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Comparative enteric viruses and coliphage removal during wastewater treatment processes in a sub-tropical environment

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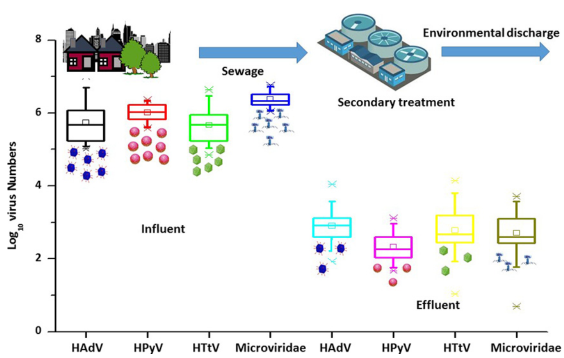
HIGHLIGHTS

- Enteric virus removal efficacy of wastewater treatment plants with activated sludge (ASP) process was determined.
- ASP in sub-tropical climate could be an effective treatment barrier with >3 log_{10} removal of enteric virus.
- Adenovirus was more resistant to removal compared to polyomavirus and torque teno virus.
- Physicochemical parameters of water quality are poor predictor of enteric virus presence.
- Adenovirus could be used as a model microorganism for determining enteric virus removal efficacy.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbiological safety of reclaimed water is one of the most important issues in managing potential health risks related to wastewater recycling. Presence and removal of human adenovirus (HAdV), human polyomavirus (HPyV), human torque teno virus (HTtV) and somatic coliphage family *Microviridae* in three wastewater treatment plants (WWTP) in sub-tropical Brisbane, Australia was investigated. All three WWTPs employ activated sludge process with added on Bardenpho process for nutrient removal. HPyV, HAdV, HTtV and *Microviridae* were consistently detected in the influent (10^5 to 10^6 Genomic copies (GC) L^{-1}) and secondary treated effluent (10^2 to 10^3GC L^{-1}). The results of this study suggest that, under appropriate conditions, WWTPs with activated sludge process in sub-tropical climate could be an effective treatment barrier with >3 log_{10} removal of enteric virus. The geometric mean of pooled data for each virus from all sites showed the highest removal for HPyV (3.65 log_{10}) and lowest for HAdV (2.79 log_{10}) which was statistically significant ($p = 0.00001$). Whereas, the removal rate of HTtV and *Microviridae* was identical (2.81 log_{10}). A poor correlation between the presence of enteric virus in influent or effluent with routinely monitored physicochemical parameters suggests limited use of physicochemical parameters as predictors of enteric virus presence. High prevalence of HAdV in influent and effluent combined with comparatively low removal suggest that it could be used as a model microorganism for determining enteric virus removal efficacy. Additional tertiary treatment may be required prior to effluent reuse for non-potable purposes or discharge into the recreational waters to prevent exposure of people to health hazards.

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1. Introduction

As increasing urban development and climate change continue to place stress on water supplies, wastewater reclamation is emerging as a viable and sustainable option to supplement limited water resources. Reclaimed water is increasingly used for non-potable applications such as irrigation water, industrial process water, and environmental enhancement in urban settings. The most significant issues of human health risk in recycled water reuse arise from the insufficient removal of enteric viruses. Enteric viruses, such as norovirus, rotavirus, adenovirus and astrovirus, are a major cause of waterborne gastroenteritis worldwide as they are highly stable in the environment and infectious at low doses (Sinclair et al., 2009).

Activated sludge process (ASP), which includes primary settling and biological degradation followed by secondary clarification, is commonly used in Australia (Keegan et al., 2013; Sidhu et al., 2017) and across the world (Kitajima et al., 2014; Nordgren et al., 2009; Simmons and Xagoraraki, 2011) for wastewater treatment and water reclamation. A number of studies have reported insufficient removal of enteric pathogens during ASP, with enteric viruses present in high numbers in the treated effluent (Albinana-Gimenez et al., 2009; Aw and Gin, 2011; Bofill-Mas et al., 2006; Kitajima et al., 2014; Nordgren et al., 2009; Simmons and Xagoraraki, 2011). Importantly, these studies typically report only enteric virus numbers in the influent and effluent, but physicochemical parameters or plant operational parameters which may influence the extent of virus removal are often not considered as part of the investigation (da Silva et al., 2007; Lodder and de Roda Husman, 2005).

The enteric virus types, numbers, and removal during the wastewater treatment may vary according to the season and geographical location due to community disease burden at different times combined with expected variation in the performance of activated sludge process in colder climatic conditions or at the time of high precipitation. Therefore, a generalization of the prevalence of pathogenic viruses in the reclaimed water is difficult (Gerba et al., 2013). The extent of human health and environmental risks associated with wastewater reuse can be effectively defined and managed if a wastewater treatment plant (WWTP) is working consistently at optimum pathogen removal efficiency.

Fecal indicator bacteria (FIB) such as fecal coliforms, thermotolerant coliforms, *Escherichia coli*, along with enterovirus are routinely monitored to assess the efficacy of wastewater treatment process in the removal of pathogens. However, the scientific consensus is that there is no correlation between the levels of bacterial indicators and enteric viruses in treated wastewater (Carducci et al., 2009; Harwood et al., 2005; Pusch et al., 2005); thus, conventional indicators may not be appropriate for evaluating treatment efficacy for enteric virus removal.

DNA enteric viruses such as human adenovirus (HAdV), human polyomavirus (HPyV), and human torque teno virus (HTtV) are prevalent in the aquatic environment and are thought to be more stable in the environment than RNA viruses such as norovirus and rotavirus (Love et al., 2010; Mena and Gerba, 2009). Consequently, the use of a DNA virus as a process indicator merits further investigation. To date, there is relatively little empirical data comparing the removal of HAdV, HPyV, and HTtV in WWTPs employing ASP from tropical and sub-tropical areas (Sidhu et al., 2017).

HAdV have been reported in wastewater in high numbers ($>10^6$ L^{-1}) worldwide (Fong et al., 2010; Maunula et al., 2012; Sidhu et al., 2013) and are important pathogens in wastewater recycling due to high thermal stability and resistance to ultraviolet light (Nwachcuku and Gerba, 2004; Thurston-Enriquez et al., 2003). HPyV is also a ubiquitous pathogen with worldwide distribution and has been proposed as indicators for the presence of human viral pathogens in contaminated water (Bofill-Mas et al., 2006). HTtV is prevalent worldwide in the general population; due to their environmental stability, they have also been suggested as an indicator of fecal contamination and process indicator for the drinking water industry (Charest et al., 2015; Griffin et al., 2008; Haramoto et al., 2005).

Somatic coliphages are non-enveloped viruses structurally similar to enteric viruses known to be present in relatively high numbers in wastewater, and their removal during wastewater treatment has been reported to be similar to removal of enteric viruses (Carducci et al., 2009; Martín-Díaz et al., 2016; Ottoson et al., 2006; Purnell et al., 2015). However, somatic coliphage is a diverse group of four distinct families (*Myoviridae*, *Microviridae*, *Siphoviridae*, and *Podoviridae*) with each family containing several genera (Lee and Sobsey, 2011). Recent studies have reported somatic coliphage belonging to the *Microviridae* to be widely prevalent in the wastewater influent and effluent (Purnell et al., 2015; Sidhu et al., 2017). Therefore, somatic coliphage family *Microviridae* may also be a useful indicator of enteric virus removal during ASP.

The aims of the study were; (i) a comparison of prevalence and removal of DNA viruses (HAdV, HPyV and HTtV) and somatic coliphage (*Microviridae* family) during wastewater treatment; (ii) diurnal variation in the selected virus numbers and existence of any correlation in presence or log reduction of enteric viruses; and (iii) correlation between routinely monitored physicochemical parameters and presence or removal of enteric viruses.

2. Materials and methods

2.1. Wastewater treatment plants and sample collection

Wastewater influent and effluent samples were collected from three major WWTPs; Luggage Point (LP), Oxley Creek (OX) and Bundamba (BU) located in Southeast Queensland (SEQ), Australia. The WWTPs vary in their influent source, treatment capacity, and specific treatment processes, but all utilize ASP and biological nutrient removal (BNR) as the secondary biological treatment step. Hydraulic Retention time (HRT) is highest at OX followed by BU and LP (Table 1).

Time separated duplicate grab samples of influent (1 L) and effluent (20 L) were collected in sterile carboy containers (Nalgene) during the southern hemisphere summer months (January to April). Wastewater samples were collected in the morning and afternoon on four occasions from LP, OX and on three occasions from BU WWTPs resulting in a total of 44 influent and 44 effluent samples. Influent and effluent samples were collected at 9:00 am and 4:00 pm on each sampling occasion. Influent samples were collected after the grit screens and effluent samples (secondary treated) were collected from the treated wastewater channel after collection from all of the clarifiers. Collected samples were immediately transferred to cooler bags and then to the laboratory for processing. All collected samples were processed within 4 h of collection.

2.2. Collection of physicochemical parameter data

A number of physicochemical parameters such as temperature, pH, electrical conductivity, and turbidity were recorded for the influent and effluent by the WWTPs operators. In addition, influent and effluent samples were sent by the management staff of WWTPs to National Association of Testing Authorities, Australia (NATA) accredited laboratories on a weekly to monthly basis for total suspended solids, total phosphorus, total nitrogen, Kjeldahl nitrogen, biological oxygen demand (BOD) and chemical oxygen demand (COD) analysis. The recorded physicochemical data (one month prior to and during the sampling period) obtained from the WWTP operators was analyzed (Table 2).

2.3. Concentration of wastewater samples

The collected influent and effluent samples were concentrated within six hours of collection by a hollow fiber ultra filtration system (HFUFS), using Hemoflow HF80S dialysis filters (Fresenius Medical

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