individuals. In addition, these inhabitants are at risk of contracting skin and ear infections as well as other infectious diseases from contact with possible opportunistic pathogenic organisms. With no significant evidence of improvement in the living conditions of residents in the RR Section, the health and safety of the residents remain a concern.

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