Original Article

Occurrence of enteroviruses, noroviruses, rotaviruses, and adenoviruses in a wastewater treatment plant

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Received: 7 April 2023 / Accepted: 16 May 2023

Published online: 6 June 2023

Abstract

The main objective of this study is to evaluate the quality of wastewater by molecular identification of enteroviruses, rotaviruses, and adenoviruses in wastewater samples collected from the ElSerw wastewater treatment facility in Damietta Governorate, Egypt. An additional objective is to assess the usefulness of these viruses as markers of viral reduction during wastewater treatment. A treatment facility's inflow and discharge were sampled 48 times. The incidence of enteric viruses was found in 29 wastewater samples (60.4%). 6.25% (3/48), 0% (0/48), 37.5% (18/48), and 20.8% (10/48) of the samples tested positive for enteroviruses (EVs), noroviruses, rotaviruses, and adenoviruses, respectively. Co-infections with two or more viruses were found in 10.4% (5/48) and 2% (1/48) of all cases, respectively. The viral burden in the wastewater treatment plant's discharge effluents dropped non-significantly when compared to intake samples. According to our findings, rotaviruses and adenoviruses have been found in 10 outlet effluent samples. The removal rates for enteroviruses, rotaviruses and adenoviruses were 39%, 61.5% and 33.3%, respectively. As a result of their high frequency and lower removal rates, both rotaviruses and adenoviruses were deemed an appropriate indicator of human enteric viral reduction during the wastewater treatment process.

Keywords Enteroviruses · Rotaviruses · Adenoviruses · Wastewater · Damietta · Egypt

1 Introduction

Egypt's water resources are becoming increasingly scarce in order to meet public drinking and agricultural water needs. Reused wastewater is increasingly being used for agricultural reasons all over the globe as an efficient way to conserve water resources [1, 2]. Pathogens such as enteric viruses and bacteria are common in repurposed wastewater, posing a health risk to subjects, land, animals, and consumers of products irrigated with treated wastewater. Over 150 distinct enteric viruses have been connected to water-borne and food-borne diseases [3]. According to up-to-date World Health Organization (WHO) figures, the annual mortality rate linked with diarrhea among Egyptian infants hit 30 deaths per 100,000. Rotavirus infection was responsible for nearly 3.9% of all documented deaths, while intestinal adenovirus was the third most common viral cause of diarrhea after rotaviruses and noroviruses [4]. There is no single suitable measure that can demonstrate complete reduction of human enteric viruses in wastewater treatment facilities [5, 6]. Conventional bacterial indicators cannot be used to identify the frequency and decline of human enteric viruses during wastewater treatment due to the negligible link between indicators and viruses [7–9]. As a consequence, identifying optimal viruses that meet all of the criteria for a viral decline measure is essential

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J.Umm Al-Qura Univ. Appll. Sci. (2023) 9:449–454 | https://doi.org/10.1007/s43994-023-00053-6

[10, 11]. Adenoviruses [12], polyomaviruses [13], F-specific RNA coliphages [14], pepper moderate mottle virus [15], and tobacco mosaic virus [16] have all been proposed as indicators of human enteric viral decline during effluent treatment. However, each of these viruses is insufficient to connect the presence of all human enteric viruses and is unsuitable for evaluating wastewater treatment efficacy [17, 18].

In particular, over 100 species of enteric viruses have been identified as common water pollutants, and the number is expanding due to the emergence of new strains. Indicators are widely used to examine the destiny of pathogenic strains due to the diversity of viruses in the environment. Previously, fecal bacterial indicators (FIB) such as *coliform bacteria*, *Escherichia coli*, *Enterococcus* and *Streptococcus* spp. were utilized to measure fecal contamination levels in water. Bacteria, on the other hand, have been proven to be significantly less resistant to wastewater treatment and far less environmentally durable than enteric viruses [19–21]. As a result, FIB are poor indicators of viral infection risk, meaning that present surveillance programs focused solely on FIB are inadequate. A suitable viral indicator for wastewater contamination evaluation should, ideally, have similar inactivation and retention to the target pathogens and be present in wastewater and wastewater-contaminated environments throughout the year. This would allow for continuous monitoring and would offer information on the level of contamination and the possibility of pathogen presence [22, 23]. Table 1 describes certain enteric viruses found in wastewater that have the potential to be used as indicators; however, not all of these viruses fit these criteria. For example, Respiratory viruses and papillomaviruses have been detected in high concentrations in wastewater but not in polluted environments, which might be due to the rapid destruction of these viruses in water. Furthermore, some enteric viruses, such as hepatitis and Rotaviruses, may be zoonotic, which means that their presence in the environment is caused by agricultural operations rather than human waste. Additionally, enteroviruses, noroviruses, and sapoviruses have substantial seasonality in temperate locations, with peaks in the summer or winter [24]. As a result, these viruses are not found in wastewater or contaminated areas throughout the year [25, 26]. Human adenoviruses (AdVs) and polyomaviruses (PyVs) are often found in polluted environments, and their use as useful fecal indicators has been suggested [27, 28]. As a result, additional research is needed to determine if a single or many suitable markers may be employed as fecal indicators. Thus, based on the results of a one-year monthly monitoring for four human enteric viruses; enteroviruses, noroviruses, rotaviruses, and adenoviruses at a wastewater treatment facility, this study evaluates dependable viruses that can indicate the reduction of human enteric viruses during the wastewater treatment process.

2 Materials and methods

2.1 Samples collection

Wastewater samples were collected at the El-Serw wastewater treatment plant (WWTP) in Damietta, Egypt's influent (raw sewage) and outflow (processed effluent). From January to December 2019, samples were collected every two weeks for a year. One litre of the influent (raw sewage) and outflow (treated effluent) was collected in a clean plastic container and sent in an ice box to Egypt's Environmental virology laboratory, Environment and Climate Change Institute, National Research Centre within 5–6 h. 48 wastewater samples were collected during the study's period.

2.2 Samples processing, nucleic acid extraction, and cDNA synthesis

Using the adsorption–elution method, each sample was filtered individually through a nitrocellulose membrane

Table 1 Primer sequences used in this study

Family	Virus type in water	Genomic structure	Zoonotic
Adenoviridae	Adenovirus types 40 and 41	dsDNA	No
Astroviridae	Astrovirus	ssRNA	Potentially
Caliciviridae	Norovirus GI, GII	ssRNA	No
Hepeviridae	Hepatitis E	ssRNA	Yes
	Hepatitis A		No
Papillomaviridae	Papillomaviruses	dsDNA	No
Picornaviridae	Cosavirus, Enterovirus	ssRNA	No
Polyomaviridae	Bk Polyomavirus	dsDNA	No
	JC polyomavirus		
Reoviridae	Rotavirus A	dsRNA	Potentially

(0.45 m pore size and 147 mm diameter). According to Katzenelson et al. [29], adsorption viruses have been isolated by 3% beef extract and re-concentrated by organic flocculation. The pellet obtained was dissolved in 1 milliliter of Na_2HPO_4 (0.14 N, pH 9) and stored at − 70 °C until use. Total viral DNA and RNA were extracted with the QIAamp DNA kit (QIAGEN, Germany) and BioZOL solution (BIOFLUX, Japan), respectively according to the manufacturer's instruction. DNA and RNA were eluted in 100 µl of elution buffer and stored at −70 °C until use. Nanodrop Spectrophotometer (A260/280 ratio) was used to determine the concentration and quality of the isolated RNA or DNA. The RevertAid RT Reverse Transcription Kit (K1691, ThermoFisher Scientific) and reverse primers specific to viral target were used to create the cDNA.

2.3 Polymerase chain reaction amplification

In a final amount of 50 µl, 100 ng of virus DNA or cDNA, 2× PCR buffer, 1.5 mM $MgCl_2$, 200 M of dNTPs, 0.5 M of each outer primer, and 5 U of Taq DNA polymerase were used for PCR amplification. The original PCR phase was 95 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s, followed by 1 cycle of 72 °C for 7 min to extend the reaction using a Bio-Rad PCR platform. Table 2 contains the primer sequences for all specific viruses.

2.4 PCR product sequencing

PCR products were purified using the QIAquick purification kit (Qiagen, Germany) and sequenced directly by used the same primers in the PCR. Consensus sequences were matched to the existing sequences in the NCBI nucleotide collection database library using BLASTN program, http://blast.ncbi.nlm.nih.gov/Blast.cgi.

3 Results and discussion

Enteric viruses, such as rotaviruses, enteroviruses, noroviruses and adenoviruses, are significant public health concerns due to their high infectivity and extended survival in the environment. Contaminated water has been identified as one of the primary transmission pathways for diarrheal diseases, highlighting the importance of reducing viral loads prior to effluent release into the environment, especially prior to water reuse [34, 35]. The purpose of this study was to look into the presence of rotaviruses, enteroviruses, noroviruses, and adenoviruses in an Egyptian wastewater treatment facility, as well as to assess the plant's ability to remove viruses. As shown in Table 3 and Fig. 1, rotaviruses were found in wastewater samples with a positive ratio of 37.5% (18/48) followed by adenoviruses with a positive ratio of up to 20.8% (10/48) and enteroviruses with a positive ratio of 6.25% (3/48); however, these ratios were found to be different with those observed in

Table 3 Incidence of enteric viruses in WWTP

Month	Enterovirus	Rotavirus	Adenovirus	Norovirus
Jan		++++	++	ND
Feb	++	+++	+	ND
March		++		ND
April		+		ND
May		++	+	ND
June	+	+	+	ND
July			+	ND
August			+	ND
September			+	ND
October				ND
November		++	+	ND
December		+++	+	ND
Total	6.25% (3/48)	37.5% (18/48)	20.8% (10/48)	ND

Table 2 Primer sequences used in this study

Virus	Region	Sequence	Reference
Enterovirus	F1	CAAGCACTTCTGTTTCCCCGG	[30]
	R1	ATTGTCACCATAAGCAGCCA	
Norovirus	NoV-ORF1-F1	ATGAATATGAATGAAGATGG	[31]
	NoV-ORF1-R1	ATTGGTCCTTCTGTTTTGTC	
	NoV-ORF1-F2	TTGACACAATCTCATCATC	
	NoV-ORF1-R2	GTACCACTATGATGCAGATTA	
Rotavirus	VP6-F	GACGGVGCRACTACATGGT	[32]
	VP6-R	GTCCAATTCATNCCTGGTG	
Adenovirus	Hexon-F	GCCGCAGTGGTCTTACATGCACATC	[33]
	Hexon-R	CAGCACGCCGCGGATGTCAAAGT	

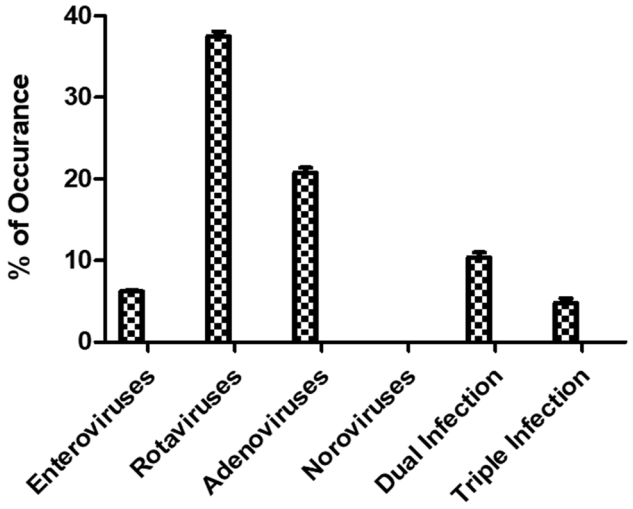

Fig. 1 Incidence of enteric viruses in wastewater treatment plant. Rotavirus was identified in 18 sample (37.5%), followed by adenovirus (20.8%), which detected in 10 samples

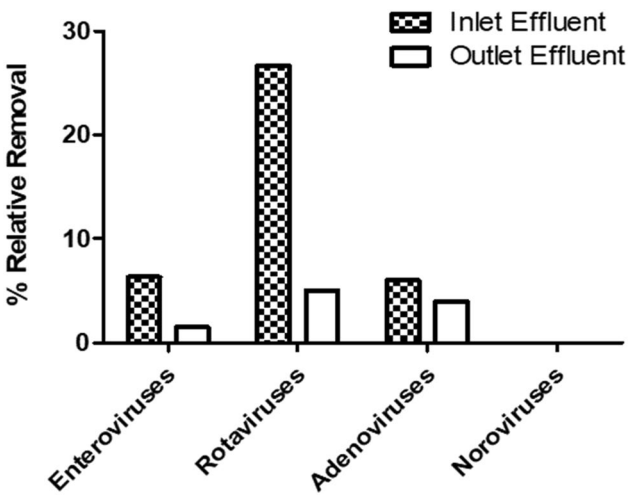

Fig. 2 Viral positive samples distribution in both inlet and outlet effluents. This figure shows the WWTP's capability in removal of viruses as next; 61.5% for enteroviruses; 39% for rotaviruses; 33.3% for adenoviruses

earlier Egyptian studies conducted in environmental or clinical samples [36–42]. Indeed, the positive ratio for rotaviruses (37.5%) in the current investigation was similar to that observed in previous Egyptian studies on clinical samples (31%), but higher than that observed in environmental samples (8.3–18.75%) [37, 38, 40]. The current study's viral positive rate (20.8%) was higher than in clinical and environmental samples (6.7–8.9%), [39, 41]. Our enterovirus detection rate (6.25%) was lower than the previous study in Egyptian sewage (22%). Variations in incidence rates may be linked to differences in geographical locations, such as rural versus urban areas, population size and society, and other environmental variables. In the current study, considerable levels of positive for enteroviruses, rotaviruses, and adenoviruses were discovered in the majority of intake samples, showing a high prevalence of enteric viruses in raw sewage. Our findings are consistent with previous research from Canada, the United States, and South Africa, where significant numbers of enteric viruses were discovered in raw sewage [18, 43, 44].

Figure 2 depicts the elimination ratios of human enteric viruses throughout the wastewater treatment process, with nearly one-third of the positive adenovirus and enterovirus ratios being adenovirus-negative and enterovirus-negative at final output discharge samples. At final discharge effluent tests, nearly half of the positive rotavirus ratio was rotavirus-negative. Although several methods for dealing with non-detects have been suggested, any of them can result in an overestimation or underestimation of the mean value [45]. The decline rates of the four human enteric viruses studied varied from 33 to 60%, which was lower than previous study findings [46]. Adenovirus removal ratios showed no notable variation, whereas enterovirus and rotavirus removal ratios were considerably higher than adenovirus removal ratios. To demonstrate that they are eliminated more effectively than the indicator virus(es) during the effluent treatment process, a virus(es) with a lower removal ratio than other viruses must be identified. Because of the high frequency of rotaviruses and the high resilience to wastewater treatment processes of adenoviruses, both rotaviruses and adenoviruses are excellent candidates to be a sign of fecal contamination, according to our findings.

4 Conclusion

The viral burden in the wastewater treatment plant's discharge effluents dropped non-significantly when compared to intake samples. As a result of their high frequency and lower removal rates, both rotaviruses and adenoviruses were deemed an appropriate indicator of human enteric viral reduction during the wastewater treatment process. Thus, rotaviruses and adenoviruses were considered suitable markers of human enteric virus removal during the wastewater treatment process.

Author contributions RS: writing, editing and visualization, reviewing. AA: conceptualization, methodology, writing, editing and visualization.

Funding There was no funding available for the study.

Data availability The data generated and/or examined during the present study are not publicly available due to an ongoing research endeavor but are available upon reasonable request from the corresponding author.

Declarations

Conflict of interest The authors state that they do not have any known competing financial interests or personal ties that could appear to have influenced the work described in this article.

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