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RESEARCH

Comparative Investigation and Trends of Respiratory Viruses Using Wastewater-Based Epidemiological Surveillance in Patras, Greece

Zoi Anastopoulou[1] · **Rafail Fokas**[1] · **Kalypso-Angeliki Koukouvini**[1] · **Angeliki Chatziantoniou**[1] · **Apostolos Vantarakis**[1]

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Abstract

The COVID-19 pandemic has underscored the importance of alternative epidemiological tools capable of providing real-time, population-level insights into infectious disease dynamics. Wastewater-based epidemiology (WBE) has emerged as a powerful, non-invasive method for tracking viral transmission within communities. While initially focused on SARS-CoV-2, WBE now offers the potential for multiplex surveillance of a broader range of respiratory pathogens. This study applied WBE to monitor the circulation of six major respiratory viruses such as Human adenovirus (HAdV), SARS-CoV-2, Influenza A and B, Respiratory Syncytial Virus (RSV) A/B, and Rhinoviruses, over a six-month period (ISO weeks 2022-40 to 2023-13) in the city of Patras, Greece. Weekly composite samples from a central wastewater treatment plant were analyzed via quantitative PCR, and viral genome concentrations were normalized per 100,000 inhabitants. The results revealed distinct circulation patterns: HAdV and SARS-CoV-2 were detected consistently throughout the period, while Influenza A peaked during the winter months, followed by Influenza B. RSV, however, appeared earlier in the season (first detectable from ISO week 2022-46) and overlapped with Influenza. Rhinoviruses displayed intermittent peaks, indicating multiple waves or persistent low-level transmission. Correlation analyses showed strong positive associations between influenza viruses, RSV, and SARS-CoV-2, suggesting synchronized seasonal trends. Hierarchical cluster analysis classified the viruses into three distinct groups: (1) an epidemic cluster including Influenza A/B, RSV, and Rhinoviruses; (2) a persistently present cluster represented by HAdV; and (3) a separate episodic cluster characterized by SARS-CoV-2. These groupings reflect differences in viral epidemiology and shedding behaviour. This study confirms the effectiveness of WBE in tracking the temporal dynamics of multiple respiratory viruses and provides evidence of its utility in supplementing traditional clinical surveillance systems. The consistent detection of underreported pathogens such as HAdV underscores the added value of environmental monitoring for public health preparedness and early warning applications. These findings advocate for the integration of WBE into routine respiratory virus surveillance frameworks.

Introduction

Since the end of the COVID-19 pandemic, the use of wastewater-based epidemiology (WBE) has grown rapidly. It has been used to detect and epidemiologically monitor the presence of SARS-CoV-2. In addition to distinguishing infectious diseases, the distinct presence of various pharmaceutical and chemical components has been used to assess drug use patterns and assess collective population exposure. The potential of WBE is amply illustrated by its ability to predict or prevent potential outbreaks in a community before patients develop clinical symptoms and up to three weeks before diagnostic clinical cases (Sims & Kasprzyk-Hordern, 2020). The economic feasibility of the methodology is underlined by its ability to provide a timely

✉ Apostolos Vantarakis
avanta@upatras.gr

Zoi Anastopoulou
zoi.anastopoulou@ac.upatras.gr

Rafail Fokas
fokasrafael@gmail.com

Kalypso-Angeliki Koukouvini
k.koukouvini@gmail.com

Angeliki Chatziantoniou
angelina.chatziantoniou@gmail.com

[1] Laboratory of Public Health, Epidemiology and Quality of Life, School of Medicine, University of Patras, 26504 Patras, Greece

snapshot of the course and presence of a pathogen in the population, contributing to the epidemiological surveillance of the disease, accompanying the clinical recording (hospital admissions or recorded cases) by the health systems of the countries. In examining the bioethical and personal-data considerations related to wastewater-based epidemiological surveillance, previous analyses have generally suggested that monitoring wastewater entering large municipal sewer networks presents comparatively low privacy concerns, as noted in recent discussions (Doorn et al. 2022; Gable et al., 2020).

Currently, several countries have established national WBE frameworks for SARS-CoV-2 enforcement, which were formalised by Commission guidance in March 2021, and efforts are now underway to integrate additional emerging pathogens or persistent pathogens, their strains or variants, into countries' epidemiological protection systems (Tavazzi et al., 2023). Furthermore, the latent period of the disease, which can extend up to two weeks before the onset of symptoms, introduces time lags between the onset of the disease and the subsequent reporting of clinical outcomes. These observations collectively suggest that urban wastewater within communities may harbour the virus at significant concentrations. Throughout the pandemic, WBE has emerged as a promising tool for providing insights into the prevalence and course of the disease, highlighting its potential as a valuable adjunct to surveillance efforts (Ahmed et al., 2020; Dumke et al., 2022).

The rationale behind the selection of specific respiratory viruses is presented in the following section. Human adenoviruses (HAdV), recognized, are prominent pathogens associated with a diverse array of human afflictions, including respiratory illnesses, gastroenteritis, conjunctivitis, and chronic systemic infections, and are particularly notable among immunocompromised individuals (Osuolale et al., 2015). Characterized by their exclusive human host tropism, adenoviruses are ubiquitously present in terrestrial and aquatic environments. Instances of adenovirus outbreaks have been documented across various settings, spanning military facilities, recreational water venues, healthcare institutions, and residential care facilities (Flint & Nemerow, 2017).

The pervasive presence of these viruses in wastewater and aquatic ecosystems underscores their heightened prevalence compared to that of other enteric viruses. Owing to their environmental stability and robust detectability, human adenoviruses are valuable indicators of wastewater contamination and offer insights into the presence of other viral pathogens (Bofill-Mas et al., 2006) Extensive research underscores the proclivity of adenoviruses to manifest in conspicuously elevated concentrations relative to their viral counterparts, rendering them promising candidates as biomarkers within aquatic ecosystems (Albinana-Gimenez et al., 2009). Temporal and geographical variations in adenovirus serotype prevalence underscore the dynamic nature of adenovirus epidemiology. Documented alterations in serotype prevalence across different regions suggest the potential emergence of novel strains and the displacement of existing strains. Despite adenovirus outbreaks predominantly occurring during winter or early spring, infections occur year-round without distinct seasonality, particularly within enclosed communities where epidemic trends tend to escalate (Lynch et al. 2016).

HAdV can be transmitted through airborne routes. Adenoviruses responsible for respiratory infections, such as types 3, 4, 7 and 21 - are known to spread through respiratory droplets expelled when an infected person coughs, sneezes or talks. These droplets can be inhaled by people nearby or sit on surfaces, leading to indirect transmission. HAdV types 3, 4, 7 and 21 have been strongly associated with outbreaks of acute respiratory disease (ARD), particularly in crowded settings such as military barracks, schools and healthcare facilities. For example, studies have shown that HAdV-3 and HAdV-7 are major causes of severe respiratory infections in children, sometimes leading to life-threatening conditions. Similarly, HAdV-21 has been implicated in severe respiratory disease epidemics (Zaki et al., 2020). Research indicates that HAdV-7 infections can result in more severe disease outcomes compared to other types. A study published in BMC Infectious Diseases found that HAdV-7 infection caused more severe pneumonia, respiratory failure, and longer hospitalization periods compared to HAdV-3. This highlights the virulence of certain adenovirus types and the need for vigilant monitoring and preventive strategies (Fu et al., 2019).

Respiratory Syncytial Virus (RSV) is a common pathogen in children and increasingly recognized in adults, especially the elderly. It primarily causes upper respiratory infections but can lead to bronchiolitis in young children and, in rare cases, progress to pneumonia, respiratory failure, apnea, or death. Treatment is mostly supportive. Passive immunization is available for at-risk children, such as premature infants or those with cardiac, pulmonary, or neuromuscular conditions. One antiviral treatment exists, but its use is limited due to cost, side effects, and limited efficacy, and is reserved for high-risk patients (Hernroth eta al 2002, Corman et al., 2012). RSV is a single-stranded, negative-sense RNA virus from the Paramyxoviridae family and Pneumovirus genus. Discovered in chimpanzees in 1955, it was soon identified in humans (Sims et al. 2020). RSV infects about 90% of children by age two and frequently affects older children and adults due to weak long-term immunity. While most infections are mild, up to 40% of primary infections in children under one cause bronchiolitis. Globally, Respiratory syncytial virus (RSV) is estimated to cause approximately 33 million episodes of acute lower respiratory infections globally in young children annually, resulting in around 3 million hospitalizations and up to 118,000–199,000 deaths, predominantly in low- and middle-income settings. (Public Health Agency of Canada, 2024). In temperate climates, RSV incidence typically peaks during winter-spring, while tropical regions exhibit more variable seasonal patterns. High-risk groups include premature infants, children with underlying health conditions, and the elderly, who face increased risk of severe outcomes and hospitalization. (WHO, 2025)

Influenza is a contagious viral infection affecting the upper and lower respiratory tract. It spreads through respiratory droplets and contaminated surfaces. Infected individuals are contagious even before symptoms appear and remain so for 5–7 days. While most recover in a few days, serious complications like pneumonia can occur, especially in young children, the elderly, pregnant women, and immunocompromised individuals. Symptoms include fever, cough, sore throat, and runny nose. Seasonal flu outbreaks occur mainly in autumn and winter, spreading rapidly across populations and varying in severity by age group (Hewitt et al., 2013; Martin et al., 2023; Rashed et al., 2022). Influenza A and B viruses cause annual epidemics worldwide. Outbreaks in the Northern Hemisphere usually occur from October to March, and from April to August in the Southern Hemisphere. In tropical regions, flu circulates year-round. Seasons dominated by H3N2 are typically more severe, particularly for children and older adults. The WHO tracks global influenza trends and virus strains monthly (WHO, 2025).

Finally, discovered in the 1950s, Human Rhinoviruses (HRVs) are mostly known as the leading cause of the “common cold”; they are ubiquitous, and HRV infections occur year-round (Prado et al., 2014) HRVs, members of the family Picornaviridae and the genus Enterovirus. HRV replicates in nasal and posterior nasopharynx mucosa (Bivins et al., 2021). Transmission is mainly attributed to hand contact between persons or through fomites. Oral or aerosol transmission seems to be rare and dependent on viral particles concentration in the droplets. Viral loads in saliva are about 30 times lower than in nasal secretions (Kumblathan et al. 2022). Studying rhinoviruses is important for several reasons, both from a clinical and public health perspective. Although typically mild, rhinovirus infections result in significant healthcare visits, school/work absences, and productivity losses, moreover, they are also frequently involved in co-infections with other respiratory pathogens, complicating clinical outcomes.

This study aims to monitor the most common and airborne-transmitted respiratory viruses, through Wastewater-Based Epidemiology (WBE), and to conduct a comparative investigation and trend analysis. The goal is to identify circulation patterns and evaluate the potential of WBE as a tool for community-level surveillance of these viruses. This study is a continuation of previous research article of Anastopoulou et al., 2024.

Materials and Methods

Wastewater Treatment Plant of Patras

The urban Wastewater Treatment Plant (WWTP) of Patras includes both municipal waste and rainwater, making the network type mixed. Considering the spatial characteristics of the city, the biological treatment system serves a total of 168.034 inhabitants, and the maximum daily wastewater supply reaches 43,000 m3/d. Based on the information of the Special Secretariat of Water, the average incoming load from sewage is 11,359 Kg BOD^5/day while the maximum is 13,207 Kg BOD^5/day. The average of the incoming sewage supply amounts to 37,745 m3/day with a maximum value of 43,075 m3/day. Primary and secondary treatment, nitrogen and phosphorus removal, biological disinfection, chlorination and sand filters are applied in these facilities (Fig. 1).

Sampling Strategy

Sampling was carried out by the technical staff of the WWTP of Municipality of Patras. The samples were composite, 24-hours, and were collected every Monday, Tuesday and Thursday, over a period of 6 months, from the beginning of October 2022 (ISO week 2022-40) until the end of March 2023 (ISO week 2023-13), covering the whole period. Total of 76 samples were collected. The selection of the specific days was based on the need to capture a variety of conditions and obtain an overall representative sampling. Moreover, based on the official epidemiological data of Greece on the presence and circulation of respiratory viruses in the community, this period of the year, covering autumn, winter and early spring months, can give us an overall image of the circulation of the viruses selected to study. The composite samples were 24-hour time-dependent composites collected using an automatic sampler provided by the Patras WWTP. The sampler obtained three equal-volume subsamples over a 24-hour period, which were then combined to form the composite sample. All samples were collected at the WWTP influent, prior to any treatment or screening. During collection, the sampler maintained the samples at approximately 5 °C. After the 24-hour composite was completed, samples were stored at 5 °C until the following morning and then transported to the laboratory in insulated coolers with ice

Fig. 1 Geographical depiction of the WWTP of Municipality of Patras

packs. Upon arrival, they were processed immediately for analysis.

Extraction and RNA Purification Method

The Total Nucleic Acid Extraction was performed using Wizard® Enviro Total Nucleic Acid Kit (Promega, Wisconsin, United States). This method was adopted, enabling direct collection and concentration of total nucleic acids from 40 ml wastewater and process carried following the protocol presented in the research article of Anastopoulou et al., 2024. Furthermore, the One Step PCR inhibitor removal kit (Zymo, Irvine, CA, USA) was employed to eliminate the inhibitory effects present in the samples.

Real-Time qPCR Assays

For the detection and quantification of viruses, q-PCR assays were conducted using a Thermocycler Stratagene Mx30005P (Thermo Scientific, Waltham, MA, USA). The protocols employed were in accordance with manufacturers' instructions. enhancing result reliability and mitigating potential inhibitory effects. A predetermined quantity of the virus, designated as the Positive Amplification Control, provided by the Greek National Public Health Laboratory (KEDY), used as the positive control sample, while PCR grade water (No Template Control) served as the negative control sample in each run.

For the HadV PCR reaction, the reaction mixture comprised 12.5 μL of TaqMan™ Universal PCR Master Mix (Thermo Fisher Scientific, Woolston, Warrington, UK), 0.5 μL of each primer, and 1 μL of RNase-free water (Jena Bioscience, Lobstedter, Germany). A total of 15 μL of reaction mix and 10 μL of the isolated sample were added to each PCR reaction tube.

For the Influenza A and B viruses, multiplexed and for the detection of RSV A/B and Rhinoviruses the One Step RT-qPCR kit (Enzyquest, Heraklion, Crete, Greece) was used.

Primer and probe sequences for the target genes are summarized in Table 1, reference indicated with slight modifications. For the SARS-CoV 2 reaction the primer sets nCoV_IP2 and nCoV_IP4 were multiplexed depicted in research article of Anastopoulou et al., 2024. Detailed cycling conditions are provided in Table 2. Multiplexed assay used for the detection of Influenza A and Influenza B. All samples were analyzed in triplicates and in 1:10 dilution for the assessment of inhibition effect on the qPCR reaction. qPCR inhibition was detected in all wastewater samples tested. To mitigate this effect, all quantification was

Table 1 Primers used for qPCR assays

Virus	Primer/Probe name	Sequence (5′–3′)	Reference
HAdV	HAdF	5’-CWTACATGCACATCKCSGG-3’	(Heim et al., 2003)
	HAdR	5’-CRCGGGCRAAYTGCACCAG-3’	
	HAdPr	5’-[FAM]-CCGGGCTCAGGTACTCCGAGGCGTCCT-[BHQ1]–3’	
Influenza A	IAV M FW (ABI)	5’-CAAGACCAATCYTGTCACCTCTGAC−3’	(Mercier et al., 2022)
	IAV M RV(ABI)	5’- GCATTYTGGACAAAVCGTCTACG-3’	
	IAV M probe (IDT)	5’-[FAM]- TGCAGTCCTCGCTCACTGGGCACG -[BHQ1]–3’	
Influenza B	IBV NS FW (ABI)	5’-TCCTCAAYTCACTCTTCGACG-3’	(Mercier et al., 2022)
	IBV NS RV (ABI)	5’-CGGTGCTCTTGACCAAATTGG-3’	
	IBV NS probe (IDT)	5’-[HEX]-CCAATTCGAGCAGCTGAAACTGCGGTG-[BHQ1]–3’	
RSV A/B	RSV A/B FW	5’-CTCCAGAATAYAGGCATGAYTCTCC-3’	(Hughes et al., 2022)
	RSV A/B RV	5’-GCYCTYCTAATYACWGCTGTAAGAC-3’	
	RSV A/B Probe	5’-[HEX]-TAACCAAATTAGCAGCAGGAGATAGATCAG-[BHQ1]–3’	
Rhinoviruses	Primer Pic-1	5’-TCCTCCGGCCCCTGAAT-3’	(Do et al.,2010)
	Primer Pic-3	5’-GAAACACGGACACCCAAAGTAGT-3’	
	Probe Pic-5	5’-[FAM]-YGGCTAACCYWAACCC-[BHQ1]–3’	

Table 2 Thermal profile of real time qPCR assays

Virus	Steps	Thermal Profile	Number of Cycles
HAdV assay	Hot Start	2 min at 50 °C	1
	Enzyme Activation	10 min at 95 °C	1
	Annealing & extension	15 s at 95 °C 1 min at 60 °C	45
Influenza A/Influenza B multiplexed, RSV A/B and Rhinoviruses assay multiplexed	Reverse Transcription (RT)	15 min at 55 °C	1
	RT inactivation/Hot Start Taq DNA polymerase activation	2 min at 95 °C	1
	Cycle denaturation, Annealing & extension	15 s at 95 °C 1 min at 60 °C	45

performed on 1:10 diluted extracts, and genome copy numbers were calculated based on the diluted template. Therefore, the final reported concentrations already incorporate the correction for inhibition.

Normalization of Data

The concentration (Genome Copies/L) of each virus detected per sample in the influent wastewater samples was normalized per 100,000 residents based on the average daily flow rate (m^3/d) of the WWTP and the estimated real-time population served by the plant. Ammonium loads were used as an anthropogenic marker to estimate the size of the real-time population served. A population equivalent (PE) of 7.0 g NH_4-N per day per person was applied, as this is the value estimated for Greek cities and used by the Greek National Wastewater Epidemiology Network (https://apth.mnss.eu/).

The real-time population served was calculated by dividing the concentration of NH_4-N that was determined in the influent samples with the average NH_4-N excreted daily per resident, according to Eq. 1.

Normalized concentrations of the individual compounds per 100,000 inhabitants were calculated as depicted in Eq. [1].

$$\mathrm{C_{norm}}\left(\frac{\mathrm{gc}}{10,000\ \mathrm{people}}\right) = \frac{\mathrm{C}\ \left(\frac{\mathrm{gc}}{\mathrm{L}}\right)\ *\ \mathrm{inlet\ flow}\ \left(\frac{\mathrm{m}^3}{\mathrm{d}}\right)\ *\ 1,000\ \left(\frac{\mathrm{L}}{\mathrm{m}^3}\right)\ *\ 10,000\ (\mathrm{people})}{\left(\frac{\mathrm{NH4\ -\ N\ excreted\ daily\ per\ person}}{7}\right)\ *\ \mathrm{real\ -\ time\ population}\ \ (\mathrm{people})} \tag{1}$$

Statistical Analysis

For the statistical analysis Mean values obtained from all samples of each iso week for each virus used to represent each iso week. Possible correlations between all viruses detected through the course of time were investigated, including SARS-CoV-2 data from previous research (Anastopoulou et al., 2024). In addition, further investigation was conducted to explore a possible correlation between the viruses and the officially reported COVID-19 cases of the city of Patras. Hierarchical cluster analysis (HCA) was applied clustering of the six monitored respiratory viruses based on their weekly concentration profiles to identify similarities in temporal circulation patterns. HCA for viruses was conducted using squared Euclidean distance and average linkage (between-groups). Correlation analyses were performed using IBM SPSS Statistics v27 and independently replicated in Python (SciPy & Matplotlib), while all graphical outputs were generated using Microsoft Excel 2016 and Python.

Results

Quantification of Viral Load

The quantification of genome copies on respiratory viruses was conducted using standard curves established for the respective target genes. The standard curve was generated based on a control sample containing 10^7 genome copies, (gc)/μl, which underwent serial tenfold dilutions from 10^7 to 10^0 gc/μl. For HAdV, the Limit of Detection (LOD) was determined to be 2509 gc/L of wastewater, for Influenza A, 2151 gc/L, for Influenza B 3819 gc/L, for RSV A/B 2397 gc/L and for Rhinoviruses 6346 gc/L. Positive Amplification Controls (PACs) used have the following quantities. For Influenza A 2,04E+07 gc/μl, for Influenza B, 2,13E+07 gc/μl (in house produced by Laboratory of Molecular Microbiology, Department of Veterinary Science, AUTH), for RSV A/B, 1,02E+06 gc/μl provided by KEDY, and for Rhinoviruses, 1,25E+04 gc/μl (Amplirun ® Rhinovirus RNA control, Vircell Molecular). The efficiency of the procedure was assessed using the equation of efficiency (Anastopoulou et al., 2024). The following values represent the mean values obtained across all plates for each virus. For HadV the slope was calculated as −3.317, with a Y-intercept value of 39.97, resulting in an efficiency of 100.2%. For Influenza A standard curve characteristics are, slope: −3,52, y intercept: 41,35, Rsq: 0,993 and Efficiency: 92,2%. For Influenza B standard curve characteristics are, slope: −3,419, y intercept: 45,58, Rsq: 0,993 and Efficiency = 96,1%. For Rhinoviruses standard curve characteristics are, slope: −3,00, y intercept: 42,92, Rsq: 0,991 and Efficiency: 115,3%. For RSV A/B standard curve characteristics are slope: −3,22, y intercept = 40,88, Rsq = 0,988, efficiency = 104,3%. Ct values were recorded for all samples and Mean Ct values per sample used for the quantification. The virus concentration was expressed as Genome Copies per Liter using the provided equations, depicted on research article of Anastopoulou et al., 2024.

Table 3 Physicochemical parameters of wastewater samples

Physicochemical parameters of wastewater samples	Mean	Range
Average daily supply (m^3/day)	36.357	35.800–36.500
PH	7,47	7,19 − 7,68
Conductivity (μS/cm)	1.399	1.223–1.653
COD (mg/L O_2)	374	228–594
BOD (mg/L O_2)	229	148–328
Total Nitrogen (mg/L N)	51	39–71
Suspended Solids (mg/L)	161	105–275
Ammonium (mg/L NH_4-N)	37	29–56

Physicochemical Parameters

To support the interpretation of viral RNA signals in wastewater-based surveillance, a set of key physicochemical parameters was measured in the influent wastewater samples. These parameters provide essential context regarding the wastewater matrix, which can influence viral particle stability, concentration efficiency, and detection sensitivity. The selected parameters included pH, electrical conductivity, chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen, suspended solids, and ammonium. These indicators were chosen for their relevance to both general wastewater quality and their potential impact on virus partitioning, adsorption, detection sensitivity and recovery efficiency during sample processing. (Bivins et al., 2020)

Physicochemical parameters of the influent wastewater samples were measured in samples taken and are shown in Table 3.

Sample Results

Throughout the six-month surveillance period, the analysis of 76 wastewater samples exhibited complete positivity for HAdV and SARS-CoV 2, for Influenza A 51,3% positivity (n = 39/76), for Influenza B 19,7% positivity (n = 15/76), for RSV A/B, 39,5% positivity (n = 30/76) and for Rhinoviruses 44,7% positivity, (n = 34/76). All mean values of viral genome copies normalized per 100.000 inhabitants per ISO week are depicted in Supplementary Table 1. The chart in Fig. 2 provides an additional dimension for visualizing the data in the course of time. The horizontal axis delineates the epidemiological weeks, as per the ISO week calendar system, while the vertical axis represents the concentration of viral genome copies per liter normalized per 100.000 inhabitants. It's important to note that the ISO week calendar system is a standardized leap week system established by the International Organization for Standardization (ISO) and can provide us with an overview of the presence of viruses in the community.

To explore potential associations between the respiratory viruses detected in wastewater samples, Spearman's rho two-tailed correlation analysis was performed. This nonparametric method was selected because it does not assume normality and is appropriate for assessing monotonic relationships between variables. The analysis identified several statistically significant associations. Influenza A showed moderate positive correlations with both RSV A/B ($\rho = 0.576$, $p = 0.003$) and Rhinovirus ($\rho = 0.467$, $p = 0.025$). In addition, a strong positive correlation was observed between Influenza B and SARS-CoV-2 ($\rho = 0.655$, $p < 0.001$). No other correlations reached statistical significance (Supplementary

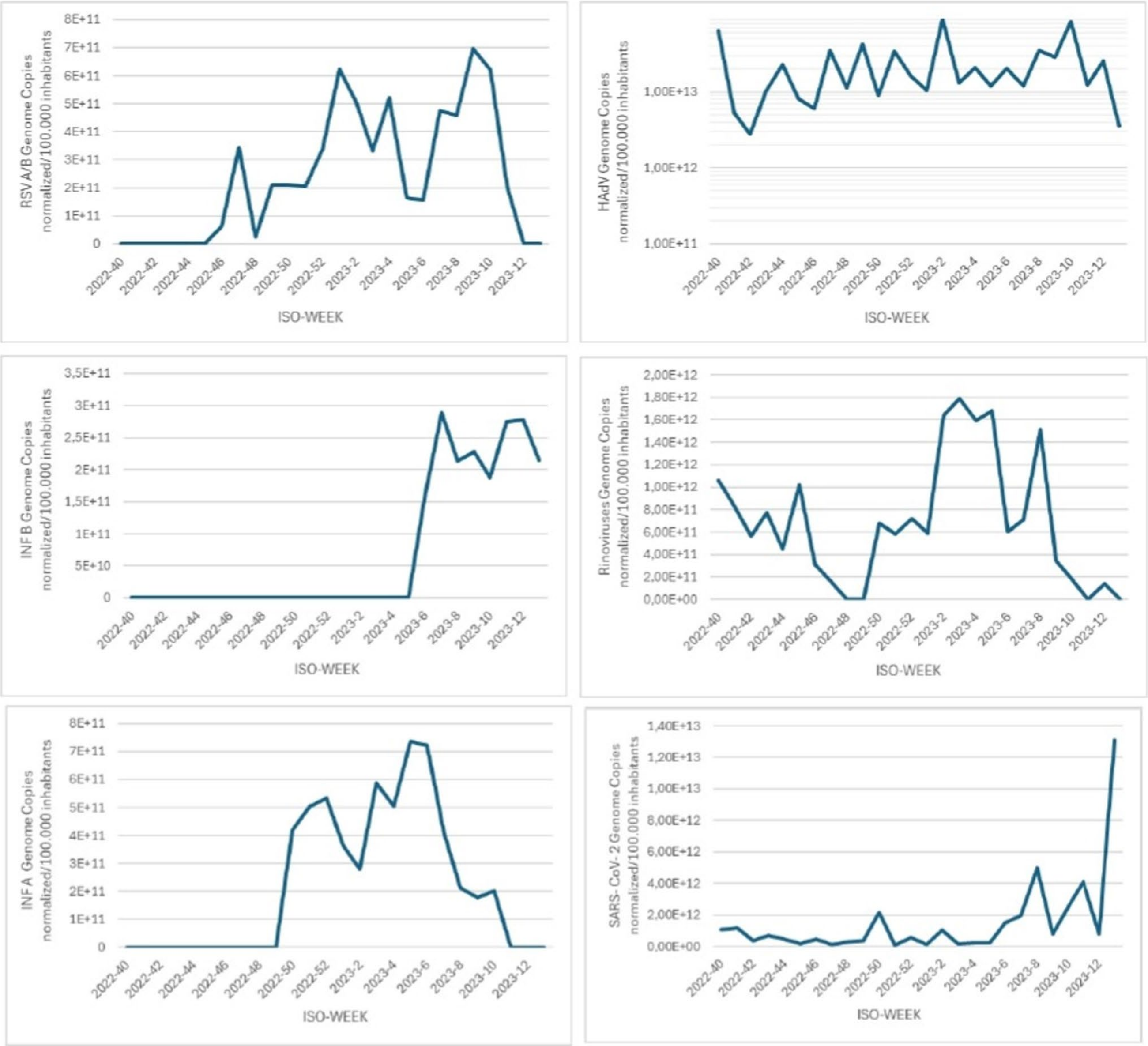

Fig. 2 Epidemiological Curves of HAdV and SARS-CoV 2, Influenza A and B, RSV A/B and Rhinoviruses, during 2022 and 2023 ISO weeks normalized per 100.000 inhabitants, based on Wastewater surveillance of city of Patras, Greece

Table S3 and Supplementary Figure S1). The hierarchical cluster analysis of respiratory viruses based on temporal concentration profiles revealed three main clusters. The first cluster included Influenza A, Influenza B, RSV, and Rhinoviruses, which showed similar seasonal behavior. SARS-CoV-2 formed a second distinct cluster due to its episodic high peaks. HAdV constituted a separate third cluster, reflecting its persistent and stable detection pattern across the entire observation period depicted in Fig. 3 and Supplementary Figure S2. Although the rescaled dendrogram, in Supplementary Figure S2 visually suggests a close proximity between SARS-CoV-2 and the members of Cluster 2, the Agglomeration Schedule provides clear mathematical justification for their separation. Specifically, a substantial numerical jump in the clustering coefficients was observed during the transition from Stage 3 to Stage 4. The coefficient increased tenfold rising at the formation of the INFA/INFB/RSV/RHINOV group when SARS-CoV-2 was incorporated. This significant increase in heterogeneity justifies the classification of SARS as a distinct cluster, as merging it with Cluster 2 would substantially compromise the internal homogeneity of that group (Supplementary Tables S4).

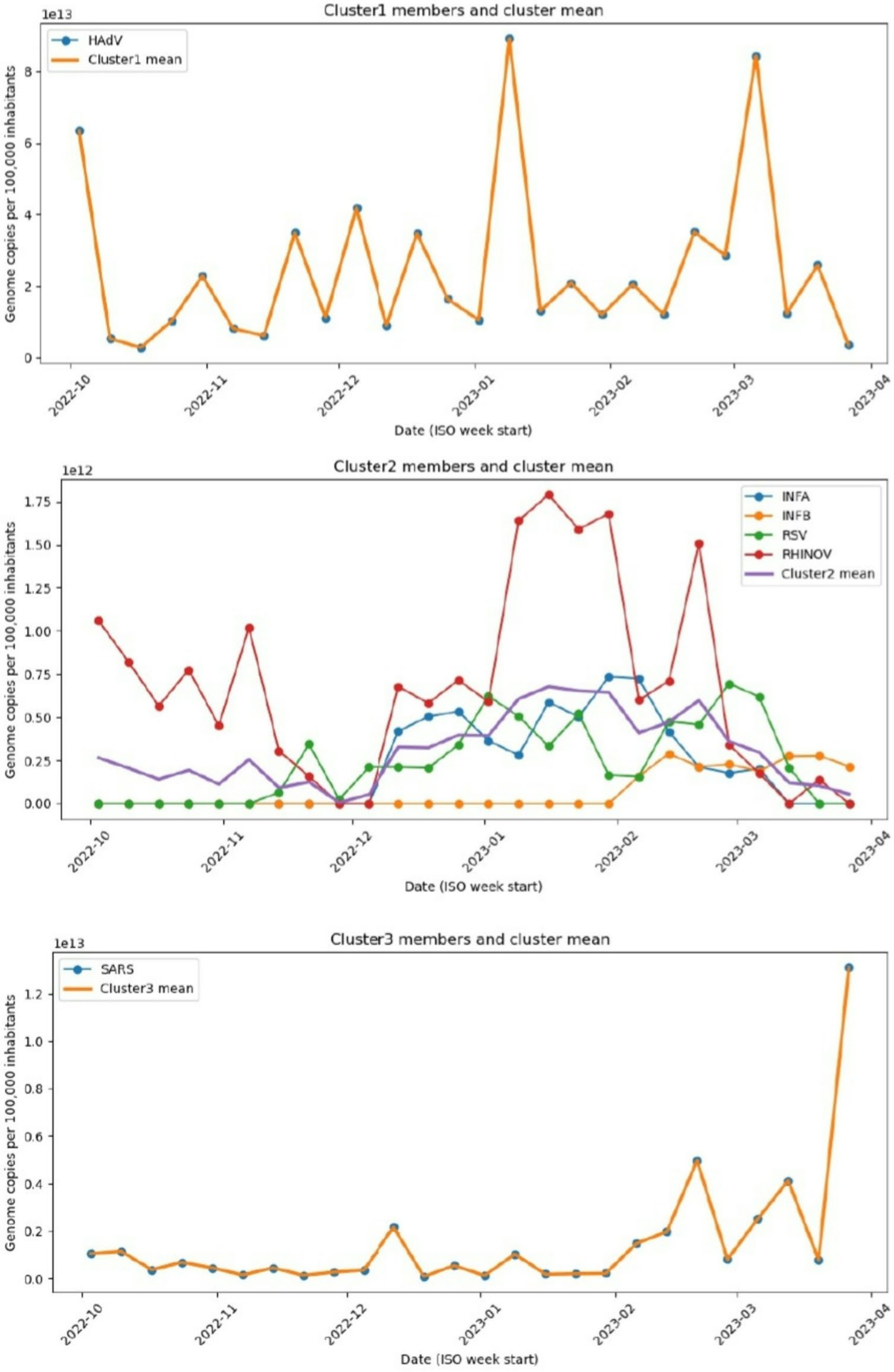

Fig. 3 Graphical representation of the hierarchical clusters of viruses, generated using Python

Discussion

Following the conclusion of the COVID-19 pandemic and up to the present, wastewater epidemiology has demonstrated considerable significance as a dual-purpose tool for both preemptive measures and immediate detection of potential community-level pandemics. The temporal trends of viral genome copies normalized per 100,000 inhabitants provide a clear view of the dynamic circulation of respiratory viruses within the community across the study period.

According to the findings of the present research, HAdV, exhibited the most persistent and elevated signal throughout the entire monitoring period, maintaining high viral loads (~ 10^{12}–10^{14} gc/100,000) even during periods of low activity for other viruses. This sustained presence indicates widespread and continuous adenovirus circulation, which may be attributed to its environmental stability and prolonged shedding. Although weekly public bulletins issued by the National Public Health Organization (NPHO) for 2022–2023 did not specifically report adenovirus activity, reflecting a surveillance emphasis on influenza, RSV, and SARS-CoV-2, our wastewater analysis from Patras revealed persistent and elevated HAdV levels. This discrepancy suggests that adenovirus transmission was likely occurring in the community but remained under-detected by conventional clinical reporting systems. Adenoviruses are commonly found in human feces and exhibit resilience against chemical or physical pollutants (Hewitt et al., 2013), partly accounting for the elevated positivity rates observed. The detection of adenovirus in wastewater during the sampling period aligns with existing literature, affirming its environmental resilience and stable presence, devoid of seasonal patterns (Lynch et al. 2016). HAdV genome copies can correlate with physicochemical parameters in wastewater samples, studies have shown significant differences in the presence of adenovirus in water and the values for nitrites, phosphates, and fixed and total solids (Silva et al., 2011). Adenovirus has been identified as the best indicator for evaluating the efficiency of wastewater treatment plants in eliminating viruses (Martin et al., 2023). Therefore, the presence of HAdV genome copies can be used as an indicator of viral pollution in wastewater samples (Rashed et al., 2022).

Influenza A and B viruses showed distinct seasonal peaks, with Influenza A peaking prominently in the winter months (weeks 2022–50 to 2023–10), followed by Influenza B, which demonstrated a delayed but similarly sharp increase during early 2023. These seasonal spikes are consistent with known influenza epidemiology and validate the sensitivity of wastewater surveillance in capturing community-level influenza activity. RSV showed elevated concentrations during late autumn and winter (peaking in week 2023–1 and 2023-9), aligning with the typical RSV seasonality. Rhinoviruses displayed a broader temporal distribution with intermittent peaks, particularly around weeks 2023–2 and 2023–5, suggesting multiple transmission waves or persistent low-level circulation. The positive correlation that was observed between HAdV and RSV A/B, is suggesting a possible concurrent circulation pattern or similar shedding dynamics in the population. Furthermore, positive significant correlations between Influenza A and Rhinovirus, Influenza A and RSV A/B, Influenza B and SARS-CoV-2 may indicate overlapping seasonal trends or shared transmission drivers, reinforcing the value of multiplex wastewater-based surveillance for tracking co-circulating respiratory pathogens.

The positive results of SARS-CoV 2 were expected when epidemiological surveillance was carried out from the beginning of autumn, continued during the winter and reached the beginning of spring. These seasons both in literature and based on the recording of clinical cases are considered peak months of the virus and they are extensively presented in research article of Anastopoulou et al., 2024.

Furthermore, the hierarchical cluster analysis grouped the six respiratory viruses into three distinct clusters based on their temporal distribution patterns across the observation period. Influenza A, Influenza B, RSV, and Rhinoviruses clustered together, indicating similar seasonal circulation and synchronized peaks during the colder months. In contrast, HAdV and SARS-CoV-2 each formed separate clusters, reflecting distinct epidemiological behavior. HAdV exhibited persistent detection with unique fluctuations, while SARS-CoV-2 demonstrated continuous presence with sharp, high-magnitude peaks, particularly in late 2022 and early 2023.

The hierarchical clustering results of the respiratory viruses offer deeper insight into the epidemiological dynamics of respiratory viruses at the community level. The grouping of Influenza A, Influenza B, RSV, and Rhinoviruses into a single cluster suggests shared seasonal drivers, likely linked to colder temperatures, increased indoor activity, and school-term transmission patterns (Polo et al., 2021; Chen et al., 2020). In contrast, the unique clustering of SARS-CoV-2 and another of HAdV reflects their divergent behavior: SARS-CoV-2 presented sharp episodic peaks likely associated with variant waves and public health policy shifts, while HAdV maintained persistent levels possibly due to environmental resistance and prolonged fecal shedding (Martin et al., 2023).

The analysis of viral genome concentrations across the study period revealed distinct temporal patterns in virus circulation. During the peak of the winter season, specifically from ISO weeks 2023-1 to 2023-5, a period of high viral activity was observed. This phase was characterized by the simultaneous presence and elevated levels of Influenza A, RSV, HAdV, SARS-CoV-2 and Rhinoviruses, reflecting a typical epidemic period where multiple respiratory pathogens co-circulate at high concentrations. Notably, from week 2023-6 onwards, there was a detectable shift with the emergence of Influenza B and a significant increase in SARS-CoV-2 concentrations, In contrast, the period between weeks 2022-40 and 2022-49 was associated with generally lower circulation for most viruses, although HAdV and SARS-CoV-2 remained consistently detectable, highlighting their persistence even during non-peak periods. These observations align with recent literature. Boehm et al. (2023) noted that wastewater-based surveillance can capture the diverse circulation patterns of multiple respiratory viruses, emphasizing that pathogens like HAdV often exhibit unique, persistent trends compared to the sharp seasonal peaks of Influenza and RSV. Similarly, Carducci et al. (2024) described cyclical temporal trends in wastewater, with the greatest differences observed between the epidemic (winter) and inter-epidemic (summer) phases. Furthermore, findings from Swiss surveillance (Baumgartner et al., 2025) confirm that while SARS-CoV-2, RSV, and Influenza A often show synchronized temporal peaks, HAdV and Rhinoviruses tend to be present at persistent background levels. Our data supports this differentiation, as Rhinoviruses demonstrated a sustained presence throughout the study, while Influenza A and RSV circulation was more strictly confined to the winter weeks. In line with typical seasonal patterns, Influenza B concentrations in wastewater peaked during late winter- early spring. This late-season emergence is a well-documented feature of Influenza B epidemiology, distinguishing it from the earlier winter peaks of Influenza A. This is supported by Caini et al. (2015), who noted that Influenza B typically exhibits late-season circulation patterns, often trailing behind the peak of Influenza A in temperate climates. Collectively, these results underscore the capacity of wastewater-based surveillance to differentiate between co-circulating viral waves and persistent background pathogens across different seasons.

Data retrieved from the National Public Health Organization of Greece (EODY) was used to explore potential correlations between community respiratory pathogen positivity and normalized viral load in wastewater samples from Patras, spanning ISO week 2022-50 to ISO week 2023-13. Specifically, the dataset included positivity rates for SARS-CoV-2, influenza viruses, and respiratory syncytial virus (RSV) derived from non-sentinel community testing conducted by EODY's Mobile Health Units (KOMY). These units perform rapid antigen testing (Rapid Ag) for SARS-CoV-2 in individuals who voluntarily present for testing, whether symptomatic or asymptomatic. For molecular surveillance of the three aforementioned pathogens, a random subset of symptomatic individuals with influenza-like illness is selected using an algorithm that ensures geographical and demographic representation across Greece. It is important to note that this sampling framework does not produce a representative sample of the general population. However, it may provide a useful indicator of percent positivity trends within the community. To assess the relationship between clinical surveillance data and wastewater-based epidemiology (WBE), Spearman's rho correlation was conducted between weekly normalized viral load in wastewater and aforementioned weekly pathogen positivity rates, for the three viruses. No significant correlations were found between the wastewater SARS-CoV-2 levels and positivity rates of SARS-CoV-2, influenza, or RSV in the community during the study period. Positivity rates are depicted in Supplementary Table S2. Reasons for lack of Significant Correlation can be explained by the following facts: The clinical testing data from the Mobile Health Units (KOMY) is based on voluntary participation and is not representative of the general population. This self-selection bias can distort observed positivity rates and limit their comparability to population-wide wastewater signals. Viral load in wastewater may precede or lag clinical case detection due to differences in viral shedding, incubation period, test-seeking behavior, and reporting delays and final (Peccia et al., 2020). Nevertheless, we observe consistency in the trends across the datasets: SARS-CoV-2 is detected at relatively stable levels in wastewater, which is reflected in the recorded test positivity rates. A similar pattern is observed for influenza (considering influenza A and B combined), where wastewater signals align with test positivity. On the other hand, RSV test positivity in the community showed a gradual decline from ISO week 2022-50 onward, whereas wastewater RSV concentrations remained elevated until ISO week 2023-11 and then dropped sharply. Therefore, the two datasets did not exhibit parallel or mirrored trends.

With regards to the normalization process, ammonium concentrations were relatively stable across the monitoring period (mean 37 mg NH_4-N/L; range 29–56), and the average daily flow also showed no extreme outliers. These observations reduce the likelihood that sudden, large non-population ammonium inputs biased the normalized viral trends. We acknowledge that, in general, factors such as industrial discharges, stormwater intrusion, or changes in wastewater composition can influence ammonium levels. However, it is important to note that no industrial wastewater is discharged into Patras WWTP, which minimizes this specific source of variability.

To summarize, the trends observed in the figure support the feasibility and effectiveness of wastewater-based surveillance in tracking the presence and intensity of multiple respiratory viruses simultaneously. These patterns mirror known seasonal behaviors while also highlighting co-circulation dynamics, which were also statistically supported by the correlation analysis. This underlines the importance of integrating wastewater surveillance into routine public health monitoring frameworks for early warning and comprehensive pathogen tracking. Furthermore, wastewater-based epidemiology (WBE) continues to demonstrate early warning potential, as temporal signals often precede or complement clinical surveillance, particularly for SARS-CoV-2 (Sims & Kasprzyk-Hordern, 2020). However, limitations persist, including virus-specific variability in shedding, environmental degradation rates, and challenges in differentiating viable versus fragmented viral genomes (Wade et al., 2022; Polo et al., 2021). Despite this, the application of WBE as a multiplex surveillance tool for monitoring co-circulating respiratory pathogens is increasingly recognized as a cost-effective and population-level approach to pandemic preparedness.

Conclusion

This study demonstrates the applicability and reliability of wastewater-based epidemiology (WBE) for the simultaneous monitoring of multiple respiratory viruses, including HAdV, SARS-CoV-2, Influenza A and B, RSV A/B, and Rhinoviruses, at the community level in Patras, Greece. The consistent detection of HAdV and SARS-CoV-2, along with the seasonal peaks observed for Influenza A, Influenza B, and RSV, confirms the capacity of WBE to reflect viral circulation trends corresponding to clinical and environmental patterns. Hierarchical clustering and correlation analyses revealed distinct epidemiological behaviors, with some viruses showing persistent detection and others displaying synchronized epidemic peaks. The study also highlights the environmental persistence of HAdV. These findings underscore the critical role of WBE in complementing conventional epidemiological tools by providing timely, population-wide insights into the dynamics of viral transmission, supporting early warning systems and informed public health interventions. Continued integration of WBE into routine surveillance frameworks can enhance preparedness and response strategies for future respiratory pathogen outbreaks.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J. W., & Mueller, J. F. (2020). First confirmed detection of SARS-CoV-2 in untreated wastewater in australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment*, *728*, 138764. https://doi.org/10.1016/j.scitotenv.2020.138764

Albinana-Gimenez, N., Miagostovich, M. P., Calgua, B., Huguet, J. M., Matia, L., & Girones, R. (2009). Analysis of adenoviruses and polyomaviruses quantified by qPCR as indicators of water quality in source and drinking-water treatment plants. *Water Research*, *43*(7), 2011–2019. https://doi.org/10.1016/j.watres.2008.11.045

Anastopoulou, Z., Kotsiri, Z., Chorti-Tripsa, E., Fokas, R., & Vantarakis, A. (2024). Urban wastewater-based surveillance of SARS-CoV-2 virus: A two-year study conducted in City of Patras, Greece. *Food and Environmental Virology, 16*(3), 398–408. https://doi.org/10.1007/s12560-024-09601-7

Baumgartner, S., Salvisberg, M., Schmidhalter, P., Julian, T. R., Ort, C., & Singer, H. (2025). Insights into respiratory illness at the population level through parallel analysis of pharmaceutical and viral markers in wastewater. *Nature Water, 3*, 580–589. https://doi.org/10.1038/s44221-025-00437-4

Bivins, A., Kaya, D., Bibby, K., Simpson, S. L., Bustin, S. A., Shanks, O. C., & Simpson, S. L. (2021). Variability in RT-qPCR assay parameters indicates unreliable SARS-CoV-2 RNA quantification for wastewater surveillance. *Water Research*, *203*, 117516. https://doi.org/10.1016/j.watres.2021.117516

Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Been, F., & Bibby, K. (2020). Wastewater-based epidemiology: Global collaborative to maximize contributions in the fight against COVID-19. *Environmental Science & Technology*, *54*(13), 7754–7757. https://doi.org/10.1021/acs.est.0c02388

Boehm, A. B., Wolfe, M. K., White, B. J., Hughes, B., & Duong, D. (2023). Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: A surveillance study. *The Lancet Microbe, 4*, e340–e348. https://doi.org/10.1016/S2666-5247(23)00052-2

Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., et al. (2006). Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. *Applied and Environment Microbiology*, *72*(12), 7894–7896.

Caini, S., Huang, Q. S., Sethi, S., Glusker, B., Hoffmann, S., Coleman, C. K., Walaza, S., Venter, M., Tempia, S., Bennett, N., Thomas, Y., Carrat, F., Paget, J., & GIHSN Investigators. (2015). Epidemiological and virological characteristics of influenza B: Results from the global influenza hospital surveillance network. *Influenza and Other Respiratory Viruses*, *9*(6), 279–286. https://doi.org/10.1111/irv.12340

Carducci, A., Federigi, I., Pagani, A., Atomsa, N. T., Conte, B., Angori, A., & Verani, M. (2024). Wastewater-based surveillance of respiratory viruses in Northern Tuscany (Italy): Challenges and added value for public health purposes. *Science of the Total Environment*, *912*, 177752. https://doi.org/10.1016/j.scitotenv.2024.177752

Chen, Y., et al. (2020). Epidemiological and meteorological factors affecting influenza virus activity. *BMC Infectious Diseases*, *20*(1), 1–9. https://doi.org/10.1186/s12879-020-05370-3

Corman, V. M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., & Drosten, C. (2012). Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Eurosurveillance*, *17*(39), 20285. https://doi.org/10.2807/ese.17.39.20285-en

Do, D. H., Laus, S., Leber, A., Marcon, M. J., Jordan, J. A., Martin, J. M., & Wadowsky, R. M. (2010). A one-step, real-time PCR assay for rapid detection of rhinovirus. *The Journal of Molecular Diagnostics, 12*(1), 102–108. https://doi.org/10.2353/jmoldx.2010.090071

Doorn, N. (2022). Wastewater research and surveillance: An ethical exploration. *Environmental Sciences Europe*, *8*(11), 2431–2438. https://doi.org/10.1039/D2EW00323H

Dumke, R., Geissler, M., Skupin, A., Helm, B., Mayer, R., Schubert, S., & Wenzel, J. J. (2022). Simultaneous detection of SARS-CoV-2 and influenza virus in wastewater of two cities in southeastern Germany, January to May 2022. *International Journal of Environmental Research and Public Health*, *19*(20), 13477. https://doi.org/10.3390/ijerph192013477

Flint, S. J., & Nemerow, G. R. (Eds.). (2017). *Human adenoviruses: From villains to vectors*. World Scientific.

Fu, Y., Tang, Z., Ye, Z., Mo, S., Tian, X., Ni, K., Ren, L., Liu, E., & Zang, N. (2019). Human adenovirus type 7 infection causes a more severe disease than type 3. *BMC Infectious Diseases, 19*, 36. https://doi.org/10.1186/s12879-019-3686-x

Gable, L., Ram, N., & Ram, J. L. (2020). Legal and ethical implications of wastewater monitoring of SARS-CoV-2 for COVID-19 surveillance. *Journal of Law and the Biosciences*. https://doi.org/10.1093/jlb/lsaa039

Heim, A., Ebnet, C., Harste, G., & Pring-Akerblom, P. (2003). Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *Journal of Medical Virology*, *70*(2), 228–239. https://doi.org/10.1002/jmv.10382

Hernroth, B. E., Condén-Hansson, A. C., Rehnstam-Holm, A. S., Girones, R., & Allard, A. K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: The first Scandinavian report. *Applied and Environmental Microbiology, 68*(9), 4523–4533. https://doi.org/10.1128/AEM.68.9.4523-4533.2002

Hewitt, J., Greening, G. E., Leonard, M., & Lewis, G. D. (2013). Evaluation of human adenovirus and human polyomavirus as indicators of human sewage contamination in the aquatic environment. *Water Research*, *47*(17), 6750–6761. https://doi.org/10.1016/j.watres.2013.09.001

Hughes, B., Duong, D., White, B. J., Wigginton, K. R., Chan, E. M. G., Wolfe, M. K., & Boehm, A. B. (2022). Respiratory syncytial virus (RSV) RNA in wastewater settled solids reflects RSV clinical positivity rates. *Environmental Science & Technology Letters*, *9*(2), 173–178. https://doi.org/10.1021/acs.estlett.2c00045

Kumblathan, T., Piroddi, N., Hrudey, S. E., & Li, X. F. (2022). Wastewater based surveillance of SARS-CoV-2: Challenges and perspective from a Canadian Inter-laboratory study. *Journal of Environmental Sciences (China)*, *116*, 229–239. https://doi.org/10.1016/j.jes.2021.03.005

Lynch, J. P., & Kajon, A. E. (2016). Adenovirus: Epidemiology, global spread of novel serotypes, and advances in treatment and prevention. *Seminars in Respiratory and Critical Care Medicine, 37*(4), 586–602. https://doi.org/10.1055/s-0036-1584923

Martin, M., Silva, M., Polo, D., et al. (2023). Adenovirus as an indicator for viral contamination in water. *Transboundary and Emerging Diseases*, *70*(2), 440–450. https://doi.org/10.1111/tbed.14472

Mercier, E., D'Aoust, P. M., Thakali, O., Hegazy, N., Jia, J. J., Zhang, Z., & Delatolla, R. (2022). Wastewater surveillance of influenza activity: Early detection, surveillance, and subtyping in City and neighbourhood communities. *Scientific Reports*, *12*, 20076. https://doi.org/10.1038/s41598-022-20076-z

Osuolale, O., & Okoh, A. (2015). Incidence of human adenoviruses and hepatitis A virus in the final effluent of selected wastewater treatment plants in Eastern Cape Province, South Africa. *Virology Journal, 12*(1), 1–9. https://doi.org/10.1186/s12985-015-0326-y

Peccia, J., Zulli, A., Brackney, D. E., Grubaugh, N. D., Kaplan, E. H., Casanovas-Massana, A., Ko, A. I., Malik, A. A., Wang, D., Wang, M., Warren, J. L., Weinberger, D. M., Arnold, W., & Omer, S. B. (2020). Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nature Biotechnology, 38*(10), 1164–1167. https://doi.org/10.1038/s41587-020-0684-z

Polo, D., et al. (2021). Making waves: Wastewater-based epidemiology for COVID-19–approaches and challenges for surveillance and prediction. *Water Research*, *186*, 116404. https://doi.org/10.1016/j.watres.2020.116404

Prado, T., Gaspar, A. M. C., & Miagostovich, M. P. (2014). Detection of enteric viruses in activated sludge by feasible concentration methods. *Brazilian Journal of Microbiology*, *45*(1), 343–349.

Public Health Agency of Canada (2024). Burden of disease of RSV in infants, children and pregnant women (Canada Communicable Disease Report, 50(1–2)). Government of Canada.

Rashed, M. K., El-Senousy, W. M., Sayed, E. S. T. A. E. S., & AlKhazindar, M. (2022). Infectious Pepper Mild Mottle Virus and Human Adenoviruses as Viral Indices in Sewage and Water Samples. *Food and Environmental Virology, 14*(3), 246–257. https://doi.org/10.1007/s12560-022-09554-5

Silva, H. D., Santos, S. F. O., Lima, A. P., Silveira-Lacerda, E. P., Anunciação, C. E., & Garcíazapata, M. T. A. (2011). Correlation analysis of the seasonality of adenovirus gene detection and water quality parameters based on yearly monitoring. *Water Quality, Exposure and Health, 3*(2), 101–107. https://doi.org/10.1007/s12403-011-0047-5

Sims, N., & Kasprzyk-Hordern, B. (2020). Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. *Environment International*, *139*, 105689. https://doi.org/10.1016/j.envint.2020.105689

Tavazzi, S., Cacciatori, C., Comero, S., Fatta-Kassinos, D., Karaolia, P., Iakovides, I. C., & Gawlik, B. M. (2023). Short-term stability of wastewater samples for storage and shipment in the context of the EU sewage Sentinel system for SARS-CoV-2. *Journal of Environmental Chemical Engineering*, *11*(2), 109193. https://doi.org/10.1016/j.jece.2023.109193

Wade, M. J., Lo Jacomo, A., Armenise, E., et al. (2022). Understanding and managing uncertainty and variability for wastewater-based epidemiology. *Water Research*, *203*, 117429. https://doi.org/10.1016/j.watres.2021.117429

World Health Organization. (2025, March 25). *Respiratory syncytial virus (RSV)*. WHO. Retrieved from WHO.

Zaki, M. E. S., Eid, A.-R., & Faried, O. A. (2020). Clinico-pathological study of adenovirus associated with respiratory infections in children. *The Open Microbiology Journal, 14*(1), 48–55. https://doi.org/10.2174/1874285802014010048