JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

Available online at www.sciencedirect.com

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Human viral pathogens are pervasive in wastewater treatment center aerosols

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ARTICLE INFO

Article history:
Received 21 February 2017
Revised 18 July 2017
Accepted 20 July 2017
Available online xxxx

Keywords:
Airborne viruses
Air sampling
Wastewater treatment plants
Viral metagenomi

ABSTRACT

Wastewater treatment center (WTC) workers may be vulnerable to diseases caused by viruses, such as the common cold, influenza and gastro-intestinal infections. Although there is a substantial body of literature characterizing the microbial community found in wastewater, only a few studies have characterized the viral component of WTC aerosols, despite the fact that most diseases affecting WTC workers are of viral origin and that some of these viruses are transmitted through the air. In this study, we evaluated in four WTCs the presence of 11 viral pathogens of particular concern in this milieu and used a metagenomic approach to characterize the total viral community in the air of one of those WTCs. The presence of viruses in aerosols in different locations of individual WTCs was evaluated and the results obtained with four commonly used air samplers were compared. We detected four of the eleven viruses tested, including human adenovirus (hAdV), rotavirus, hepatitis A virus (HAV) and Herpes Simplex virus type 1 (HSV1). The results of the metagenomic assay uncovered very few viral RNA sequences in WTC aerosols, however sequences from human DNA viruses were in much greater relative abundance.

Introduction

Wastewater treatment centers (WTCs) are, unsurprisingly, highly contaminated environments. Concentrations of viruses in effluent waters can be extremely high, sometimes reaching 10^{11} viruses/mL (La Rosa et al., 2010). Several studies have reported higher symptom and disease rates among this group of workers (Khuder et al., 1998). They experience respiratory symptoms, fevers, gastrointestinal symptoms, and headaches more often than the non-exposed population (Khuder et al., 1998; Smit et al., 2005; Van Hooste et al., 2010). The term "sewage worker's syndrome" was used for the first time in 1973 to describe the assemblage of these symptoms (Rylander et al., 1976).

Despite the fact that most WTC occupational symptoms can be associated with viral infections, only a few studies have investigated viruses as an occupational risk in these environments. Previous studies have demonstrated the presence of human pathogenic viruses in influent water, including noroviruses (Pouillot et al., 2015; Qiu et al., 2015) and rotaviruses (Baggi et al., 2001; Qiu et al., 2015) that cause gastroenteritis; adenoviruses (Osuolale and Okoh, 2015; Qiu et al., 2015),

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http://dx.doi.org/10.1016/j.jes.2017.07.015
1001-0742/

rhinoviruses and enteroviruses (Baggi et al., 2001, Qiu et al., 2015) that are responsible for the common cold, and even herpes simplex viruses, which can cause oral and genital sores and blisters (Bibby and Peccia, 2013). Among the viruses detected at WTCs, many of these pathogens are transmitted by aerosols (Tseng et al., 2010; Bonifait et al., 2015). Despite the importance of this route of transmission, surprisingly few studies have examined the potential exposure of WTC workers to pathogenic viruses through the air. Moreover, in these studies, only one or two viruses were analyzed (Romano et al., 1999; Uhrbrand et al., 2011; Masclaux et al., 2014). In Denmark WTCs, noroviruses (Noro) GI and GII were detected for the first time in air samples using personal samplers (Uhrbrand et al., 2011). Adenovirus (AdV), hepatitis E virus (HEV) and norovirus were also investigated in air samples from 31 Swiss WTCs. The researchers found AdV, noroviruses and HEV in 100%, 2% and 0%, respectively of the WTC aerosols tested (Masclaux et al., 2014). Enterovirus and reovirus were identified in 3.4% of the air samples from an aerosol study of WTCs in Italy (Romano et al., 1999).

To date, metagenomics has been used to characterize viral communities in aerosols in only three studies (Whon et al., 2012; Hall et al., 2013; Be et al., 2015). In all of these studies, RNA viruses were excluded, despite the fact that most human respiratory viruses have RNA genomes such as coronavirus, influenza virus, and metapneumovirus. To the best of our knowledge, this is the first time that a metagenomic approach has been used to characterize viral communities from WTC aerosols.

In Eastern Canada, WTC secondary treatment can differ from one plant to another. For example, some centers perform biological removal of residual matter (also called biofiltration) as the water exits the early stages of the treatment pipeline, while others conduct a secondary decantation as an alternative to biofiltration. Every day, WTC employees work in rooms or locations where different processing steps are taking place, some of which increase the risk of exposure to bioaerosols. In this study, we used various air samplers to evaluate the presence and abundance of some human pathogen viruses in aerosols in different locations of WTCs using qPCR. We also applied a viral metagenomics approach in one of the participating WTCs.

1. Material and methods

1.1. WTCs and site selection

Since very few reports exist on occupational airborne viral exposure, there is no consensus sampling strategy for the collection and purification of viral nucleic acids from aerosols. We therefore used both low and high flow rate sampling approaches.

Air samples were collected from four different WTCs from Eastern Canada during summertime. Indoor sites where wastewater treatment occurs and workers daily tasks are occurring were sampled. A total of 11 sites (or sampling locations) distributed among four WTCs are presented in this study. Pertinent details for each site are presented in Table 1.

1.2. Air sampling methods

1.2.1. Sampling for qPCR analysis

In this study, two different samplers were used to collect samples for qPCR detection (Coriolis®μ and Marple). The samplers were positioned at the same location at each water treatment site. Although the sampling duration was dependent on the sampler model, sampling was always performed during the same day over the same 6-hour shift.

The Coriolis®μ (Bertin Technologies, Montigny-le-Bretonneux, France) is a liquid cyclone that collects particles in 15 mL of Phosphate Buffer Saline (PBS). The flowrate was 200 L/min for 10 min, for a total of 2 m^3 of air/sample. The volume of the recipient was readjusted to 15 mL after sampling to compensate for evaporation. Five mL of the total were concentrated in a final volume of 200 μL using tangential flow filtration devices (100 kDa, Millipore, Darmstadt, Germany) used to conduct viral qPCR analyses (0.67 m^3 of air).

The second sampler, a size fractionating collector, the Marple Personal Cascade Impactor (Thermo Fisher Scientific, Waltham, USA) was used to collect information about aerosols size distribution of viruses containing bioaerosols. Due to material limitation, a maximum of two sampling locations per WTC were selected. In this sampler, air is accelerated by going through six radial slots of a first impactor stage in which each of the 8 stages impacts a subfraction of particles ranging from 0.5 to 21 μm of aerodynamic diameters that are ultimately collected on filters. After the 8th stage, another filter collects the remaining fine particles. It was used at a flowrate of 2 L/min for 5 hr, for a total of 0.6 m^3 collected. Each of the 9 filters was eluted in 5 mL of PBS and 500 μL were concentrated in a final volume of 25 μL using tangential flow filtration devices (100 kDa, Millipore, Darmstadt, Germany). Nucleic acids were extracted for this subsample and then used for qPCR.

1.2.2. Viral metagenomics

For viral metagenomics approach, the sampling was accomplished with the SASS 2300 sampler (Research International,

Table 1 – Description of wastewater treatment centers (WTCs) and sites of the study.

WTCs	Sites	Tasks
WTCs 1 and 2	Screening	Removal of big objects (*e.g.*,: plastics and paper)
	Grit/fats, oils and greases (FOGs) removal)	Removal of granular matter and FOGs
	Biofiltration	Biological degradation of residual organic matter
WTC3	Primary screening	Removal of big objects
	Secondary screening	Removal of residual big objects
WTC4	Screening	Removal of big objects
	Grit/fats, oils and greases removal	Removal of granular matter and FOGs
	Secondary decantation	Removal of residual particles by decantation