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Human enteric bacteria and viruses in five wastewater treatment plants in the Eastern Cape, South Africa

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ABSTRACT

Monitoring effluents from wastewater treatment plants is important to preventing both environmental contamination and the spread of disease. We evaluated the occurrence of human enteric bacteria (faecal coliforms and *Escherichia coli*) and viruses (rotavirus and enterovirus) in the final effluents of five wastewater treatment plants (WWTPs) in the Eastern Cape of South Africa. Human viruses were recovered from the effluent samples with the adsorption–elution method and detected with singleplex real-time RT–PCR assays. Rotavirus was detected in several effluents samples, but no enterovirus was detected. At WWTP-C, rotavirus titre up to 10^5 genome copies/L was observed and present in 41.7% of the samples. At WWTP-B, the virus was detected in 41.7% of samples, with viral titres up to 10^3 genome copies/L. The virus was detected once at WWTP-E, in 9% of the samples analysed. The viral titres at WWTP-A were below the detection limit in all 25% of the 1.25 L samples in which the virus was detected. Rotavirus was not observed at WWTP-D. Faecal coliform bacteria and *E. coli* were detected in all the WWTPs, but no correlation was established between the enteric bacteria and viruses studied. The occurrence of rotavirus in effluent samples discharged into surface waters highlights the importance of assessing viral contamination in the water sources used for domestic water use.

Introduction

Freshwater is essential for the daily life of all aquatic and terrestrial organisms, including humans. It is an important resource for human survival and deserves proper monitoring to protect it [1]. Every nation undertakes to protects its various waterbodies with water policies, monitoring, and treatment strategy [2]. Although water is normally a recyclable resource, it requires careful management and protection because it is vulnerable to overexploitation and pollution [3]. Avoiding the contamination of water assets and ensuring human well-being by protecting water supplies against the spread of pathogenic organisms are the two principal purposes behind the treatment of wastewater. The deteriorating state of the municipal wastewater and sewage treatment infrastructure in South Africa continues to constitute the greatest cause of the various contamination issues faced in many regions of the country, and is a particularly real threat to the well-being of deprived communities [4].

It is well known that microorganisms play many beneficial roles in wastewater systems [5], and are useful in reducing the volumes of sludge sewage effluent in both wastewater treatment plants (WWTPs) and *on-site* wastewater treatment systems, such as septic tanks [6]. However, studies have shown that a number of exceptional organisms are dangerous and have contributed to several water-borne disease epidemics [7]. As a case in point, wastewater effluent has been shown to contain a mixture of anthropogenic substances, a large proportion of which have endocrine-disrupting properties [8]. Faecal coliform bacteria and more specifically *Escherichia coli* are the most commonly used bacterial indicators of faecal pollution. This indicator group is used to evaluate the quality of wastewater effluents, rivers, sea beaches, raw water for drinking, treated drinking water, water used for irrigation, aquaculture sites, and recreational water (DWAF: Department of Water Affairs [9]). Other indicators used to test effluent quality include human enteric viruses, which are also considered indicators of faecal contamination [10].

It has become increasingly obvious that viruses are a leading cause of waterborne gastroenteritis [11,12]. Various studies have demonstrated that enteric viruses are present at high levels in treated wastewater [13]. Norovirus was detected in the final effluent of a wastewater treatment plant [14]. Human enteric viruses are currently listed on the United States Environmental Protection Agency Contaminant Candidate List (USEPA CCL) as emerging contaminants. To date, no regulations have been imple-

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Table 1
Description of the treatment systems in five wastewater treatment plants (WWTPs), the sampling sites for rotavirus occurrence in the Eastern Cape, South Africa

WWTP	Flow rate (m^3 day^{-1})	Inhabitants	Wastewater treatment technology	Sampling sites (ID)
A	8000	16 600	Activated sludge	Final effluents (FE) Discharge point (DP)
B	5000	43 100	Bio-filter/PETRO (pond enhanced treatment and operation) process treatment system	Final
C	40 000	141 000	Activated sludge system	efflu-
D	12 000	111 621	Bio-filter and activated sludge system	ents
E	1800	20 000	Bio-filter system	(FE)

mented to monitor viral concentrations in wastewater before it is discharged into a water body. Human enteroviruses, human adenoviruses, norovirus, rotavirus, and hepatitis A virus (HAV) are some of the enteric viruses causing main infections. These infections are associated with several water-borne ailments, including severe gastroenteritis, conjunctivitis, and respiratory disease, in both developed and developing nations throughout the world. There are several ways in which the general community can become contaminated by pathogens, including by direct contact (faecal–oral route or dermal contact) and through food-borne contaminants and pollution [12,15]. A combined sewage overflow was reported to release significantly high concentrations of viruses into the receiving waterbodies, and the occurrence was greater during wet weather than in periods of dry weather [16,17]. The release of infectious enteric viruses in final effluents has also been demonstrated [15,18,19]. Insufficiently treated wastewater is also a wellspring of human enteric viruses in the environment [20].

The aim of this study was to assess the final effluents of five selected WWTPs in the Buffalo City Local Municipality for contamination by enteric viruses and bacteria which can give rise to public health problems. The human enteric pathogens studied were rotaviruses, enteroviruses, *E. coli*, and faecal coliforms. The presence of these viruses have never been studied in these areas.

Materials and methods

Sample collection

Samples were collected monthly from five WWTPs for a 1 year, from September 2012 to August 2013. The sampling period covered the four seasonal time of the year. The spring (August–mid-October), summer (October–February), fall (February–April) and winter (May–July). The details of the treatment plants are summarized in Table 1. WWTP-A had two sampling points: the final effluent point (FE), just after chlorination, and the discharge point (DP), immediately before the effluent is discharged into the river. The two points were 136.2 m apart. WWTP-B, WWTP-C, WWTP-D, and WWTP-E were only monitored at FE because their DP were inaccessible. The effluent samples were collected in sterile 1.7 L Nalgene bottles containing sodium thiosulfate to dechlorinate the samples. A cooler box was used to store all samples and transport them to the laboratory for processing within 2 h. The effluent samples were collected as part of the routine surveillance of enteric viruses at each WWTP. The samples were collected once a month at each WWTP (n = 12). Because of unfavourable climatic conditions, no samples were collected from WWTP-A (DP) in December 2012 or from WWPT-E in September 2012, so a total of 70 samples were processed.

Concentration of water samples for viral detection

The effluent samples were concentrated with the adsorption–elution method, as described by Haramoto et al. [21], with some modifications.

Control strains

The prototype strains of rotavirus (strain WA, ATCC VR-2274) and Coxsackievirus A2 (strain Fleetwood, ATCC VR-1550) used in this work were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Nucleic acid extraction procedure

RNA was extracted from 100 μL of each ATCC stock culture (control strains) with the extraction protocol of the ZR Viral RNA Kit™ (Zymo Research Corporation, 17062 Murphy Ave. Irvine, CA 92614, U.S.A). Nucleic acids were extracted from all the concentrated environmental samples with the same extraction kits, according to the manufacturer's instructions.

Detection of enterovirus and rotavirus

The two extracted RNA viruses were reverse transcribed to complementary DNA (cDNA). Before the reverse transcription reaction, the rotavirus RNA was denatured by heating at 95 °C for 5 min, and then incubated on ice for 2 min to denature its double-stranded RNA [22]. The eluted RNA (20 μL) was reverse transcribed in a reaction containing 2 μL of random hexamer primer, 2 μL of dNTP mix, 4 μL of diethylpyrocarbonate (DEPC)-treated water, 8 μL of 5 × RT buffer, 1 μL of RiboLock RNase Inhibitor, and 2 μL of RevertAid Premium Reverse Transcriptase (Fermentas Life Sciences, Life Technologies, 200 Smit Street, Fairland, South Africa). The reaction was incubated at 25 °C for 10 min and then at 60 °C for 30 min, and then terminated by heating at 85 °C for 5 min. The resultant cDNA was used as the template for quantitative TaqMan real-time PCR (StepOnePlus PCR™ Real-Time PCR System;, Applied Biosystems) with TaqMan probes in a 96-well plate. The wells were loaded with 20 μL of reaction buffer containing 12.5 μL of 2 × TaqMan® Universal PCR Master Mix (Applied Biosystems), 400 nM forward primer, 400 nM reverse primer, 250 nM TaqMan probe (Table 2), and PCR-grade water. Aliquots (5 μL) of the sample cDNA were added to the mixture to total reaction volumes of 25 μL. The thermal cycling protocols used for the viruses were as follows: enterovirus: activation

Table 2
Probes and primer pairs for rotavirus and enterovirus quantification.

Enteric virus	Primers and labelled TaqMan probe	Reference
Rotavirus	JVK (F): 5′-CAGTGGTTGATGCTCAAGATGGA-3′ JVK (R): 5′-TCATTGTAATCATATTGAATACCCA-3′ JVK (P):5′-FAM-ACAACTGCAGCTTCAAAAGAAGWGT-MGBNFQ –3′	[22]
Enterovirus	EV1 (F): 5′-CCCTGAATGCGGCTAAT-3′ EV1 (R): 5′-TGTCACCATA AGCAGCCA-3′ EV-BHQ (P): 5′-FAM-ACGGACACCCAAAGTAGTCGGTTC-MGBNFQ-3′	[60,61]

Abbreviations: F, forward/sense; R, reverse/antisense; P, probe; FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/non-fluorescent quencher. The primers and probes for rotavirus were designed to detect the five major VP7 serotypes of epidemiological importance (i.e., G1–G4, and G9).

of Taq DNA polymerase at 95 °C for 10 min, 45 cycles of denaturation at 94 °C for 15 s, annealing at 58 °C for 1 min, and extension at 72 °C for 20 s; rotavirus: activation of Taq DNA polymerase at 95 °C for 15 min, 45 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s [23].

cDNA standard

The RNA of stock viruses was extracted and purified with the Zymo Viral RNA Extraction Kit. The cDNAs were prepared and their concentrations determined spectrophotometrically with the Qubit® 1.0 Fluorometer (Life Technologies), according to the manufacturer's instructions. The cDNA was serially diluted ten-fold with nuclease-free water. Standard curves were generated from the dilution over 7 log range of the cDNA. To minimize potential contamination, the cDNA was prepared in a separate room, and the PCR plates containing the cDNA standards were not taken into the RT–PCR set-up laboratory.

PCR specificity, sensitivity, and detection limits

The specificity of each real-time primer and probe set used in this study was examined. The cDNA standards were included in all the real-time PCR assays. No cross-reactivity of the primers and probes was observed when the cDNA standards were used as the templates. To validate the real-time PCR assays before their application to environmental samples, the detection limit and amplification efficiency of each reaction were determined. Standard curves were constructed with ten-fold serial dilutions of cDNA, assayed in triplicate. The resulting standard curves had strong correlation coefficients ($r^2 = 0.98$), indicating strong linear relationships. The PCR amplification efficiencies for the assays were calculated from the slopes of the standard curves, and were 82% and 94% for the enterovirus and rotavirus assays, respectively. The detection limit was 10 copies of target RNA per reaction for all PCR assays, indicating the high sensitivity of the assay.

Faecal coliform detection

Faecal coliform bacteria were detected and counted with a membrane filtration method, and the filtrates were then transferred onto m-FC agar and incubated at 44.5 °C for 24 h. The positive target colonies, blue or magenta in colour, were counted and reported in colony-forming units (CFU)/100 mL. Sterile water blanks were analysed during each sampling period and were always negative for total coliforms and *E. coli* [24].

E. coli *detection*

E. coli–coliform selective agar (Conda, Madrid) was used to isolate and enumerate *E. coli.* It differentiates *E. coli* from other Enterobacteriaceae chromogenically by staining it a dark blue–greenish colour. *E. coli* was examined as described above. The filters were placed on the *E. coli*–coliform chromogenic agar and incubated at 37 °C for 24 h. The target colonies were counted and reported as CFU/100 mL (SABS, 2011).

Statistical analysis

The data were analysed with the SPSS (IBM SPSS Statistics version 22, Armonk, NY: IBM Corp).

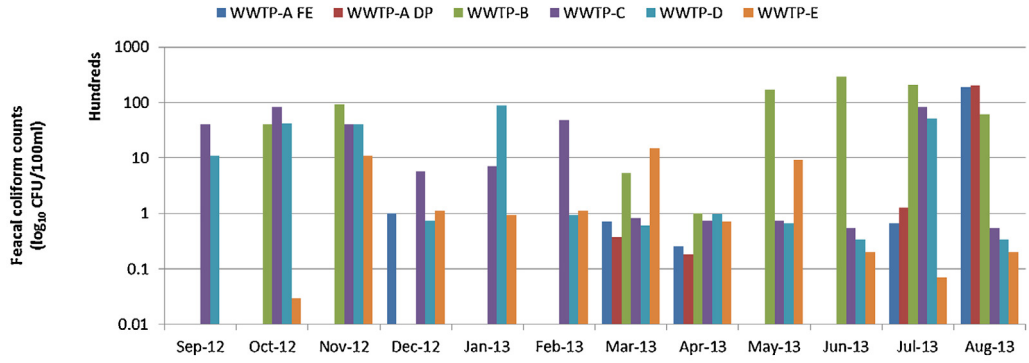
Results

Faecal indicators in effluent samples

Culturable faecal coliforms were detected in the effluents samples for all the WWTPs. The average of each triplicate plate counts (CFU) for the month are shown in Fig. 1. Two limits are set by the South Africa regulatory guidelines for effluent quality discharge: a general limit of 1000 CFU/100 mL and a special limit of 0 CFU/100 mL (DWAF, 2013). Seventeen (24.3%) of the effluent samples analysed met the DWAF special limit guideline for effluent discharge (0 CFU/100 mL), 33 (47.1%) were within the general limit (1000 CFU/100 mL), and the remaining 20 (28.6%) were above the general limit. The faecal coliform counts were 0–1.9×10^4 CFU/100 mL at WWTP-A FE and 0–2.0×10^4 CFU/100 mL at WWTP-A DP; 0–9.3×10^3 CFU/100 mL at WWTP-B; 55–8.4×10^3 CFU/100 mL at WWTP-C; 34–9.0×10^3 CFU/100 mL at WWTP-D; and 3–1.5×10^3 CFU/100 mL at WWTP-E.

E. coli *in effluent samples*

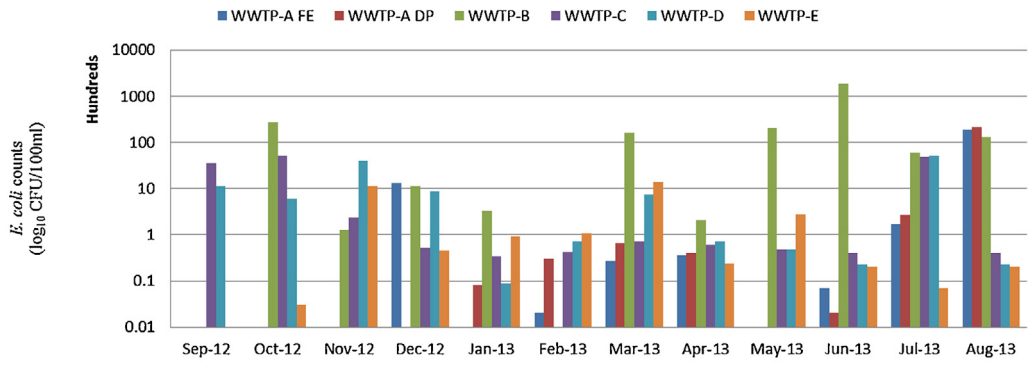
The *E. coli* counts recorded in this study, shown in Fig. 2, ranged between 0–1.86×10^4 CFU/100 mL at WWTP-A FE and



	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	May-13	Jun-13	Jul-13	Aug-13
WWTP-A FE	0	1	0	1.00E+02	0	0	71	26	0	0	6.70E+01	1.90E+04
WWTP-A DP	0	0	0		0	0	38	18	0	0	1.30E+02	2.00E+04
WWTP-B	0	4.00E+03	9.30E+03	0	0	0	5.40E+02	1.00E+02	1.70E+04	2.90E+04	2.10E+04	6.10E+03
WWTP-C	4.00E+03	8.40E+03	4.00E+03	5.80E+02	7.20E+02	4.80E+03	82	75	74	55	8.20E+03	55
WWTP-D	1.10E+03	4.20E+03	4.00E+03	74	9.00E+03	95	61	96	68	34	5.20E+03	34
WWTP-E		3	1.10E+03	1.10E+02	93	1.10E+02	1.50E+03	71	9.30E+02	20	7	20

Sampling months (n = 12)

Fig. 1. Occurrences of faecal coliforms in effluent from five WWTPs. There was a period of no sampling at WWTP-E (September 2012) and WWTP-A DP (December 2012).



	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	May-13	Jun-13	Jul-13	Aug-13
WWTP-A FE	1	0	0	1310	1	2	27	36	0	7	167	18600
WWTP-A DP	0	0	0		8.3	30	66	40	1	2	267	21600
WWTP-B	0	28000	130	1100	333	0	16000	210	20900	185000	6000	12900
WWTP-C	3600	5100	240	52	35	43	71	62	47	41	5000	41
WWTP-D	1100	600	4000	860	9	72	750	73	47	23	5200	23
WWTP-E		3	1100	46	93	110	1400	24	280	20	7	20

Sampling months (n = 12)

Fig. 2. Occurrences of *E. coli* in effluent from five WWTPs. There was a period of no sampling at WWTP-E (September 2012) and WWTP-A DP (December 2012).

0–2.16 × 10^4 CFU/100 mL at WWTP-A DP, with both points having the highest *E. coli* counts for the month of August 2013. However, the *E. coli* counts were higher at DP than at FE. At WWPT-B, the *E. coli* counts ranged between 0–1.85 × 10^5 CFU/100 mL and were highest in June 2013. The counts for *E. coli* at WWTP-C were 35–5.1 × 10^3 CFU/100 mL, and were highest in October 2012. At WWTP-D, the counts were 9–5.2 × 10^3 CFU/100 mL, and were highest in July 2013. At WWTP-E, the counts were 3–1.4 × 10^3 CFU/100 mL, and were highest in March 2013.

Rotavirus and enterovirus concentrations in samples from the five WWTPs

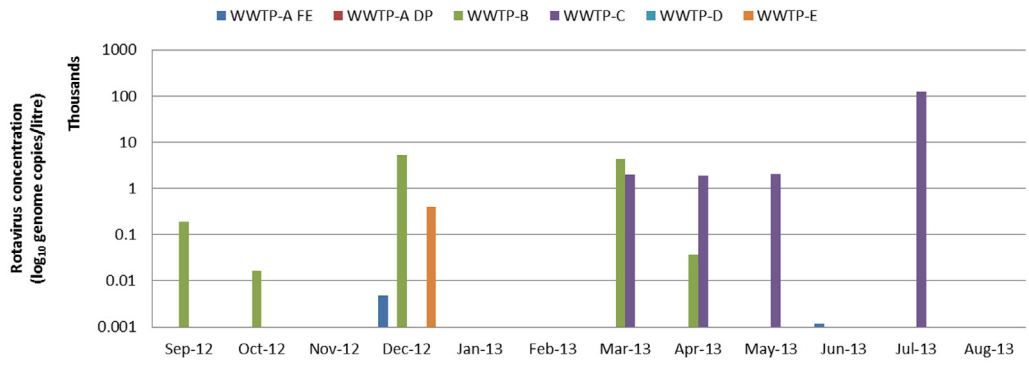
Rotavirus and enterovirus numbers in the effluent samples from the five WWTPs were quantified monthly with real-time RT–PCR in samples collected from the facilities between September 2012 and August 2013 (Fig. 3) in suburban (WWTP-A and WWTP-E) and urban areas (WWTP-B, WWTP-C, and WWTP-D). All the WWTPs were negative for enterovirus. The concentrations of rotavirus genome in the effluent samples per location per month are shown in Fig. 3. At WWTP-C, viral titres of up to 10^5 genome copies/L were observed and 41.7% of the samples were positive for the virus: the viral concentrations ranged from 1.9 × 10^3 to 1.2 × 10^5 genome copies/L. At WWTP-B, the virus was detected in 41.7% of samples and the viral titres were up to 10^3, in the range 1.6 × 10^1–5.2 × 10^3 genome copies/L. The virus was detected once at WWTP-E, in 9% of the samples analysed. The viral titres recorded at WWTP-A were below the detection limit in all 25% of the samples in which the virus was detected. WWTP-D samples were all negative for the virus. The failure to detect the virus in most samples (79%) suggests that the rotavirus concentrations in the effluents were relatively low or absent or as a result of inhibition, so that it was undetectable in the effluents.

Seasonal occurrence of faecal coliforms, E. coli, *rotavirus, and enterovirus*

The seasonal occurrence of faecal coliforms in the effluent samples is shown in Fig. 1. High faecal counts were observed at all the plants between autumn (March 2013) and winter (August 2013). The highest average monthly concentrations of coliforms were recorded in August (7.5 × 10^3 CFU/100 mL), July (5.8 × 10^3 CFU/100 mL), June (4.9 × 10^3 CFU/100 mL), and May 2013 (3.0 × 10^3 CFU/100 mL), exceeding the set general limit (1000 CFU/100 mL) by factors of 3–7 in the effluent discharges. These data emphasize the focus of faecal coliform bacteria in the winter period. The presence of coliforms was recorded at WWTP-C, WWTP-D, and WWTP-E in all seasons. Summer months also showed high faecal coliform counts (between September to November 2012 and in January 2013), but these were lower than those recorded in winter. Based on the annual average per WWTP, WWTP-B (7.3 × 10^3 CFU/100 mL) had the highest coliform counts, with the highest counts in winter (May–July 2013) at log 4 CFU/100 mL. This was followed by WWTP-C (2.6 × 10^3 CFU/100 mL), with high coliform counts in summer (October 2012) and winter (November 2013) of log 3 CFU/100 mL; and WWTP-D (2.0 × 10^3 CFU/100 mL), which recorded its highest counts in summer (January 2013). WWTP-A (1.6 × 10^3 CFU/100 mL at FE; 1.8 × 10^3 CFU/100 mL at DP) recorded it highest counts in winter (August 2013), and WWTP-E (360 CFU/100 mL) recorded its highest counts (the lowest maximum of all plants) in autumn (March 2013).

E. coli was detected in all months, except at WWTP-A, where bacteria were not detected in some months (Fig. 2). The highest concentrations of *E. coli* were observed in winter (May–August 2013), summer (October 2012), and autumn (March 2013). It must be noted that WWTP-B displayed very high concentrations in certain months, which influenced its average monthly counts (Fig. 2). Of all the WWTPs, WWTP-A recorded the highest *E. coli* counts in winter (August 2013) and WWTP-B recorded high counts in summer (October 2012), autumn (March 2013), and winter (May–August 2013). High *E. coli* counts were recorded in summer (September and October 2012) and winter (August 2013) at WWTP-C; in summer (November 2012) and winter (July 2013) at WWTP-D; but no high counts were recorded in any season at WWTP-E.

Fig. 3 shows the comparative seasonal profiles of rotavirus at the WWTPs. Rotavirus was detected once in summer (December 2012) and twice in winter (June 2013 and August 2013) at WWTP-A. At WWTP-B, the virus was detected in summer and autumn between September 2012 and April 2013. At WWTP-C, the virus was detected in late autumn and winter (March 2013–July 2013). The virus did not occur at WWTP-D and was detected only once in summer (December 2012) at WWTP-E. The average annual concentration of rotavirus in the final effluents was the highest



	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	May-13	Jun-13	Jul-13	Aug-13
WWTP-A FE	0	0	0	4.85	0	0	0	0	0	1.19	0	0.01
WWTP-A DP	0	0	0		0	0	0	0	0	0	0	0.10
WWTP-B	1.91E+02	1.62E+01	0	5.24E+03	0	0	4.37E+03	3.77E+01	0	0	0	0
WWTP-C	0	0	0	0	0	0	1.95E+03	1.87E+03	2.10E+03	0.21	1.24E+05	0
WWTP-D	0	0	0	0	0	0	0	0	0	0	0	0
WWTP-E		0	0	4.05E+02	0	0	0	0	0	0	0	0

Sampling months (n = 12)

Fig. 3. Occurrences of rotavirus in the effluent from five WWTPs. There was a period of no sampling at WWTP-E (September 2012) and WWTP-A DP (December 2012). Samples in which no virus was detected are marked with zeros viral concentrations were below the detection limit.

at WWTP-C (2.6×10^4 genome copies/L), followed by WWTP-B (2.0×10^3 genome copies/L) and WWTP-E (4.1×10^2 genome copies/L). However, the highest incidence of rotavirus was recorded in winter (July 2013), with a viral titre of 1.2×10^5 genome copies/L.

Discussion

Because wastewater systems are an important avenue for the transmission of water-borne human enteric pathogens, the study of rotaviruses, enteroviruses, and faecal indictor bacteria in treated wastewater is important for public health, especially in regions in which there is no surveillance of these organisms. A year-long study of the quality of the effluent discharged by five WWTPs was conducted in the Eastern Cape Province of South Africa.

The results of this study indicate that the occurrence of faecal coliforms and *E. coli* was higher than that of rotaviruses or enteroviruses in the treated effluents from the five WWTPs (Figs. 1–3). No enterovirus was detected at any of the plants. A good treatment regimen was observed at WWTP-A, where 91.6% of the faecal coliform counts were below the 1000 CFU/100 mL limit and on certain occasions, no faecal coliform was detected. However, the last month sampled (August 2013) was characterized by very high coliform counts, which was attributed to the unavailability of chlorine disinfectant. No enterovirus was detected at the plant and no seasonal effect on the treatment processes was observed but rotavirus was detected in December 2012, June and August 2013 at a very low concentration below the set detection limit. Our monthly monitoring of the remaining WWTPs showed that the occurrence of coliforms was high at all WWTPs, and that WWTP-B had the highest concentrations in its effluent. The detected faecal coliforms at each WWTPs followed no seasonal pattern. The month of July 2013, which is winter, was characterized by high concentrations of coliform at all WWTPs except WWTP-E (Fig. 1). High coliform concentrations were also recorded in August 2013, with the highest concentration at WWTP-A (Fig. 1), which greatly influenced the total coliform concentration level observed. Faecal coliforms are one of the most commonly used indicators of microbial water quality and are frequently used in human health risk assessment [25] because they correlate with the presence of several organisms that cause water-borne diseases [26,27]. This study has shown that these five treatment plants are sources of the faecal coliforms in the environments surrounding them. However, none of the WWTPs complied fully with the effluent standards. Very high concentrations of faecal coliforms have also been reported in the rivers downstream from the WWTPs in the Eastern Cape [28,29]. In another study, effluent from another province was reported to be the source of faecal pollution in the downstream river into which it was discharged [30]. Thus, several previous studies of the Eastern Cape Province have reported surface waters with high levels of faecal coliforms, indicating microbial contaminants in the effluents discharged into them [31]. The failure of South African WWTPs to produce effluents of high microbiological quality has been shown to be responsible for the contamination and pollution of water resources [30].

E. coli was detected in 66.7% of the samples analysed from WWTP-A, but in 83.3% of those samples, the *E. coli* counts were less than 1000 CFU/100 ml. Therefore, based on the coliform counts at this treatment plant, we infer that the treatment regimen is efficient. However, it must be noted that no specific limit has been set for *E. coli* like that for faecal coliforms, which was used as the standard limit against which to compare *E. coli* concentrations. At WWTP-B, bacteria were detected in 83.3% of the samples, and 50% of those samples had very high counts. *E. coli* was detected in all the samples from WWPT-C, WWPT-D, and WWPT-E, and 25% of these exceeded the concentration limit at WWPT-C and 16.7% at WWPT-D. Characterization of effluent from WWTPs has shown that poorly treated wastewater can be a source of *E. coli* [32,33], pathogenic *E. coli*, and antibiotic-resistant *E. coli* [34–36]. However, other studies characterizing effluents have not detected *E. coli*, especially when the treatment processes are efficient, although faecal coliforms have been found [37,38]. Studies in several regions of South Africa have identified *E. coli* in poorly treated effluents discharged into the environment [30,39] and its presence in the environment, especially in surface waters, has been reported [4,40,41].

Rotavirus was also detected at some WWTPs. The highest viral titres were at WWTP-B and WWTP-C, at which the virus was, detected five times each at average concentrations of 3 log and 4 log genome copies/L, respectively. The virus was only detected once at WWTP-E and not at all at WWTP-D. The occurrence of rotavirus at the WWTPs showed no seasonal pattern. The presence of rotavirus and enterovirus in the Eastern Cape rivers of South Africa was reported by Chigor and Okoh [42]. A similar study by Sibanda

and Okoh [43] only detected rotavirus, and no enteroviruses were present in any river samples. The prevalence of rotavirus, an aetiological agent of viral gastroenteritis, is under-investigated in the South African aquatic environment. However, clinical infections have been reported, especially among infants [44,45]. Because these enteric viruses were detected at various concentrations in the final effluent samples here, subsequent studies must be undertaken to ascertain how the presence of these viruses correlates with human disease. The presence of rotavirus in the wastewater effluent was observed once at all WWTPs except WWTP-D, suggesting the possible circulation of rotaviruses in the human environment in this province. The survival strategy of rotaviruses across seasons could not be clarified because their rates of occurrence were low at the WWTPs. Li et al. [46] reported that summer is epidemiologically important for rotaviruses because the virus is inactivated by the high temperatures and UV in sunlight during summer. Our monthly monitoring results show that the occurrences and concentrations of rotaviruses were low generally, and in most cases, no rotavirus was detected. At WWTP-B, the virus was detected most frequently in summer, because the wastewater treatment regimen was poor, and once at WWTP-E. Rotavirus is considered a winter virus because it is commonly found in winter [47]. Winter occurrences of the virus have been reported by Zuccotti et al. [48] and Li et al. [46]. However, no rotavirus was detected in winter at any WWTP except WWTP-C, where it was detected in May–July 2013. Nakajima et al. [49] reported that the occurrence of rotavirus did not increase significantly in winter, and rotavirus has been reported all year round in most parts of the world [47,50].

In this study, we also evaluated the relationship between the occurrences of faecal coliforms and rotavirus at the WWTPs. However, the utility of faecal coliforms as a predictor of rotavirus was not established because there was no correlation between these pathogens. There was a very weak correlation between faecal coliforms and the environmental circulation of rotaviruses in a study by Grassi et al. [51]. In contrast, Li et al. [46] correlated the presence of rotavirus and bacterial pathogens in their study. Kittigul et al. [52] detected coliform bacteria but no rotaviruses in their study, demonstrating that the two organisms are poor indicators of the presence of the other. The high prevalence and occurrence of faecal coliforms and the low concentrations of rotavirus observed in our study suggest that the use of these viruses as indicators of faecal pollution could cause wrong conclusions to be drawn on the extent of faecal contamination [53]. The results of this study support previous findings regarding the prevalence of rotavirus in the final effluents of WWTPs.

The correlation between faecal coliforms and *E. coli* was also very weak in this study. Therefore, the presence of faecal coliforms was not good predictor of *E. coli* in the effluent samples. In their review, Pachepsky and Shelton [54] attest strongly to the weak correlation between faecal coliforms and *E. coli*, and a similar study by Hachich et al. [55] reported no correlation between faecal coliforms and *E. coli*. In contrast, studies of environmental samples and food samples have identified a correlation between faecal coliforms and *E. coli* [56–59]. These results and the absence of a statistical correlation between *E. coli* and faecal coliform counts suggest that the regulation of effluent samples known to contain faecal coliforms, as in WWTPs in South Africa, may be insufficient to prevent environmental and surface water contamination.

This study provides entirely new information on the prevalence of rotaviruses at different WWTPs in this province. It also demonstrates the impact of poorly treated wastewater discharge on the quality of the receiving surface water in terms of the potential spread of infectious diseases caused by rotaviruses and enteric bacteria. Further studies are required, conducted on a larger scale and over a longer period, to monitor the presence of rotaviruses in different WWTP effluents and receiving streams. These should extend our understanding of the geographic fate and transport of rotaviruses through wastewater treatment processes and their impact on public health.

The results of our study are consistent with the limited data available on wastewater quality in terms of viral contamination, and reveal the benefits of environmental surveillance in clarifying the molecular epidemiology of the viruses circulating in a community. We emphasize the need for environmental surveillance programmes in countries such as South Africa with limited epidemiological surveillance systems for viral gastroenteritis and no environmental surveillance system currently in place. We suggest that similar long-term studies will be valuable and complementary tools in the establishment of epidemiological surveillance systems.

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Competing interests

None declared.

Ethical approval

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