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Original Article

Molecular Detection and Characterization of Human Adenoviruses in Wastewater and Air Samples of Aeration Tanks

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Abstract

Background and Aims: Human adenoviruses (HAdV) are worldwide distributed pathogens that cause a variety of illnesses, including gastrointestinal infections. These viruses are considered as important pathogens in wastewater, reclamation, and reused water. We aimed to investigate the molecular prevalence and characterization of HAdV in wastewater samples as well as air samples of aeration tanks of a wastewater treatment plant (WWTP) in Tehran, Iran.

Materials and Methods: From November 2017 to April 2018, a total of 14 wastewater and 9 air samples were collected and analyzed for the presence of HAdV by an integrated cell culture/polymerase chain reaction (ICC/PCR) technique. The samples were collected from the Ekbatan WWTP in the west of Tehran. To capture bio-aerosols, a liquid impingement biosampler was used. Typing of HAdV was performed by sequencing analysis.

Results: Out of nine untreated wastewater samples, one sample (11.1%) was positive for the presence of HAdV cytopathic effect (CPE), while two (40.4%) of five treated wastewater samples were positive for the HAdV CPE. The results of PCR assay also showed that 44.4 % (four out of 9 samples) of the untreated wastewater and 60% (three out of 5 samples) of the treated wastewater samples were positive for HAdV genome. Regarding the air samples of aeration tanks, four (44.4%) of nine samples were positive for the presence of the HAdV genome. HAdV in the wastewater samples were type B (40%), type C (40%), and type D (20%) and all four positive air samples for HAdV were type C.

Conclusion: The human adenovirus detected in 50% of the wastewater and 44.44% of the air samples of the wastewater treatment plant of the Ekbatan, Tehran, Iran.

Keywords: Human adenovirus, Waste Water, Molecular typing

Introduction

Human adenoviruses (HAdV) are worldwide distributed, and it has been estimated that around 90% of the human population is exposed to multiple serotypes of HAdV around the world (1). HAdV are transmitted by close person-to-person contact, aerosolized virus, oral-fecal route, and contaminated water (2, 3). They can infect a wide variety of organs such as the respiratory tract, gastrointestinal tract, eye, and bladder (4, 5), causing a wide spectrum of clinical diseases like pharyngitis, pneumonia, conjunctivitis, and hemorrhagic cystitis. It has been well-documented that HAdV, especially types 40 and 41, are the second most common etiology of viral gastroenteritis in children after rotavirus (6).

The reclamation and reuse of water from wastewater is an important issue in water resource management (7, 8). The reuse of wastewater can be beneficial for agriculture because of nutritional value (9, 10), however, several limitations such as the presence of toxic and infectious agents have raised

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concerns regarding the process (11, 12).

By considering the recent developments in wastewater reclamation, the consumption of these resources can be dangerous for human health in the developing countries (13).

Wastewater contains various types of human pathogens including viruses, bacteria, and parasitic protozoans. Enteric pathogens are released into wastewater from human or animal fecal wastes. Among wastewater pathogens, viruses constitute a major threat to public health. Enteric viruses such as norovirus, adenoviruses, hepatitis A, and rotavirus are considered as the major viral pathogens in wastewater, which are closely associated with several diseases in humans such as gastroenteritis, hepatitis, and meningitis (14-19).

HAdV are more resistant to different physical and chemical agents and ultraviolet (UV) irradiation during the wastewater treatment compared with other waterborne enteric viruses and fecal indicator bacteria, making them a serious public health concern (20).

Given the importance of HAdV in the wastewater and reclamation of the water, the current study was aimed to investigate the molecular epidemiology and characterization of HAdV in wastewater samples as well as air samples of aeration tanks of a wastewater treatment plant (WWTP) located in Tehran, Iran.

Methods

Sample collection and concentration: From November 2017 to April 2018, samples including 14 treated and untreated wastewater and 9 air samples from aeration tanks were collected from the Ekbatan WWTP, located in the west of Tehran. All wastewater samples were collected in sterilized tubes and transported under refrigeration to the laboratory.

A liquid impingement biosampler was also used to capture air samples from aeration tanks. The concentration of samples was performed based on the method described by Minor et al (21), with some modifications.

Briefly, samples were centrifuged at 4000 rpm for 25 minutes at 4°C, and the resulting supernatant was then transferred to a new tube. Thereafter, 3.9 ml Dextran, 3.5 ml NaCl, and 2.84 ml polyethylene glycol 6000 were added to each 500 mL of the supernatant. The final mixture was centrifuged at 4000 rpm for 2 hours at 4℃, the supernatant was discarded, and the pellet was suspended in phosphate-buffered saline (PBS), and stored at −80°C until use (22, 23). The samples were then subjected to the detection of HAdV by the integrated cell culture-polymerase chain reaction (ICC-PCR).

Cell culture: In this study, the HeLa cell line was used for ICC-PCR. The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin at 37°C and 5% CO2 atmosphere. Before inoculation into the HeLa cell line, the samples were filtered using 22 µm filters. The presence of cytopathic effect (CPE) read on day 7. The cultures were then subjected to freeze-thaw cycles to release viral particles from the cells. The lysate was then centrifuged, and the supernatant was used for viral DNA extraction.

DNA extraction: DNA extraction was performed by Viral Nucleic Acid Extraction Kit (Yekta Tajhiz Azma, Iran), according to the manufacturer's protocols. The concentration of the extracted DNA was determined using NanoDrop (ND-1000, Thermo Scientific, USA).

HAdV detection by PCR: To detect all possible HAdV from the extracted DNA, PCR was used to amplify a 243 bp region of the hexon gene. The forward and reverse primers were 5'-GCTTCGGAGTACCTGAGYCC-3' and 5'-GGCCATRTCCAGCACTCKGT-3', respectively. PCR was performed in a thermal cycler machine model 9700A (Applied Biosystems, USA). The total volume of the reaction mix was 20 µL, and it contained the following components: 10µL of master mix (Yekta Tajhiz Azma, Iran), 0.5 µL of each primer, 3µL of template, and 6 µL of double-distilled water (ddH2O). Thermal PCR cycles were as follows: The initial denaturation step was carried out at 95°C for 5 minutes, followed by 35 cycles of 95°C (30 seconds), 60°C (30 seconds), and 72°C (30 seconds), and an extension of 72°C for 5 minutes. PCR products

were separated on a 1.5% agarose gel stained with safe stain and visualized under UV light.

Sequencing and phylogenetic tree: The PCR products were sequenced directly by Sanger sequencing. The obtained sequences were aligned with the HAdV reference sequence by the CLC Main Workbench 5.5 software (CLC bio, Boston, MA, USA). The phylogenetic analysis was performed using MEGA version 10. The phylogenetic tree was constructed using the Tamura three-parameter model with a bootstrap test of 100 replicates.

Statistical analysis: Statistical analysis was performed using SPSS version 22 software (SPSS Inc., Chicago, IL). The Chi-squared test was used to assess the statistical differences between groups. A P-value of less than 0.05 was considered statistically significant.

Results

A total of 14 wastewater and 9 air samples were taken at different locations in the Ekbatan WWTP, located in the west of Tehran, and examined for the presence of HAdV. Among 14 wastewater samples, 9 were row (untreated) and 5 were treated wastewater. The results of cell culture assay showed that among 9 untreated wastewater samples, 1 sample (11.1%) was positive for the presence of HAdV CPE, while the CPE was observed in 2 samples (40.4%) of 5 treated wastewater samples. Also, the PCR assay showed that 44.4 % (four out of 9 samples) of the untreated wastewater samples and 60% (three out of 5 samples) of the treated wastewater samples were positive for HAdV genome. For the detection of HAdV in air samples from aeration tanks, PCR assay was used. We found that among 9 air samples, 4 (44.4%) were positive for the presence of the HAdV genome. Table 1 shows the results of HAdV detection at the sampling points by cell culture and PCR assays.

The analysis of the sequence alignment and the phylogenetic tree showed that the detected HAdV in the wastewater samples were type B (40%), type C (40%), and type D (20%). Furthermore, all four positive air samples for HAdV were type C. The phylogenetic tree is illustrated in figure 1.

Discussion

Some viruses can maintain their infectivity for a long time in a humid environment (24). Noroviruses, hepatitis A virus, hepatitis E virus, rotaviruses, astrovirus, enteroviruses, and adenoviruses can remain infectious after weeks in the wastewater treatment (14-19). Some of these viruses could be found in wastewater treatment plants (25). One of the wastewater treatment aims is to reduce the circulating dangers pathogens for humans (26). The row wastewater could be consumed as a source for the human enteric pathogens (27). The non-enveloped viruses are more stable in the humid environments and some of them can be transmitted through oral-fecal transmission. Until the current time, there is no study for the evaluation of the circulating adenoviruses in the wastewater of Tehran, Iran. The current study was aimed to investigate the adenovirus distribution and molecular characterization in the wastewater from Tehran, Iran. The study results indicated that the adenovirus distribution in wastewaters was 50% and in the air samples was 44%.

Also, the typing of the viruses shows the B, C, and D types are detectable. Conducted studies indicated that the HAdV type B could be associated with the respiratory and urinary infections. Also, type D could be associated with gastrointestinal diseases which could justify the presence of these two types in the wastewater samples. Also, type C was reported in the air samples, and the virus mostly associated with respiratory disease.

By considering the importance of respiratory disease by type C of the HAdV the personal protection equipment for the wastewater treatment plant could be helpful (28, 29). The molecular techniques could not differentiate the infective viral particles from non-infective ones. This point should be considered, and it could be concluded that the detection of the adenoviruses genome in the wastewater samples does not reflect the infectious particles (30).

Table 1. the culture and PCR results of the wastewater and air samples							
Type of samples		**CPE in cell culture**			**PCR**		
		Positive, n (%)	**Negative, n (%)**	**Total (%), n (%)**	**Positive, n (%)**	**Negative, n (%)**	**Total (%), n (%)**
Wastewater	Row	1 (11)	8 (89)	9 (39)	4 (44)	5 (56)	9 (39)
	Treated	2 (40)	3 (60)	5 (22)	3 (60)	2 (40)	5 (22)
Air sample		-			4 (44)	5 (56)	9 (39)

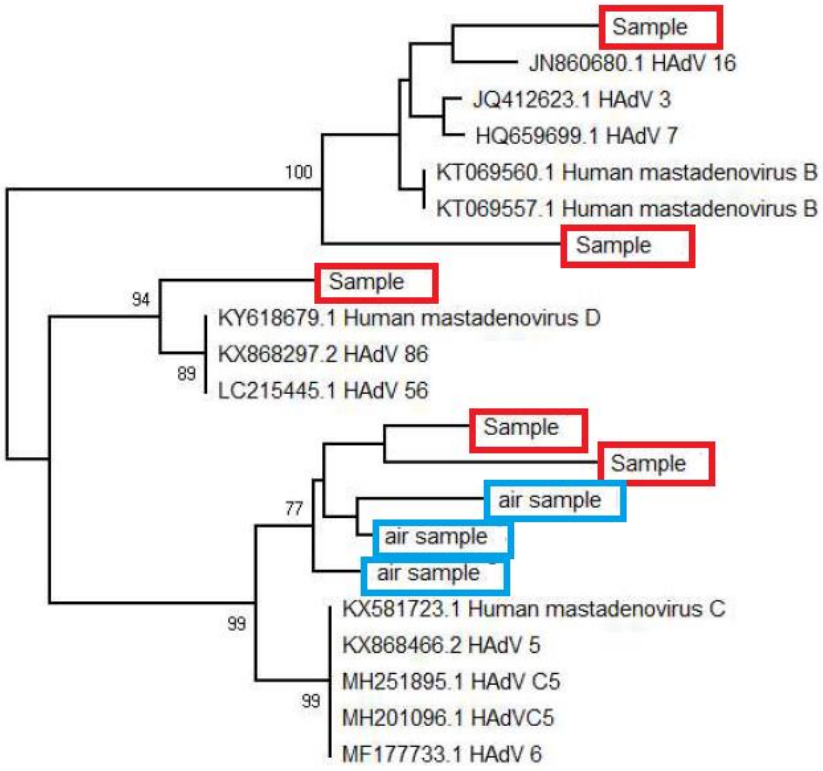

Fig. 1. the phylogenetic tree for the partially hexon sequence of the HAdV by 160bp nucleotides, the wastewater samples shows with the red and the air samples shows with the blue color.

In Morocco, Amdiouni et al. (23) investigated the adenoviruses and enteroviruses in the wastewater samples. The Amdiouni's study result indicated the adenoviruses are detectable in 45.5% of the tested samples. Also, the major determined types were D and B. one of the major complications in the assessment of the virtues by the molecular technics in the wastewater is the presence of inhibitory factors, which the ICC-PCR could be helpful in this case (31). Also, the Xagoraraki et al. (32) suggested that the ICC-PCR could be helpful in the detection of the viruses when the direct PCR result is negative. The current study method was similar to Amdiouni's study and the results could be confirmed by this study. Although, the geographical differences should be considered between these two studies. In the other study conducted in Switzerland, the adenoviruses were the dominant detected virus in aerosols and detected in 60% of the samples (33). The current study results show the adenovirus is detectable in 44.44% of air samples from aeration tanks. The differences between these two studies by considering the geographical differences and sample size could be justified. The major limitation of the current study was the limited sample number. Also, the

investigation of the presence of other gastro-intestinal viruses could be suggested.

Conclusion

In conclusion, the study result indicated that the HAdV detected in 50% of the wastewater and 44. 4% of the air samples of the wastewater treatment plant of the Ekbatan, Tehran, Iran. The investigation of the treated and non-treated wastewater could not show any statistically significant differences, which leads to the suggestion of further investigation for the more effective techniques for the wastewater treatment.

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Conflict of interest

No conflict of interest is declared.

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