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ARTICLE

Assessment of the removal efficiency of pathogenic viruses in three urban wastewater treatment plants in Palestine

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ABSTRACT

Using technologies and enforcing national reuse guidelines helps reduce health risks from recycled water used in irrigation. Analysing viral presence in raw and treated sewage is crucial for preventing pandemics. This study used qPCR to evaluate three urban wastewater treatment plants in Ramallah: Al-Tireh MBR, Al-Bireh AS, and Rawabi MBBR. Samples collected in March 2019 were treated and analysed using real-time PCR with specific primers and probes. Results showed Al-Tireh MBR achieved 100% removal of enterovirus, adenovirus, norovirus, and rotavirus. Al-Bireh WWTP achieved 33% removal for adenovirus and rotavirus. Rawabi MBBR achieved varying removal rates: 100% for adenovirus, 33% for norovirus, and 66% for rotavirus. Chlorinated samples showed no direct correlation between faecal coliforms and viral presence. These findings suggest retrofitting Al-Bireh WWTP and Rawabi MBBR with ultrafiltration or MBR units to mitigate viral outbreaks.

KEYWORDS

Wastewater treatment; viruses; qPCR; recycled water; water reuse

Introduction

Waterborne diseases worldwide cause the deaths of 2.2 million people every year, of which 1.4 million are children. Waterborne pathogens and related diseases are major public health concerns, and since they impose a burden on governments, economic losses of nearly US$1 billion, mainly in the US, have been estimated. These high costs are necessary for treatment and prevention [1]. Human enteric viruses, such as noroviruses (NoVs), adenoviruses (AdVs), and enteroviruses (EVs), are excreted at high concentrations in the faeces of infected individuals (up to 10^{11} virus/g faeces) with or without symptoms and are transmitted via the faecal-oral route, including contaminated food and water. Recent investigations [2] based on qPCR have revealed that the abundances of some viruses, such as noroviruses (NoVs) and saproviruses (SaVs), increase in winter, which is the epidemic period. The enteric viruses, such as AdVs and EVs, are abundant throughout the year. The infectious agents found in wastewater are bacteria, protozoa, viruses and helminth eggs. According to Castillo [1], improving water quality will reduce the global government burden for treatment of waterborne diseases by 4%.

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Epidemiology of pathogenic human viruses in treated wastewater

Enteric human viruses are shed in faeces and vomit, and the more voluminous the fluid output is, the greater the environmental contamination caused. Gastrointestinal human viruses tend to be strong enough to withstand extreme and difficult conditions more than respiratory viruses.

Water scarcity and wastewater reuse in Palestine

Many people suffer from outbreaks caused by consuming contaminated food or water, especially from contaminated salads, raw shellfish, or drinking unsafe river water. Outbreaks are common in impoverished regions with poor hygiene and with limited education [3]. Reusing treated wastewater and managing water demand, especially in irrigated agriculture, are among the most recommended strategies to alleviate severe water shortages in Palestine. Water scarcity significantly constrains the economic, social, and environmental sustainability of the agricultural sector in arid and semi-arid regions of the Palestinian Territories. The water deficit is projected to worsen in domestic and industrial sectors, leading to increased water demand. Palestine was expected to face a severe water deficit, estimated at approximately 271 million cubic metres by 2020 [4]. This is only going to increase, whatever the outcome of the current destruction (2024) by Israel of the Gaza Strip.

Palestinians face numerous challenges in the water sector, necessitating innovative research and capacity building. Limited access to water sources and insufficient financial support to establish or rehabilitate old sewerage systems and improve services prompt some farmers to use partially treated wastewater, a risky practice. The primary drawback is the presence of pathogens, viruses, and parasites, posing health risks to farmers, soil, nearby communities, and consumers of irrigated products. Treatment technologies capable of meeting effluent discharge limits set by the Ministry of Agriculture (MoA) and the Palestinian Water Authority (PWA), considering human health and water quality criteria for recycled water, include activated sludge systems (ASS), membrane bioreactors (MBR), constructed wetlands (CWs), trickling filters (TFs), and integrated fixed film activated sludge (IFAS) system (known as AGAR system). These wastewater treatment technologies have been implemented across the West Bank and Gaza, with technology selection based on location and population served [4,5]. This comparative study evaluated three urban WWTPs with different technologies: ASS, MBR, and IFAS systems in respect to their pathogen removal efficiency, particularly viruses.

Pathogens of concern

The four groups of microorganisms identified in treated and untreated wastewater include bacteria, protozoa, helminth eggs, and viruses. Episodes of cholera in the United Kingdom during the 19th C are not fully explained, but do appear to have been herald waves, i.e. small epidemics that heralded larger outbreaks. These herald waves are not necessarily linked to a specific origin but rather to the introduction of new cholera strains [6]. There were apparently more complex epidemiological patterns, with non-seasonal introductions of cholera. In Arizona, investigation

over a 12-month period (August 2011 to July 2012) into the occurrence of Cryptosporidium, Giardia, and Cyclospora at two wastewater treatment plants showed that these protozoan pathogens are prevalent in the study area and that efficacy of the conventional wastewater treatment processes at physically removing (oo)cysts is limited [7].

Viruses are obligate intracellular parasites that replicate exclusively within host cells and exhibit host specificity. Enteroviruses comprise a diverse family including polioviruses, echoviruses, coxsackieviruses, hepatitis A viruses, rotaviruses, and examples such as noroviruses [7]. Noroviruses and other viruses have been identified through reverse transcriptase RT-PCR, and electron microscopy [8]. Noroviruses are known to cause gastroenteritis. Rotaviruses are responsible for acute gastroenteritis, particularly among children, and contribute significantly to infant mortality in developing countries. Rotaviruses are commonly found in rivers, lakes, tap water, and municipal wastewater, transmitted via the oral-faecal route [9].

Adenoviruses are resilient to disinfectants and are not effectively removed by conventional treatments, making them persistent contaminants in the environment [8,9]. There are 47 types of adenoviruses, with types 40 and 41 identified as causative agents of gastrointestinal illness, especially in children [10]. Municipal wastewater treatment plants can effectively remove pathogens but also serve as potential source of environmental pathogen contamination [11,12]. Symonds *et al.* [13] reported the detection of adenoviruses and picobirnaviruses in 100% of raw sewage and 25% and 33% of final effluent samples. Enteroviruses and noroviruses were detected in 75% and 58% of raw sewage samples, respectively, and their presence in 8% of final effluent samples was proposed as potential markers of faecal contamination.

Wastewater treatment technologies for pathogens removal and resources recycling

Worldwide, most countries are currently facing not only water scarcity caused by climate change but also serious challenges in wastewater management. Yet there is an increase in water demand because of the rapid growth of the world population, which is expected to reach 11 billion by 2050. Therefore, urban wastewater treatment plants (WWTPs) should have the ability to remove pathogens and biological nutrients [14]. This study investigated the efficacy of three large-scale wastewater treatment plants (WWTPs), established in Al-Bireh/Ramallah governorate, using different technologies for the biological treatment of municipal wastewater and water reuse:

- Al-Bireh Wastewater Treatment Plant (Al-Bireh WWTP) uses the activated sludge system (ASS) with simultaneous nitrogen removal and aerobic sludge stabilisation.
- Al-Tireh Membrane Bioreactor Facility (Al-Tireh MBR), a modified ASS, applies immersed ultrafiltration (UF) membranes for tertiary treatment with separate aerobic sludge stabilisation.
- Rawabi Wastewater Treatment Plant (Rawabi IFAS) uses an integrated fixed film activated sludge (IFAS) process for biological nutrient removal with aerobic sludge digestion.

The three treatment technologies examined are of particular interest, as they represent the most common examples of full-scale urban wastewater treatment plants (WWTPs) in Palestine. These WWTPs are designed specifically to treat municipal wastewater, ensuring public health and environmental protection, while facilitating water recycling for agricultural irrigation in the Al-Bireh/Ramallah governorate.

Following the introduction of diverse wastewater treatment technologies with recycled water schemes in Palestine, there is a need to avoid including enteric viruses causing severe diarrhoea in farmers and their children, who consume recycled water-irrigated vegetables [14,15]. The selection and type of treatment technology for the removal of pathogens from recycled water, which is destined for food production, has become an important public health need [16]. This study aimed to analyse the viral presence in raw and recycled water from three selective municipal WWTPs, which is crucial for preventing pandemics and safe water recycling in Palestine.

Materials and methods

Urban wastewater treatment plants: treatment schemes and sampling locations

The aim was to assess the potential removal efficacy of waterborne human enteric viruses in three full-scale wastewater treatment plants (WWTPs) with different treatment processes, where all WWTPs use the activated sludge process with its modifications. Established in 2000 to serve 50.000-population equivalent (PE), Al-Bireh WWTP (Figure 1), a single-stage activated sludge system with simultaneous nitrogen removal and aerobic sludge stabilisation, is currently an overloaded oxidation ditch with partial nutrient removal. Table 1 shows that the UV light units are non-functional. This is because they are aged and should be replaced. Therefore, the treated water (6000 m^3/d) is currently discharged without disinfection into a nearby Wadi; the aerobically stabilised sludge is transported for landfilling after centrifugal dewatering.

Figure 2 depicts the flow scheme diagram with sampling sites for Al-Tireh WWTP, a suburb area of Ramallah city in the West Bank, which is connected to a membrane bioreactor (MBR). Put into operation in 2010 to serve about 25,000 capita, Al-Tireh MBR

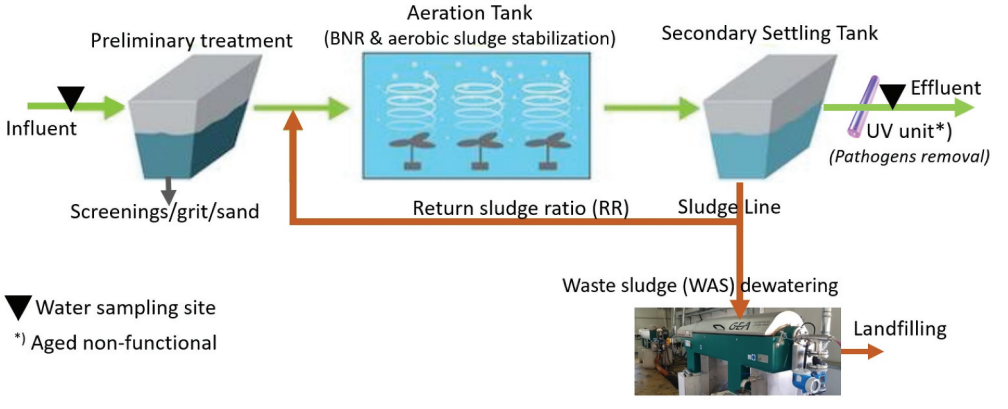

Figure 1. Al-Bireh wastewater treatment plant.

Table 1. Design and operational parameters of investigated urban wastewater treatment plants.

Plant Identifier/Parameter	Al-Bireh WWTP	Al-Tireh MBR	Rawabi-IFAS
Daily flow rate (m^3/d)	6000	2200	800
Membrane type/configuration	mixed liquor sludge flocs	Hollow-fibre/immersed	fixed film media
Sludge age (days)	20–25	18–20	22–25
Bioprocess configuration	Simultaneous NN/DN	Pre-Denitrification	BNR fixed film
Waste sludge stabilization	Simultaneous aerobic	Separate aerobic	Separate aerobic
Disinfection units	UV light [non-functional]	Chlorination	Chlorination

NN: Nitrification DN: Denitrification BNR: Biological nutrient removal.

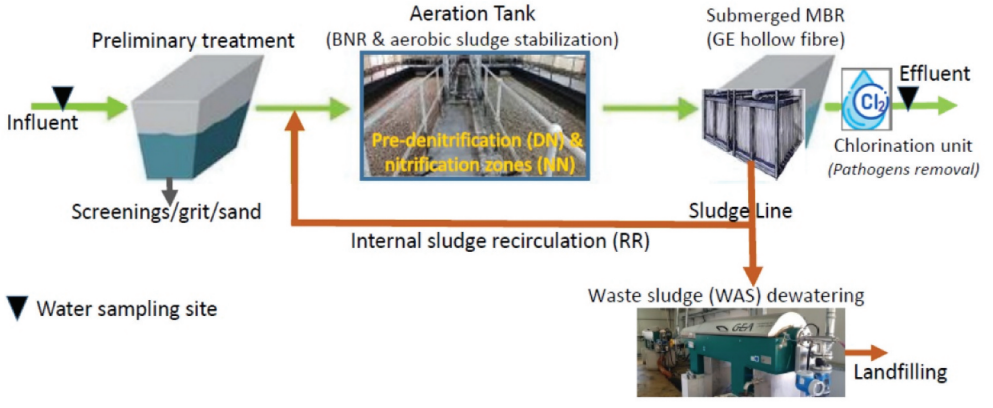

Figure 2. Al-tireh membrane bioreactor (MBR) facility.

facility uses a modified activated sludge with immersed hollow-fibre membranes (ultra-filtration: nominal pore size 0.045 μ). After preliminary treatment stage (fine screens and aerated grit chamber), the municipal wastewater is biologically treated in anoxic (pre-denitrification) and oxic MBR (nitrification) zones (Table 1). The MBR system operated at a flux rate of 18 L/(m^2.h) during the study. The membrane filtration cycle was set at 9 min followed by a relaxation period of 1 min.

After separate aerobic sludge stabilisation of the resulting waste sludge, the biosolids are disposed of at a municipal landfill site. Applying a zero-liquid discharge strategy, the reclaimed water undergoes disinfection in a chlorination system for restricted agricultural irrigation (Figure 2). Since early 2023, Al-Tireh MBR facility is being improved to provide a total treatment capacity for 50,000 PE.

Figure 3 presents the unit operations of Rawabi WWTP, which is a modified ASS depicting an integrated fixed film activated sludge process (IFAS). The process operation either can follow as a moving bed bioreactor (MBBR) without sludge recirculation or with sludge recirculation as an IFAS process. Serving about 8000 PE in its first planning phase, Rawabi IFAS system is built as compact stainless steel modules, designed for biological nutrient removal with separate aerobic sludge digestion. Reclaimed water is first disinfected, and then chlorinated (Table 1).

Table 1 shows the process specifications and operational parameters of the three urban WWTPs used in the research study. As shown in Figure 3, Al-Tireh MBR facility employed membrane geometries (hollow-fibre), fouling control strategies (relaxation and backwash) and employed GE membranes with an age of 5 years.

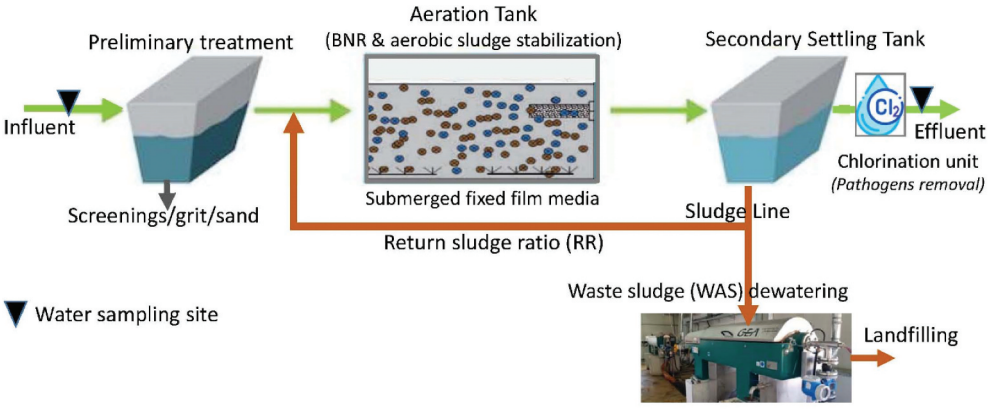

Figure 3. Process flow scheme with sampling locations at rawabi IFAS system.

Collection of water samples and lab analysis effluent quality

The lack of an autosampler requires the collection of composite water samples from both the influent and effluent of each WWTP. The sampling programme entailed collection of 3 grab water samples every 3 hours (early morning at 10:00 a.m., noon at 13:00 p.m. and afternoon at 15:00 p.m.), followed by a thorough mixing. Grab samples (72 samples of 0.5 litres each) collected at three different time intervals from the three WWTPs yielded 18 composite samples [3 time intervals x 2 (influent and effluent) x 3 WWTPs].

All water samples were collected and stored in plastic bottles on ice. The samples were delivered to the PWA Central Laboratory in Ramallah within a day after sampling and immediately processed for faecal coliforms (FCs) and virus concentration upon arrival. All of the samples were assayed for FCs on Difco M-FC agar medium and incubated at 44.5°C for 24 h according to American Public Health Association Standard Methods for Examination of Water and Wastewater [17]. The routine tests included BOD_5 (biological oxygen demand) using the Oxitop method, COD (chemical oxygen demand) using Hach kits, the total suspended solids (TSS) and total nitrogen (TN) [17]. Analyses of the chemical and biological parameters were performed to assess the compliance of three WWTPs treated water with the Palestinian effluent guidelines for agricultural irrigation.

Viral DNA/RNA extraction and qPCR analysis

The composite samples were sent to the laboratory for enterovirus testing using the quantitative real-time polymerase chain reaction (qPCR) analysis. Detailed laboratory procedures and analysis can be found in previously published works [18,19].

An important step was the protocol used for the concentration and preparation of the samples using polyethylene glycol (PEG) and sodium chloride [18,19]. After adding NaCl to the samples, the samples were homogenised for an hour using an automatic stirrer and stored at 4°C overnight for the next step. A total number of 18 wastewater samples (nine influent and nine effluent sites) were prepared for the next two lab steps: the extraction of DNA and RNA using automated Nuclisens Easy Mag followed by real-time PCR analysis using an automated Applied Biosystem 7500 [20,21]. Degenerate reverse and forward

primers and probes for each of the targeted viruses were added to the master mix tube, and 5 µl of nucleic acid was added to the extracted nucleic acid from the previous step.

Enterovirus RNA was amplified and detected using TaqMan technology on the ABI Prism 7500 system (Applied Biosystems, Foster City, CA, USA), following established protocols [20,21]. Specific primers and probes are added for the different types of viruses. For adenovirus, qPCR was performed with Taq polymerase. For enterovirus, rotavirus, and norovirus, reverse transcriptase was used first to generate complementary DNA (cDNA) at 500°C for 30 minutes prior to the amplification step. The virus-specific probes had fluorescent labels at the 5' end. A CCD camera on a 7500 instrument [20,21] detected the generated fluorescent signals.

Water samples were collected and stored in plastic bottles on ice to preserve integrity. Within 24 hours of collection, the samples were transported to the Palestinian Water Authority (PWA) Central Laboratory in Ramallah, where they were immediately analysed for faecal coliforms (FCs) and viral concentrations. Faecal coliforms were quantified using Difco M-FC agar medium, with incubation at 44.5°C for 24 hours, following the procedures outlined by the American Public Health Association (APHA) [17]. Additionally, grab samples from both the influent and effluent of each sewage treatment facility were analysed for physical parameters (total suspended solids, TSS) and chemical parameters (biochemical oxygen demand, BOD; chemical oxygen demand, COD; and total nitrogen).

Results and discussion

Previous studies in Palestine [14–16] analysed microbial pathogens, including faecal coliforms (FC), protozoa, and trophozoite, in raw and treated wastewater from Upflow Anaerobic Sludge Blanket (UASB) septic tanks at the Al-Bireh WWTP using microscopy and culture media. Samhan *et al.* [14] reported Salmonella presence in 30% of influent samples, with 15–39% detection, but none in the effluent. Table 2 provides the quality parameters of treated effluent produced by three WWTPs. The data include measurements of critical water quality indicators, such as biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and total nitrogen and Phosphorus), and the faecal removal rates as $\log_{10}$ FC, which are essential for assessing the suitability of the effluent for reuse applications. The values are compared to relevant national standards for effluent reuse, particularly in agricultural irrigation. All WWTPs produced recycled water that met the Palestinian effluent guidelines [22] for physical and chemical parameters, making the treated water suitable for agricultural irrigation. However, only the Al-Tireh MBR facility complied with the faecal coliform (FC) standards required for unrestricted agricultural irrigation [22].

Table 2. Effluent quality of treated water from the three wastewater treatment plants (WWTPs).

Plant Identifier/Parameter	Al-Bireh WWTP	Al-Tireh MBR Facility	Rawabi-IFAS System	PSI Effluent Guidelines [22]
TSS (mg/L)	30	10	22	30
BOD_5 (mg/L)	20	10	17	20
COD (mg/L)	90	30	70	100
TN (mg/L)	22	10	44	45
FC removal (Log_{10})	2.57	6.24	2.92	3.00

The enterovirus (EV) genus belongs to the Piconarviridae family and includes poliovirus, coxachievirus A&B echovirus and other enteroviruses. Most EVs are asymptomatic, and some of them can cause a wide variety of illnesses, including aseptic meningitis, dermatomyositis, polymyositis, and dilated cardiomyopathy. Rotavirus and astrovirus are difficult to multiply in cell culture [13].

Quantitative real-time polymerase chain reaction (qPCR) using Taqman chemistry was performed, and the results were analysed at Caritas Baby Hospital in Bethlehem. qPCR was performed on the extracted samples to check for enteric human viruses present in the raw and treated wastewater samples. The viruses were Enteroviruses, Adenoviruses, Noroviruses, and Rotaviruses. From each treatment unit, two samples were collected (influent and effluent) to compare the presence of the enteric viruses to determine the removal efficiency of the viruses in the treated wastewater from the three different wastewater plants. This permitted us to determine whether there is a correlation between the removal efficiency of viruses and the removal of faecal coliform bacteria.

Table 3 presents the cycle thresholds of the four viruses in the influent and treated effluent determined by real-time PCR using an automated 7500 Real-Time PCR thermal system. The composite collected samples labelled from A to R for every WWTP technology included three influent samples and three effluent samples. The three samples had similar components and the same sampling conditions.

An automated system (AB 7500 thermal system) was used to detect the enterovirus in the influent of the Al-Bireh WWTP at three different cycle threshold (Ct) numbers, 41.6, 46.8, and 38.9, as shown in Table 3. The Ct value is the cycle during which the fluorescence level starts to increase above the background level. For the effluent from the Al-Bireh WWTP, sample with label J, the system detected the adenovirus at Ct 39.1 and the rotavirus at Ct 31.7. The three samples from the Al-Tireh influent were negative for enterovirus (J-K-L) but were positive for adenovirus, norovirus, and rotavirus (Table 3). For adenovirus, there was negative detection of the virus in the three effluent samples of the Al-Tireh-labelled group (D-E-F), and the table shows 100% removal efficiency of viruses since the results were negative.

Table 3. Cycle thresholds of the four viruses in the influent and effluent samples.

Sampling Site	N	Enterovirus	Adenovirus	Norovirus	Rotavirus
A Effluent of Al-Bireh WWTP	4	Negative	39.1	Negative	31.7
B Effluent of Al-Bireh WWTP	4	Negative	Negative	Negative	Negative
C Effluent of Al-Bireh WWTP	4	Negative	Negative	Negative	Negative
D Effluent of Al-Tireh MBR	4	Negative	Negative	Negative	Negative
E Effluent of Al-Tireh MBR	4	Negative	Negative	Negative	Negative
F Effluent of Al-Tireh MBR	4	Negative	Negative	Negative	Negative
G Effluent of Rawabi IFAS	4	Negative	36.0	Negative	29.9
H Effluent of Rawabi IFAS	4	Negative	38.6	Negative	Negative
I Effluent of Rawabi IFAS	4	Negative	35.4	33.6	21.4
Sample Site		Enterovirus	Adenovirus	Norovirus	Rotavirus
J Influent of Al-Tireh MBR	4	Negative	31.0	Negative	27.6
K Influent of Al-Tireh MBR	4	Negative	30.0	32.5	27.9
L Influent of Al-Tireh MBR	4	Negative	29.7	35.0	25.4
M Influent of Al-Bireh WWTP	4	41.5	26.1	30.9	26.2
N Influent of Al-Bireh WWTP	4	46.8	25.9	28.6	28.3
O Influent of Al-Bireh WWTP	4	38.9	26.2	34.2	28.1
P Influent of Rawabi IFAS	4	Negative	35.7	34.4	32.8
Q Influent of Rawabi IFAS	4	Negative	35.2	Negative	35.5
R Influent of Rawabi IFAS	4	Negative	33.6	Negative	17.8

N: number of samples at three different sampling intervals.

For the influent of Rawabi, it was clear from the results (Table 3) that the labelled influents (p-Q-R results were negative for enterovirus detection), and for adenovirus. The PCR system detected adenovirus in three influent samples (I) at three different Ct values (35.7, 35.2, and 33.6). One sample from the three effluent samples (I) at CT 33.6 was positive for norovirus. Three influent samples were positive for rotavirus, and three effluent samples at Rawabi were negative for enterovirus.

The three effluent samples were positive for adenovirus, one positive and two negative for norovirus, and one negative for rotavirus. Analysis of the Rawabi WWTP effluent revealed one positive and one negative result. The removal efficiency of the Al-Tireh WWTP was 100%. Al-Bireh WWTP showed a removal percentage of 33% for both adenoviral rotaviruses. As shown in Table 3, adenovirus was not removed from the Rawabi effluent (G-H-I), the removal efficiency of norovirus was 33%, and the removal efficiency of rotavirus in the effluent of Rawabi effluent was 66%.

Figure 4 shows the fluorescence signal when the number of cycles (J-K-L) of adenovirus in the influent of the Al-Tireh MBR system was 31.0, 30.0, and 29.7. This indicated that the Al-Tireh influent had positive adenovirus results compared to those of the effluent, which were negative for adenovirus.

Figure 5 shows the peak with a fluorescent signal when the cycle number of norovirus in the Rawabi WWTP was 33.6, which means that the effluent had a positive result for norovirus, and the cycle threshold for norovirus was below 40.

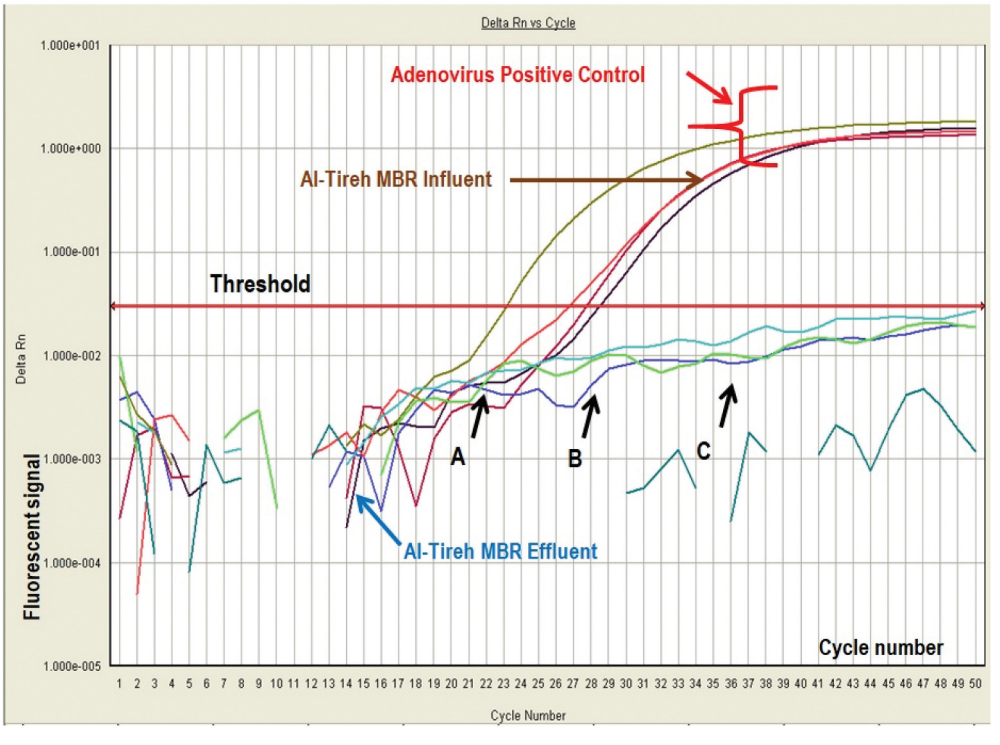

Figure 4. Real-time PCR positive amplification curve for the Al-tireh influent (red) adenovirus. Al-tireh effluent **(black colour)** adenovirus real-time PCR negative real-time PCR amplification curve.

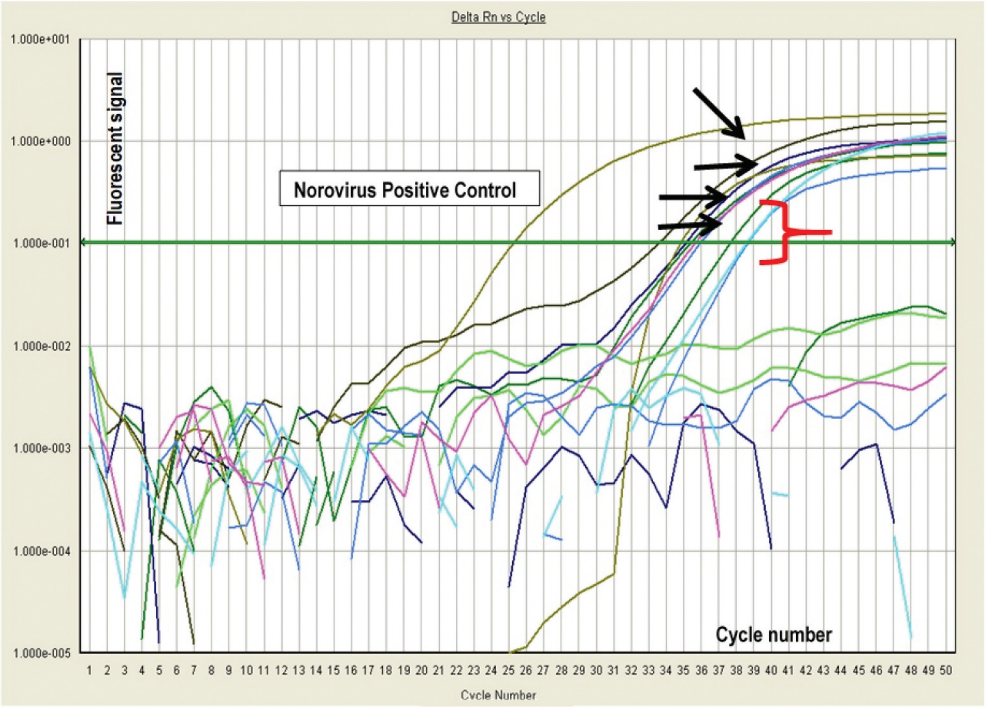

Figure 5. Rawabi effluent [red colour] Norovirus real-time PCR positive amplification curve of the wastewater treatment plant. Positive real-time PCR amplification curve for the norovirus influent (black colour).

The results of this study revealed that, despite effective reduction of physical, chemical, and biological parameters, enteric virus removal was incomplete. In Rawabi, PCR detected enteric viruses even with zero faecal coliform, highlighting the lack of correlation between faecal coliform elimination and virus removal [2,8,23,24]. In contrast, the Al-Tireh membrane bioreactor (MBR) achieved 100% virus removal, likely because of its 0.045-micron membrane, which effectively blocks viral particles [25–27]. Conversely, enteric viruses persisted in Rawabi and Al-Bireh plants, indicating the need for enhanced tertiary treatments to improve virus removal [14–16,26]. This highlights the public health risk posed by residual enteric viruses, aligning with findings from recent studies on fate of virus in treated wastewater destined for unrestricted agricultural irrigation, as shown by quantitative PCR methods [28–32].

Conclusions

This study assessed pathogenic viruses in wastewater from Al-Bireh and Rawabi WWTPs, revealing a 33% removal efficiency for adenovirus and rotavirus in Al-Bireh. The cycle threshold (CT) values for adenovirus and rotavirus in Al-Bireh's effluent were below 40. Rawabi's effluent showed CT values below 40 for adenovirus and norovirus and below 30 for rotavirus. These findings highlight the need for further investigation into the installation of post-treatment units at both plants, particularly the repair of UV units at

Al-Bireh. Implementing advanced filtration and disinfection stages could enhance reclaimed water quality, ensuring compliance with national reuse guidelines.

The results showed no correlation between faecal coliform removal and virus elimination. Despite zero faecal coliform in Rawabi, the PCR system detected enteric viruses at a specific cycle threshold. The membrane bioreactor (MBR) demonstrated a remarkable 100% removal efficiency for enteric viruses, including Adenovirus, Enterovirus, Norovirus, and Rotavirus, attributed to its submerged membrane with a nominal pore size of 0.045 microns. This effectively served as a tertiary treatment, preventing the passage of small viruses (0.01–0.3 μm). In contrast, the Rawabi and Al-Bireh wastewater treatment plants detected enteric viruses, highlighting the need for additional tertiary treatments to enhance virus removal efficiency. Although physical and chemical parameters, as well as faecal coliform bacteria, were adequately reduced, the persistence of enteric viruses poses significant public health risks.

This study found that faecal coliform concentration is not a reliable indicator of virus contamination, suggesting the need for alternative indicators. MS2 was identified as a suitable external control and a strong indicator for virus presence. Real-time PCR, because of its high specificity and 100% sensitivity emerged as the most effective method for virus detection. Quantitative real-time PCR (qPCR) amplifies and detects viral DNA or RNA in samples but cannot differentiate between live and dead viruses. As a result, qPCR can detect viral genetic material even if the virus is inactive or non-infectious.

Surveillance of infectious viruses in sewage can control them and so help to protect a community against the risk of an outbreak. The findings suggest that regular genetic screening and mapping should target these viruses by RT–qPCR to protect human health from these infectious viruses. Surveillance of COVID-19 and other infectious viruses in wastewater treatment plants will help to lower the disease burden on governments.

To reduce the global burden of waterborne diseases, selecting the adequate wastewater treatment technology is crucial. WWTPs can effectively lower the transmission of waterborne viruses, which spread through exposure to infected human waste, by disinfection of secondary effluent, and partially treated primary effluent.

Recommendations

Based on the results obtained in this study, the following recommendations can be made:

- Water safety plan (WSP) measures are necessary to improve the epidemiological surveillance of waterborne diseases and improve water quality in water catchments for health protection.
- Membrane treatment units such as ultrafiltration (UF), microfiltration, or nanofiltration (NF) are recommended as posttreatment methods to enhance the removal efficiency of pathogens, especially viruses, in treated water from the Al-Bireh and Rawabi WWTPs.
- Regular monitoring of the different WWTPs, particularly those in the Ramallah governorate under investigation and other WWTPs on the west bank, was performed by performing regular weekly, monthly or seasonal assessments of three physical, chemical, and microbiological parameters, including special indicator tests

for pathogenic viruses in wastewater, not only using routine faecal coliform indicators.
- In the phase of construction and design of large and costly WWTPs, the right technology is chosen according to the end uses of the reclaimed water, whether for unrestricted irrigation, restricted irrigation and safe industrial use or discharge into the receiving environment.
- Safety measures should be taken, especially among employees in wastewater plants in close contact with wastewater because this water contains hazardous pathogens that threaten human health.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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