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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).