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Original Research

Data-driven systematic analysis of waterborne viruses and health risks during the wastewater reclamation process

Jia-Xin Ma [a, b, c], Xu Wang [b, d, *], Yi-Rong Pan [a, b, c], Zhao-Yue Wang [b], Xuesong Guo [a, **], Junxin Liu [a], Nan-Qi Ren [b], David Butler [d]

[a] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China
[b] State Key Laboratory of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology, Shenzhen, Shenzhen, 518055, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China
[d] Centre for Water Systems, College of Engineering, Mathematics and Physical Sciences, University of Exeter, Exeter, EX4 4QF, United Kingdom

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ABSTRACT

Waterborne viral epidemics are a major threat to public health. Increasing interest in wastewater reclamation highlights the importance of understanding the health risks associated with potential microbial hazards, particularly for reused water in direct contact with humans. This study focused on identifying viral epidemic patterns in municipal wastewater reused for recreational applications based on long-term, spatially explicit global literature data during 2000–2021, and modelled human health risks from multiple exposure pathways using a well-established quantitative microbial risk assessment methodology. Global median viral loads in municipal wastewater ranged from 7.92×10^4 to 1.4×10^6 GC L^{-1} in the following ascending order: human adenovirus (HAdV), norovirus (NoV) GII, enterovirus (EV), NoV GI, rotavirus (RV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Following secondary or tertiary wastewater treatment, NoV GI, NoV GII, EV, and RV showed a relatively higher and more stable log reduction value with medians all above 0.8 (84%), whereas SARS-CoV-2 and HAdV showed a relatively lower reduction, with medians ranging from 0.33 (53%) to 0.55 (72%). A subsequent disinfection process effectively enhanced viral removal to over 0.89-log (87%). The predicted event probability of virus-related gastrointestinal illness and acute febrile respiratory illnesses in reclaimed recreational water exceeded the World Health Organization recommended recreational risk benchmark (5% and 1.9%, respectively). Overall, our results provided insights on health risks associated with reusing wastewater for recreational purposes and highlighted the need for establishing a regulatory framework ensuring the safety management of reclaimed waters.

1. Introduction

Water scarcity stands as one of the most pressing global challenges facing humanity. To address this issue, the treated effluent from wastewater treatment plants (WWTPs) constitutes an alternative water source for agricultural and landscape irrigation, groundwater recharge, or recreational purposes [1]. Numerous practices have demonstrated the feasibility of water reclamation and recirculation [2]. However, the reclaimed water from WWTPs is often undermined by the inevitable accumulation of pathogenic pollutants. According to statistics from *The Lancet* between 2010 and 2019 [3], exposure to unsafe water sources remains a major human health risk, mainly due to waterborne viruses. These viruses exhibit a heightened public health risk potential resulting from their high environmental survival rates compared to other pathogenic contaminants. For example, between 1951 and 2006, 55 water recreation-associated viral disease outbreaks were recorded, even when the water sources had undergone treatment [4]. This highlights an urgent need for not only monitoring common

* Corresponding author. State Key Laboratory of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology, Shenzhen, Shenzhen, 518055, China.
** Corresponding author.
E-mail addresses: wangxu2021@hit.edu.cn (X. Wang), gxs0122@rcees.ac.cn (X. Guo).

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waterborne viruses and their transmission trajectories during water treatment and reuse processes but also their environmental behaviour and potential human health risks in reclaimed water. Such surveillance could facilitate the prompt identification and control of prospective viral disease outbreaks.

The acceptable quality of disinfected secondary effluent for reuse depends heavily upon the concentration of viruses in the reclaimed water. This concentration is determined by a combination of factors, including the load of human viral pathogens in municipal wastewater, their persistence, and the effectiveness of wastewater treatment measures. Human pathogenic viruses commonly found in WWTPs include enveloped viruses such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other non-enveloped enteric viruses, such as norovirus (NoV), rotavirus (RV), human adenovirus (HAdV), and enterovirus (EV) [5]. High concentrations of SARS-CoV-2, NoV, HAdV, EV, and RV were found in WWTPs' inflows of many countries, such as the USA [6], France [7], Italy [8], Japan [9], and Tunisia [10]. Besides their high incidence in wastewater, NoV, HAdV, and EV can survive for more than a year, RV remains active from one month to a year [11], and SARS-CoV-2 can persist for several days [12,13], substantially increasing their health risks. Over the years, relevant studies on viral incidence in reclaimed water have been conducted on a regional scale; however, global-scale systematic investigations are currently scarce, impeding progress in this discipline. Furthermore, despite the consensus that WWTPs are long-term active viral reservoirs, knowledge about their capacity for viral removal remains limited. A decline in viral load (removal efficiency ranging from 0 to 6.5-log) has been reported in secondary effluents of WWTPs [14,15]. Given that viral genetic material and extracellular proteins are hard to denature, conventional WWTPs cannot provide a safe barrier to waterborne viral dissemination. In contrast, treating waterborne viruses with disinfection techniques has shown excellent performance in damaging viruses. Ultraviolet (UV) irradiation mainly affects genomic material, either through the formation of pyrimidine dimers that destroy nucleic acids or through photoproducts that lead to direct photolysis of photosensitive viral components [16]. Viral inactivation by ozone is mainly associated with the direct oxidation of capsid proteins through the formation of free radicals [16,17], while inactivation by chlorine or chlorine dioxide disinfection is the result of genetic or protein damage [18]. In summary, disinfection of secondary effluents before reuse could be pivotal for ensuring reliable viral inactivation and minimising dissemination [19]. Thus, deciphering the effects of different treatments on removing waterborne viruses in WWTPs is vital for controlling viral dissemination and reducing the health risks associated with wastewater reclamation.

Recently, the health risks arising from the recreational reuse of water, such as in fully developed water-based recreational sites for swimming, fishing, and boating, have attracted growing attention. For example, the mean probability of adenoviral infection per single event associated with wastewater reuse was reported to be high (>0.90) for all these recreational activities [20,21]. Furthermore, the estimated annual median risk for exposure to recreational disinfected secondary effluent in California was reported to be approximately 1.1×10^{-3} per person per year (pppy) for NoV, an order of magnitude higher than the acceptable level of 10^{-4} ppy [22]. Similarly, the annual infection probability by RV per person using recreational impoundments in Sweden was at the same level [23]. Unfortunately, studies using the health outcome value as the assessment endpoint remain limited. Only one study reported that the disease burden caused by HAdV and RV during swimming was approximately 10^{-3} disability-adjusted life years (DALYs) pppy [24]. In brief, the above studies suggested that the current evidence advises against deeming reused water as a safe option for replenishing recreational water sources. However, their range of application is narrow as they focus on individual aspects of the problem, such as the viral pathogen, exposure pathway, or assessment endpoints. A more comprehensive consideration of combinations of viral exposure pathways is required to improve reality simulation by modelling studies. Evaluating the probability of illness and the value of health outcomes can also provide a complete understanding of viral exposure risks during water reclamation and reuse.

Accordingly, our study emphasised the identification of viral epidemic patterns across cases of municipal wastewater reclamation. Our approach involved a comprehensive analysis of long-term, spatially explicit global literature data and modelled human health risks originating from multiple exposure pathways during recreational activities. Specifically, we conducted a quantitative microbial risk assessment (QMRA) analysis to evaluate the impact of reclaimed recreational water on personal health risks and determine the performance of alternative treatment processes. Hence, our study offers valuable insights for future viral monitoring, risk assessment, and implementing regulations to prevent the spread of viral pandemics due to wastewater reclamation.

2. Methods

2.1. Data collection and handling

Tracing the viral migration and load reduction from municipal wastewater to reclaimed water relied on extensive data from historical epidemic patterns. To gather relevant data on NoV GI, NoV GII, HAdV, EV, RV, and SARS-CoV-2 in municipal wastewater, as well as their removal in secondary and tertiary processes with subsequent disinfection units, we conducted a comprehensive review of English-language peer-reviewed literature published between 2000 and 2021 in the Web of Science database. We integrated these into a database attached to the Appendix, categorised for easy reference. Individual data points, including minimum, median, maximum, mean, standard deviation, or distribution types and parameters, were extracted from texts, tables, or digitised from figures using WebPlotDigitizer version 4.5 [25]. Overall, our dataset included 212 articles from 34 countries, all meeting the criteria regarding scope, data availability, and study quality [26]. The specific screening procedure is shown in Fig. S1.

The simple substitution method using LOD/2 and LOQ/2 (LOD: limit of detection; LOQ: limit of quantification) is the most widely used method for handling left-censored data. It is suitable for cases where the data is characterised by substantial sample sizes, high positive rates, and large geometric standard deviations [27–30]. Although not all individual studies included in this study met this criterion, concentrations that were undetectable or non-quantifiable were set to half the LOD or LOQ, respectively [31], primarily due to computational complexities. In the rest cases, the removal efficiencies of viruses in the studied WWTPs were expressed as $\log_{10}$ reduction values (LRV), directly extracted from the study or indirectly calculated based on the concentrations of a specific virus in the influent (secondary, tertiary processes or disinfection influent according to the treatment processes adopted) and effluent (secondary, tertiary processes or disinfection effluent according to the treatment processes adopted). LRV values were calculated according to equation (1):

$$LRV = \log_{10}\left(\frac{C_0}{C_t}\right) \quad (1)$$

where C_0 and C_t are viral concentrations at times 0 and t, respectively.

Numerous studies underscored the importance of using probability distribution functions instead of point values to describe raw wastewater viral concentrations. Additionally, the clearance of viruses during wastewater treatment was noted in numerous studies. Triangular and normal distributions are recommended due to their ability to capture a measure of central tendency for describing data more realistically. Therefore, non-distributed data, characterised by parameters such as "Mean-Standard Deviation" and "Minimum-Median/Mean-Maximum", were assumed to follow a normal and triangular distribution, respectively, whereas with " Minimum-Maximum" as the parameter, they were considered to follow a uniform distribution [32]. To obtain municipal wastewater concentrations, the LRV data of secondary, tertiary treatment, and disinfection units representing the global level of each virus were subsequently compared with several parametric distributions. These distributions included triangular, normal, Poisson, inverse Gaussian, uniform, Weibull, beta, gamma, log-normal, exponential, hypergeometric, binomial, and lognormal distributions. Then, the best-fitting probability distribution function selection was based on ranking derived from the Anderson-Darling, Kolmogorov-Smirnov, and Chi-square tests. Additionally, the LRV of the entire treatment was obtained by adding the LRV of the secondary or tertiary treatment and that of the disinfection unit. The calculated treatment efficiencies were used to estimate viral effluent.

2.2. Overview of scenario modelling

In this study, we conducted risk assessment scenario modelling to elucidate the effect of viruses and different exposure behaviours on human health in reclaimed water and to investigate potential control strategies from a water sector perspective. Once introduced to wastewater, waterborne viruses might be readily dispersed, ending up in reclaimed water [33]. More importantly, residual viruses greatly threaten public health as they are usually transmitted to susceptible individuals via wastewater-associated routes during recreational activities. Therefore, one of our modelling scenarios focused on viral transmission from municipal wastewater and intermediate water to reclaimed water, while the other examined viral transmission from reclaimed water to humans.

To investigate the water transmission routes of viruses from municipal wastewater to reclaimed water, we established a scenario simulating the current most representative water reuse practices in urban regions globally. This scenario specifically emulated the replenishment of recreational water in metropolitan parks solely with disinfected secondary or tertiary effluent supplies transferred from WWTPs through pipelines without any input from stormwater runoff. Given the global scope of our study, the scenario was assumed to handle municipal wastewater with a viral load obtained from section 2.1. NoV, HAdV, EV, and RV were selected as the study viruses because they are responsible for the most commonly reported recreational water-associated gastrointestinal (GI) illness and acute febrile respiratory illness (AFRI) attributed to viruses (all can induce GI illness, while HAdV and EV can also induce AFRI). SARS-CoV-2 was excluded because current removal procedures are considered sufficient for its control [34], and no reported cases of waterborne infection are available to date [35,36]. To better reflect real-life conditions, the scenario implemented disinfection, using chlorine, UV, and ozone, in combination with various common secondary treatment approaches, such as the Bardenpho, oxidation ditch, and conventional active sludge processes. Additionally, we included a tertiary treatment approach, i.e., membrane bioreactor process. Detailed information regarding the evaluated concentrations of target viruses in disinfected effluent is presented in section 2.1.

Modelling of the transfer process of pathogenic viruses from reclaimed water to the public focused on human contact with recreational water in metropolitan parks, often during human activities such as exercise, fishing, cleaning, dabbling at the water's edge, boating, and swimming (Fig. 3a). Different assumptions about specific recreational aspects, such as duration, frequency, and volume ingested, were made and outlined in Table S2. Notably, direct oral ingestion and indirect nasal inhalation constitute wastewater-associated viral transmission routes, while other potential routes of exposure were not considered. During all these recreational activities, indirect nasal inhalation of virus-loaded wastewater aerosols generated and transported by wind can occur [19], causing accidental droplet ingestion. However, the likelihood of such occurrences during exercising around water is relatively low. This is primarily attributed to the fact that individuals typically exercise at a considerable distance from the water body, thus minimising the possibility of direct oral ingestion.

2.3. Target virus-associated health risks in reclaimed recreational water

This study used a step-by-step QMRA to model individual health risks associated with each target virus following exposure to water bodies replenished with reclaimed water during recreational activities [37]. To this end, we performed an exposure assessment to estimate the viral ingestion and inhalation doses of individuals engaged in activities around water bodies replenished with reclaimed water (Section 2.3.1). Based on the doses, we calculated the probabilities of infection per exposure using dose−response data from an epidemic-causing strain (Section 2.3.2). Following oral or nasal infection, we used two established risk characterisation evaluation endpoints to provide robust information on health outcomes, which were expressed as illness risk probability per person per event (pppe) from the traditional perspective of the presence or absence of disease and annual disease burden (DB) analysed on a time scale, that is, DALY developed by the World Health Organization (WHO) [31]. The former was calculated using the estimated morbidity of GI illness or AFRI, whereas the latter was the sum of the years of life lost (YLLs) and years lived with disability (YLDs) resulting from a given health problem (Section 2.3.3). Moreover, 10,000 Monte Carlo iterations were performed using the Oracle Crystal Ball software. Microsoft Excel 2019 was used to represent the spread of heterogeneity in QMRA and to stabilise the distribution of human health risk assessment results.

2.3.1. Exposure assessment

The exposure model describes how pathogens translocate from reclaimed water through different paths to specific receptors, considering specific exposure conditions. The dose of ingested pathogens was calculated according to equation (2) [38]:

$$D_{i,j} = C_{1,i} \times I_i \times 10^{-DR} \times V_j \quad (2)$$

where $D_{i,j}$ is the dose of pathogens (in gene copies (GC) pppe), $C_{1,i}$ is the virus concentration in the effluent of the tertiary wastewater treatment plant (in GC L^{-1}), i equals NovGI, NovGII, hAdV, EV, or RV, I_i is the detection fraction of infectious viral particles, DR is the viral deactivation rate under sunlight UV in typical waters of imperfect clarity (40−80 turbidity units (NTU)) under cool weather with a temperature range from 8 to 18 °C, V_j is the volume ingested during activities (in L pppe), and j equals angling, boating, cleaning, swimming, dabbling, or exercise.

The dose of pathogens received through aerosol inhalation was calculated according to equation (3) [39]:

$$D_{i,j} = C_{1,i} \times I_i \times 10^{-DR} \times AT_j \times RR \times IR \times ED_j \quad (3)$$

where AT_j is the atomization ratio used to convert viral concentrations in water to air during recreation, RR is the aerosol lung retention ratio, IR is the inhalation rate (in L h^{-1}), and ED_j is the exposure duration during activities (in h pppe).

2.3.2. Dose-response assessment

The mathematical relationship between the exposure dose of viral pathogens derived from the exposure assessment and the probability of infection response depends on the biological response mechanism. It can be described by either the following exponential model [38]:

$$P_{\mathrm{inf},i,j} = 1 - \mathrm{e}^{-r_i \times D_{i,j}} \quad (4)$$

where $P_{\mathrm{inf},i,j}$ is the probability of infection (in % pppe), and r_i is the exponential model parameter for the virus (HAdV in this study), or by the β-Poisson distribution model [38]:

$$P_{\mathrm{inf},i,j} = 1 - \left(1 + \frac{D_{i,j}}{\beta_i}\right)^{-\alpha_i} \quad (5)$$

where α_i, β_i are the β-Poisson distribution model parameters for the virus (NoV, EV, and RV in this study).

2.3.3. Risk assessment characterisation

The health outcome expressed as the probability of illness ($P_{\mathrm{ill},i,j}$) was calculated according to equation (6) [40]:

$$P_{\mathrm{ill},i,j} = P_{\mathrm{inf},i,j} \times P_{(\mathrm{ill}|\mathrm{inf}),i} \quad (6)$$

where $P_{(\mathrm{ill}|\mathrm{inf}),i}$ is the conditional probability of the virus to cause illness given the occurrence of infection.

Additionally, to express the health outcome in the annual DB, equation (7) was implemented [41]:

$$DALY_{i,j} = \left(1 - \left(1 - P_{\mathrm{inf},i,j}\right)^{f_{\exp,j}}\right) \times P_{(\mathrm{ill}|\mathrm{inf}),i} \times \frac{DALYs}{ill_i} \times f_{s,i} \quad (7)$$

where $DALY_{i,j}$ is the disability-adjusted life years (in DALYs pppy), $f_{\exp,j}$ is the frequency of activities (in events year^{-1}), $\frac{DALYs}{ill_i}$ is the DB per virus-induced illness (in DALYs per illness), and $f_{s,i}$ is the viral susceptibility fraction.

2.4. Sensitivity analysis

The statistical variability and uncertainty in the QMRA model inputs were estimated using local sensitivity analysis [36]. To uniformly consider the effects of both distributed- and point-value model inputs, the medians of underlying probability distributions (or values of point estimates) were adopted as baseline point values for each input parameter and output variable DB. Subsequently, a differential value for each output variable was calculated by decreasing the baseline input parameter value by 10%, characterised as sensitivity between the two. Positive or negative values reflected positive or negative correlations between the effect of model input and DB, whereas the absolute value determined the strength of the monotonic relationship between model inputs and outputs, with a higher absolute value indicating a greater contribution to the variability of DB, and vice versa.

2.5. Recommended levels of treatment for waterborne viruses from a human-health perspective

Despite the availability of epidemiological studies providing estimations of human health risks arising mainly from water reuse in landscape recreational recharge, viral risks have been largely neglected in criteria establishment, except for a few promulgated criteria defining requirements for systematic viral monitoring [35]. Furthermore, most approaches regulating the use of reclaimed water through water quality parameter thresholds are flawed in terms of technical guidance [42]. To alleviate this, we attempted to formulate standards for the level of treatment of viruses in wastewater, considering a new human health-oriented perspective using the reverse QMRA approach [43]. Based on the benchmark values set by WHO for ensuring a good or very good recreational water environment, which consists of the probability of GI illness of 0.05 and probability of AFRI of 0.019 [44], we proposed the use of probabilistic risk-based recommended values as requirement values for viral removal of reclaimed water used in the replenishment of recreational water. The overall calculation principle was based on inverting the equivalent dose based on the benchmark value and then inverting the log reduction target (LRT) of this equivalent dose.

For viruses whose dose-response model followed the exponential model, the equivalent dose was calculated as:

$$D_{i,j}\,\mathrm{eq} = \frac{\ln\left(\frac{P_{(\mathrm{ill}|\mathrm{inf}),i}}{P_{(\mathrm{ill}|\mathrm{inf}),i} - \mathrm{target}\ P_{\mathrm{ill},i,j}}\right)}{r_i} \quad (8)$$

where $D_{i,j}$ eq is the equivalent dose of pathogens (in GC pppe) and target $P_{\mathrm{ill},i,j}$ is the benchmark for the probability of disease (in % pppe).

Whereas, for viruses whose dose-response model followed the β-Poisson distribution model, the equivalent dose was calculated as:

$$D_{i,j}\,\mathrm{eq} = \left(\left(\frac{P_{(\mathrm{ill}|\mathrm{inf}),i}}{P_{(\mathrm{ill}|\mathrm{inf}),i} - \mathrm{target}\ P_{\mathrm{ill},i,j}}\right)^{\left(\frac{1}{\alpha_i}\right)} - 1\right) \times \beta_i \quad (9)$$

Finally, for viral exposure through ingestion, the LRT was calculated as:

$$LRT_{i,j} = \log_{10}\left(\frac{C_{0,i} \times I_i \times V_j}{D_{i,j}\,\mathrm{eq}}\right) - DR \quad (10)$$

whereas, for viral exposure through inhalation, the LRT was calculated as:

$$LRT_{i,j} = \log_{10}\left(\frac{C_{0,i} \times I_i \times AT_j \times RR \times IR \times ED_j}{D_{i,j}\,\mathrm{eq}}\right) - DR \quad (11)$$

where $LRT_{i,j}$ is the $\log_{10}$ reduction target of the virus throughout the treatment process.

3. Results

3.1. Concentrations of typical viruses in municipal wastewater

The worldwide distribution and range of concentrations of typical viruses in municipal wastewater from 1999 to 2021 are depicted in Fig. 1a. We found that HAdV exhibited the highest incidence rate in the influent, with a median concentration of 1.4×10^6 GC L^{-1}. NoV GII, EV, NoV GI, and RV followed closely with median concentrations in influent samples higher than 10^5 GC L^{-1}, whereas the concentrations of SARS-CoV-2 were up to an order of magnitude lower than these viruses, with a median concentration of 7.92×10^4 GC L^{-1}. On a continental scale, however, we observed alarming levels of SARS-CoV-2 concentrations as high as 1.01×10^7 GC L^{-1} in Africa, where, in combination with HAdV and EV, exhibit the highest potential for waterborne epidemics, by far surpassing those on other continents. Comparisons of data on waterborne viruses between countries revealed the simultaneous prevalence of NoV GI, NoV GII, HAdV, EV, RV, and SARS-CoV-2 in municipal wastewater water only in Italy, France, Brazil, the USA, China, and Japan, showing a clear geographical bias towards Europe, the Americas, and Asia (Fig. 1b). However, we observed that the concentration patterns of major waterborne viruses in municipal wastewater differed between the above six representative countries (Fig. 1c). In particular, we determined that comparable levels of HAdV concentrations in municipal wastewater were found in Italy and France with the mean concentrations being as high as 2.96×10^6 and 2.03×10^6 GC L^{-1}, respectively. We also found that NoV GI and NoV GII were significantly more prevalent in France, with mean concentrations of 8.39×10^6 and 9.01×10^6 GC L^{-1}, respectively. Whereas the highest viral concentration of EV was detected in Brazil, with mean concentrations of 1.12×10^9 GC L^{-1}, much higher than any other virus in any country. Interestingly, we determined that the prevalence of waterborne viruses in the USA and China was generally lower than that in other countries, except for HAdV in the USA and EV in China, which exhibited comparable mean concentration values of 1.38×10^6 and 1.26×10^6 GC L^{-1}, respectively. The prevalence of EV in Asian countries was also

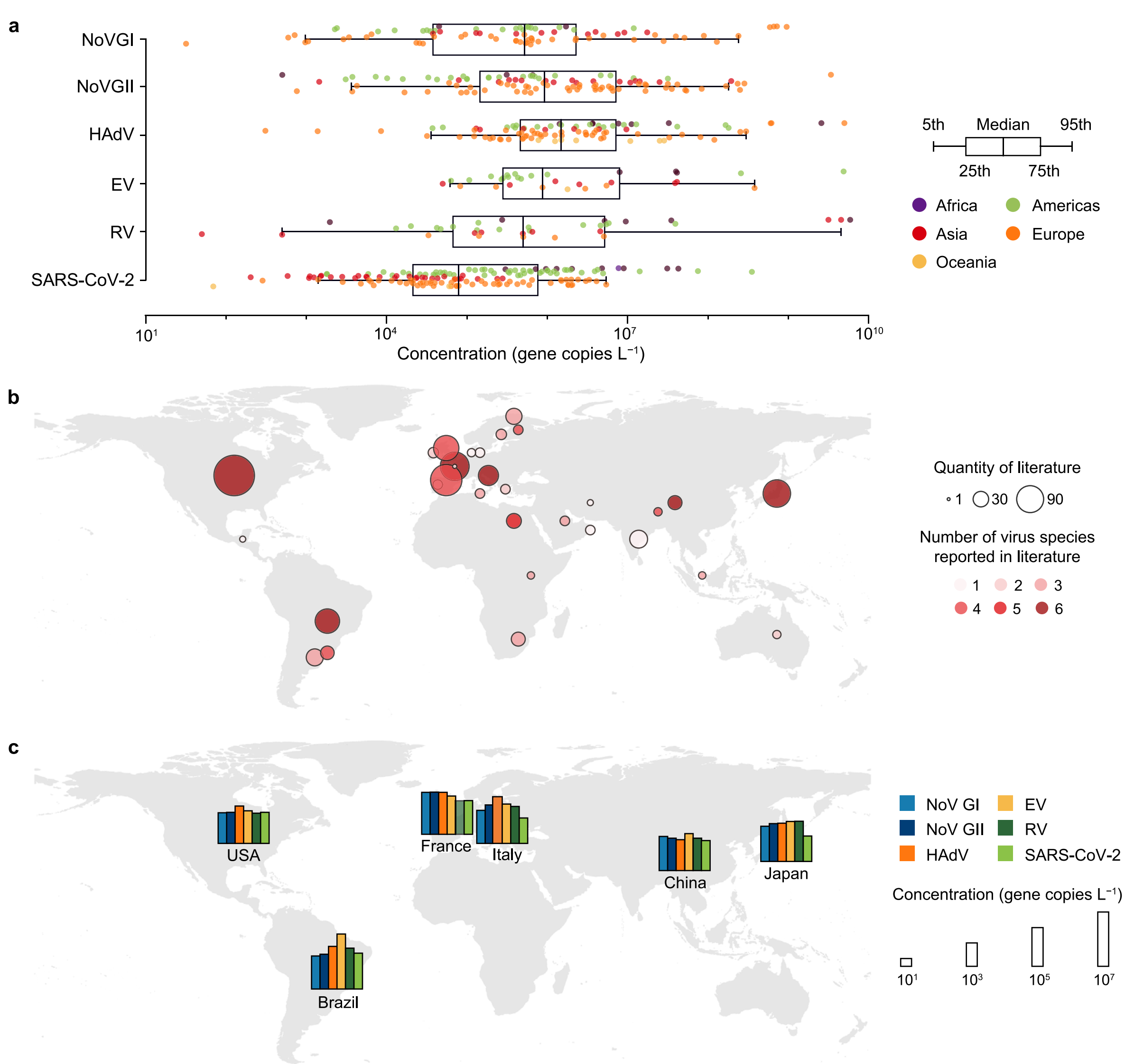

Fig. 1. Quantification of typical viruses in municipal wastewater derived from systematic literature review. a, Worldwide geographical distribution of viral concentration. **b**, The selected literature between 1999 and 2021. **c**, Viral concentrations in representative countries. Our study analysed six viruses (NoV: norovirus; HAdV: human adenovirus; EV: enterovirus; RV: rotavirus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2).

evident in Japan, where its mean concentration was 4.16×10^6 GC L^{-1}. It is also worth noting that Japan was the only country where RV was the prevalent waterborne virus, with a mean concentration of 4.34×10^6 GC L^{-1}.

3.2. Removal efficiency of waterborne viruses in WWTPs

The removal efficiencies of viruses in the studied WWTPs are closely related to the characteristics of each virus (Fig. 2). From this, certain conclusions can be drawn regarding the potential and shortcomings of traditional WWTPs in reducing viral pathogen concentrations. They also provide compelling evidence for the necessity of disinfection after secondary and tertiary treatment to prevent viral spread to environmental surroundings.

Although most of them were not designed to eliminate viruses, we found that many conventional secondary and tertiary WWTPs unit operations reduced the concentrations of viral pathogens in reclaimed water, achieving over 0.8-log (84%) median removal of NoV GI, NoV GII, EV, and RV. However, the current secondary treatment technology or tertiary membrane bioreactor process could not completely inactivate all viruses, while we only observed a low removal of SARS-CoV-2 (median LRV = 0.33). Similarly, we detected HAdV was less effectively removed than NoV GI, NoV GII, EV, and RV, with a relatively poor median LRV of 0.55 and a high variability in general removal efficiencies. This instability in the efficiency of viral removal caused by differences in specific secondary or tertiary processes is demonstrated in Fig. S3.

Hence, additional disinfection processes following secondary and tertiary treatment are conducive to achieving further viral inactivation. Specifically, we found that NoV GI (median LRV = 1.23), NoV GII (median LRV = 1.40), HAdV (median LRV = 0.89), EV (median LRV = 1.35), and RV (median LRV = 1.00) were efficiently removed in the final effluents of tertiary treatment. Due to the lack of available data, SARS-CoV-2 was not investigated. All common chlorine-based disinfectants, ozone, or UV light, used as the major barrier against the transmission of viral diseases, can oxidise the viral cell material (except for UV-C), significantly improving the quality of reclaimed water. The efficiency of viral removal by specific disinfection processes is shown in Fig. S4.

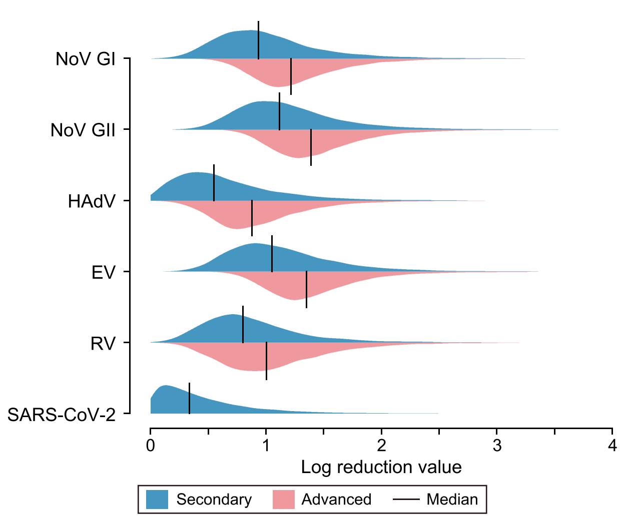

Fig. 2. The efficiency of removal of six typical waterborne viruses. Secondary treatment consisted of the Bardenpho, oxidation ditch, conventional active sludge processes, etc., while tertiary treatment referred to membrane bioreactor. Advanced treatment combines secondary or tertiary treatment with chlorine, ultraviolet, or ozone disinfection.

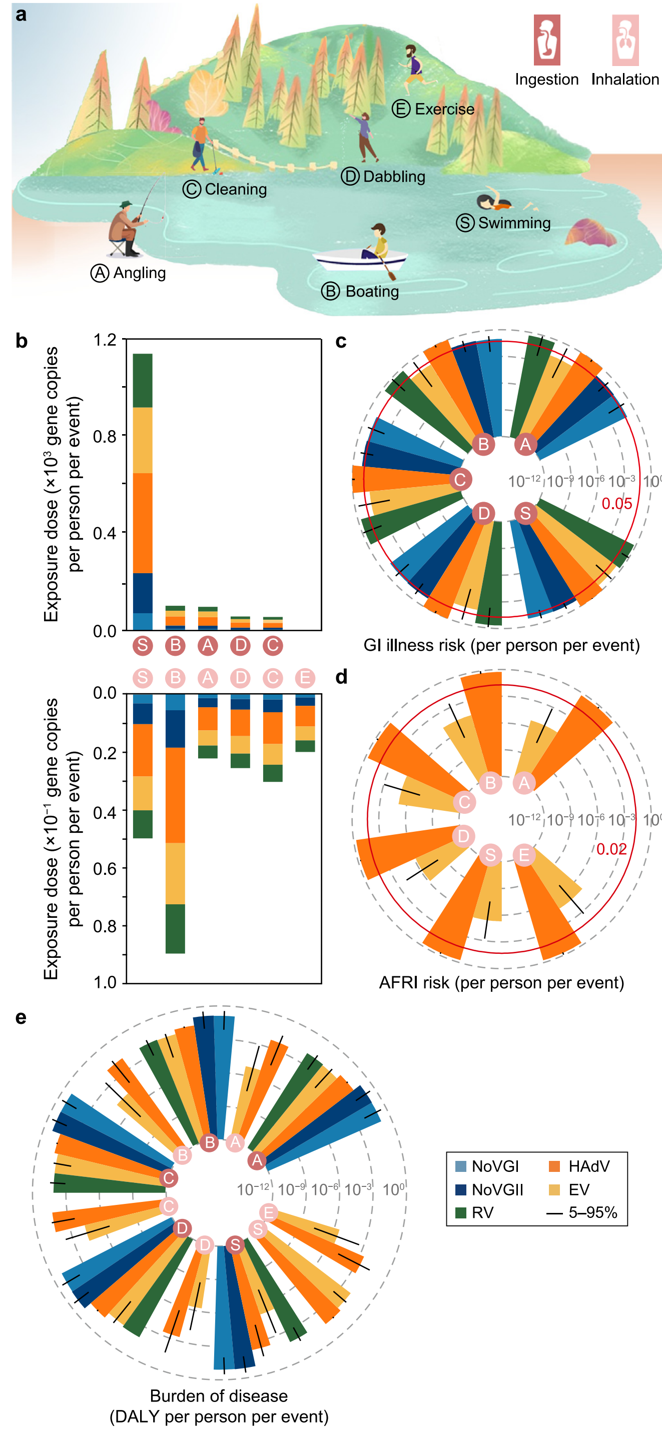

Fig. 3. Exposure assessment and risk characterisation. a, Exposure scenario of human contact with reclaimed recreational water. **b**, Exposure dose per event, with the median value as the characteristic value. **c**, The probability of the gastrointestinal (GI) per event. Column heights represent median values, with whisker lines in the range of 5–95%; Red circles represent the WHO-recommended recreational risk benchmark for event probability of GI. **d**, The probability of the acute febrile respiratory illnesses (AFRI) per event. Column heights represent median values, with whisker lines in the range of 5–95%; Red circles represent the WHO-recommended recreational risk benchmark for event probability of AFRI. **e**, The annual burden of disease. Column heights represent median values, with whisker lines in the range of 5–95%.

3.3. Risk characterisation on target viruses in reclaimed recreational water

The estimated probabilities of GI illness and AFRI for different single exposure events under the scenario of conventionally disinfected secondary and tertiary effluent are presented in Fig. 3c and d. We observed a small variation in the probabilities of GI illness and AFRI among different exposures to recreational water, with swimming in the river exhibiting the highest median event probabilities (from 0.13 to 1). We estimated slightly lower probabilities for boating in the river (from 0.02 to 1), angling (from 0.01 to 1), dabbling (from 0.01 to 1), and cleaning (from 0.01 to 1) around the surface water. The lowest values were found in exercise (from 0.001 to 0.83). Interestingly, the prevailing disease risk of swimming over other recreational activities in reclaimed water closely agreed with the levels of human exposure to virions according to each activity. In particular, we determined that the direct oral exposure to viral particles during swimming was up to 10^3 GC pppe, five orders of magnitude greater than indirect nasal exposure. This resulted in a total exposure dose during swimming that was one order of magnitude greater than other activities. Owing to the distance between the recreational water and those exercising, the number of viral particles inhaled through indirect nasal exposure alone was far lower than that in individuals performing other activities (Fig. 3b). Nonetheless, regardless of the recreational activity type, the probability of GI illness was almost clearly above 0.05 (tolerable level of GI illness for a good or very good recreational water environment).

Furthermore, we observed that the highest rate of disease probability was caused by HAdV, whereas the lowest by EV. More specifically, HAdV exposure during activities resulted in levels of disease probability that were well above the 0.019 tolerable levels of GI illness (values close to 1) and AFRI (from 0.50 to 0.53). In contrast, the infection risks of EV were considerably lower than HAdV's, with generally lower than 0.05 probabilities of GI illness (except for swimming at 0.21) and median probabilities of AFRI considerably below 0.019 (from 0.000001 to 0.00002). These findings highlighted the importance of strengthening surveillance, removal, and alert for waterborne HAdV.

As DALY analyses for recreational water are scarce, our study (Fig. 3e) can provide valuable input for enhancing health risk management. We observed that the annual risk was increased in most recreational activities to levels comparable to swimming. For example, median DALYs induced by dabbling, swimming, boating, angling, and cleaning were 2.8×10^{-1}, 2.9×10^{-1}, 3.0×10^{-1}, 3.5×10^{-1}, and 3.4×10^{-1} DALYs pppy, respectively. Conversely, low exposure during exercise accounted for the lowest DALY induced, at 2.58×10^{-3} DALYs pppy. We also determined that the contribution of individual viruses to the total annual DALY varied considerably within each activity. At the same time, a similar distribution was observed between different viruses, with the DALYs for NoV GI and NoV GII being higher than others. However, their disease probabilities were not the highest. Analysis of the construction of DALY revealed that a high proportion of infectious particles and susceptible individuals for NoV GI and NoV GII (Table S2) indicated a non-negligible risk to individuals exposed to all recreational water activities ($>1.1 \times 10^{-1}$ DALYs pppy).

4. Discussion

4.1. Eliminating viruses from reclaimed water for protecting public health

The variability of the annual DB caused by different viruses is reflected by the extension of the whiskers (Fig. 3e), indicating high variability in public health risk caused by all target viruses. These results provide information about the preliminary feasibility of conducting risk control. Sensitivity analysis of the final risk summarised in Fig. 4 provides a context for the barrier and contribution of viral removal. It can be used as a reference for validating the various solutions employed for viral removal from reclaimed water to protect public health. Overall, as the variability of the annual DB was relatively sensitive to concentration-related factors, followed only by risk-related pathological factors, the mentioned solution can still be considered a prospective prerequisite for water reuse. Especially for swimming, boating in the river, and angling along the river, the treatment efficiency and raw wastewater concentration of HAdV and EV significantly correlated with the annual DB, confirming that improving treatment efficiency is a robust controlling method of public risk, at least for HAdV and EV. Regarding other target viruses, including NoV GI, NoV GII, and RV, the treatment efficiency and raw wastewater concentration did not significantly impact the annual DB due to their capacity for trace infectivity [45]. However, it should be noted that the 10% fluctuation range above and below the median value was smaller than the range of the original data distribution. Such a substitution might underestimate the treatment efficiency and raw wastewater concentration effects. The above results highlighted the need for measures against the viral pathological capacity and the implementation of new removal strategies for waterborne viruses to ensure safe and efficient water reuse.

4.2. Guidelines for water system design in the context of viral risk management

Approximately 20% of the global reclaimed water is used for landscape improvement, including several recreational applications [46]. As municipal wastewater is always expected to contain viral pathogens, it is important to reduce viral loads to non-infectious levels when reused for recreational applications to minimise their impact on public health. Currently, the efficacy of methods for disinfecting waterborne viruses in post-secondary treatment is limited, further hindered by regulatory constraints in developing effective public health technologies to remove viruses from wastewater. Under this context, driven by a trend towards increasing water reuse, global consensus addressed any concerns about waterborne viruses by implementing initiatives and regulations in compliance with proposed regulatory limits. Despite the enactment of several sanitary regulations based on bacterial indicators in aquatic environments, the inefficiency of bacterial indicators for detecting viruses highlights the need to develop new prevention strategies based on viral analyses. Due to inconsistencies in locally determined water quality and chlorination/disinfection standards, which can lead to outbreaks and spread of waterborne recreational diseases [4], governments and organisations have proposed a separate assessment and management of recreational water environments with high levels of microbial contamination [44,47]. Microbial risk assessment can be used to draw insights on minimising public health risks. It might aid in resetting water quality standards that meet specific objectives of water reuse in recreational applications. This way, the safe development of alternative water resources will be ensured, and health benefits will be maximised. At the same time, production costs and energy demand will be reduced by eliminating unnecessary treatments and long-distance transportation.

In the present study, a data-driven, system-modelling approach that couples waterborne viruses and their disease risk was employed to explore ways for embedding synergetic principles of biosecurity into the standard-setting phase of reclaimed water management. Focusing on the effect of replenished disinfected

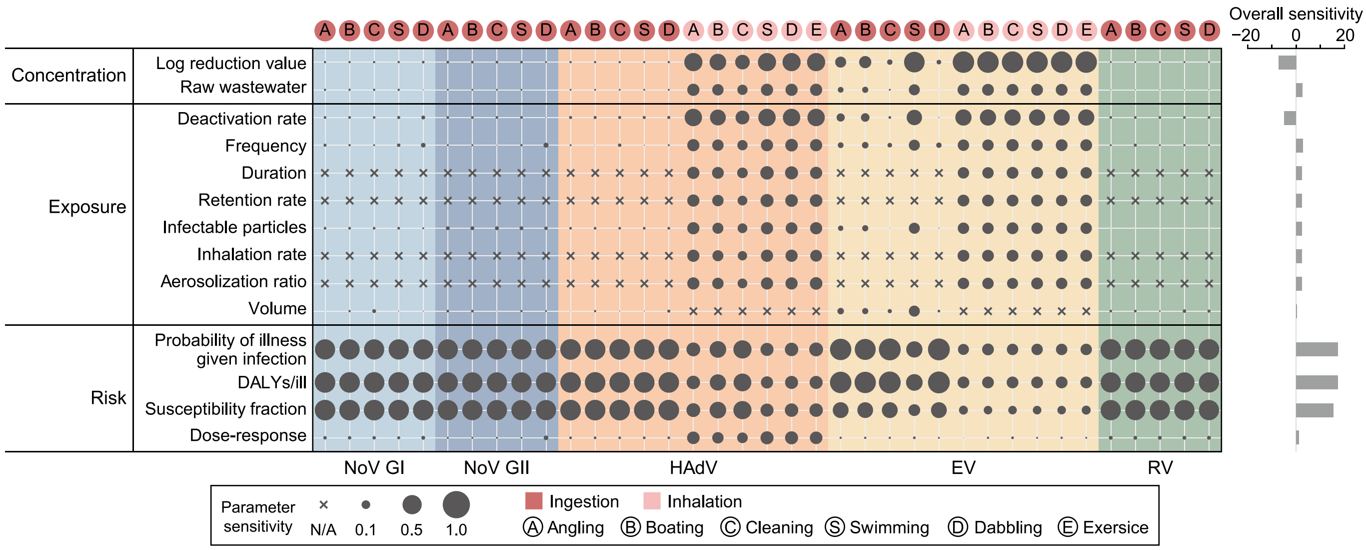

Fig. 4. Sensitivity analysis of annual burden of disease. The size of each dark grey circle is proportional to the magnitude of the absolute sensitivity value, with the light grey bars representing the overall sensitivity.

secondary and tertiary effluent used in recreational water on human health, the level of treatment required for different viruses was estimated considering the WHO-specified health standards. The LRT was 1.99–3.49 for NoV GI, 2.47–3.97 for NoV GII, −0.96 to 4.41 for HAdV, 2.9–4.4 for RV, and 0.78–2.27 for EV in concentrations at the current global municipal wastewater level (Fig. 5). However, to keep the probability of pathogen infection following contact with recreational water below 95%, the viral removal rate of approximately 1.5 log in reclaimed water should be further increased [48]. The estimated LRTs in this study suggested a need to tighten the standards, according to the notion that standard settings are influenced by management objectives. As shown in Fig. 5, the applicability of these new LRTs was further tested in terms of their compliance in some real cases; details can be found in the Appendix. The current advanced treatment was less effective than the minimum requirement for RV and NoV removal, hence challenging biosecurity. Another limitation regarding biosecurity concerns was the disinfection efficiency. The combination of the secondary/tertiary treatment process and chlorine disinfection sometimes fails to meet the minimum requirements for EV removal, whereas the combination with ultraviolet disinfection usually does. In contrast, UV disinfection techniques hardly inactivated HAdV to compliance levels, probably due to the UV resistance of this virus.

Overall, we provided a reference to be used as a guideline for municipal wastewater treatment based on new health-target perspectives. Furthermore, the proposed LRTs could be improved by using national or regional data on raw wastewater concentrations, the treatment effect of reclaimed water, water exposure during different activities, disease burden per case, and the susceptibility fraction of the population. The selection of corresponding appropriate technology should also align with the specific contextual factors of the local conditions.

4.3. Uncertainty analysis

Based on the dataset of 212 published articles, this study provided an assessment of viral concentrations in municipal wastewater and the viral removal efficiency of WWTPs worldwide. Undeniably, the analysis methods conducted in these articles are different, leading to contradictory results. However, it is precisely because of the differences and contradictions of research on a small scale that the amalgamation of these studies becomes imperative for a comprehensive analysis and the generation of broadly applicable conclusions. Regarding the uncertainty caused by the integration process, we arrived at the following conclusions. Although the maximum number of samples tested for a single concentration-related article was 1751, with a median sample size of 13 for all articles, not all single articles met the requirements for large data sample sizes, positivity rates, and geometric standard deviations for

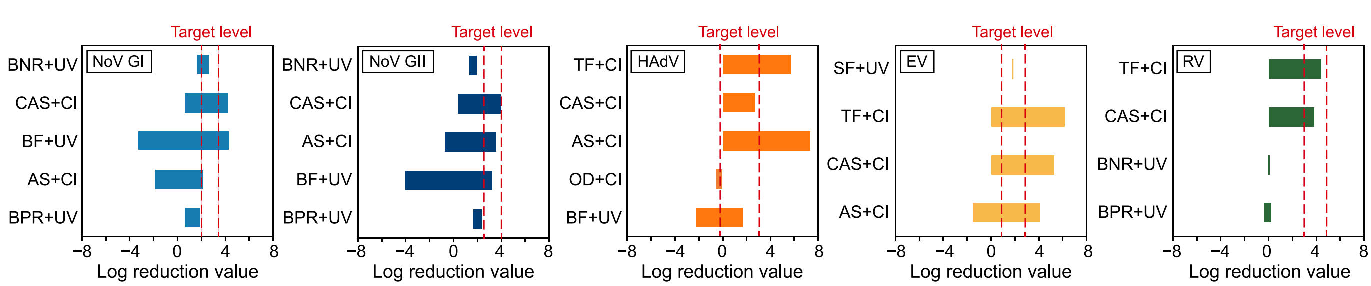

Fig. 5. Health-target treatment requirements in comparison with literature. The width between dotted vertical lines was determined by the minimum and maximum values of treatment levels required to meet public health standards for all recreational activities. The treatment levels of the listed processes were derived from the literature. BNR: biological nitrogen removal; UV: ultraviolet disinfection; CAS: conventional activated sludge; CI: chlorine disinfection; AS: activated sludge; BF: biofilter; BPR: biological phosphorus removal; TF: trickling filter; OD: oxidation ditch.

the simple alternative methods (LOD/2 and LOQ/2) specified for handling left-censored concentration data cases. Although 38% (124/327) of the removal rate data were obtained directly from articles, the remaining LRV data were obtained indirectly through virus density data in wastewater samples, possibly also involving the effect of the simple substitution method. In addition, limited research is available regarding the viral removal efficiency of disinfection, with only 21 articles included in our study. Thus, uncertainties exist when extrapolating the results of this study on the typical levels of viruses found in municipal wastewater and the typical rates of viral removal efficiency in WWTPs.

In addition, the uncertainty of the QMRA model was mainly attributed to the following two reasons. First, the inherent limitations of the present studies include the attendant uncertainty with variations in model input parameters. To address this challenge, we incorporated these variables using a probability-based method (ranges and factor values are provided in the Supplementary Materials), with 10,000 Monte Carlo iterations performed in Oracle Crystal Ball to account for the effects of these parameter distributions on the overall QMRA results. Second, it is undeniable that some details of the exposure model might be missing. A scenario that features the recreational reuse of reclaimed water was represented, along with the public health issues of GI illness and AFRI. Furthermore, virus concentration data in reclaimed recreational water are derived from molecular methods, such as RT-qPCR or qPCR, and do not discern viability; hence, QMRA conducted in this study may probably overestimate the risk extent, despite taking into account virus survival and infection possibilities. Given that waterborne viruses are more likely to attack the gastrointestinal and respiratory tract through droplet ingestion and aerosol inhalation [49], the skin contact exposure route was not considered in our scenario, as its impact was regarded insignificant. Moreover, due to constraints posed by data availability, we approximated the atomization ratio (AT) based on the characteristics of the fountain (Section 2.2 in Supporting Information), making the calculation significantly less complex. Although it introduces uncertainty, it remains a valuable quantitative method for evaluating recreational water aerosols. Overall, the exposure models in this study can be considered adequate for providing a rough estimate of the overall virus-related disease burden in reclaimed water management.

5. Conclusion

In this study, we have unveiled the following key findings:

(1) A growing body of monitoring evidence in municipal wastewater has consistently revealed the prevalence HAdV as a dominant waterborne viral threat, which constitutes a waterborne viral threat across Africa and a spatial heterogeneity of prevailing viral strains among countries. These results serve as valuable early warning indicators for potential viral disease outbreaks.
(2) Water infrastructure played a major role in reducing the viral spread. Waterborne viruses (NoV GI, NoV GII, EV, and RV) can be moderately removed by secondary and tertiary WWTPs, except for HAdV or SARS-CoV-2. Moreover, more than 0.89-log (87%) of viruses were effectively removed in the final effluents.
(3) In the environmental context, the possibility of viral pathogenicity due to reclaimed recreational water constitutes a major concern. The health risk assessment results estimated by QMRA revealed that all probabilities of virus-induced GI illness and AFRI exceeded the reference value, except for EV, which was below the benchmark level of AFRI. HAdV and NoV posed the most substantial safety risks of recreational water regarding disease probability and DB, respectively. Water exposure, especially direct oral ingestion, should also be evaluated for all recreational activities.

Based on these findings, there is a pressing imperative to incorporate health-related criteria into the guidelines governing the utilisation of reclaimed water, with a primary focus on robust virus inactivation before discharge. This proactive approach is essential for ensuring the health and safety of reclaimed recreational water. A higher LRT of treatment for viruses is required (RV: 2.9–4.4, HAdV: −0.94 to 4.41, NoV GI: 1.99–3.49, NoV GII: 2.47–3.97, EV: 0.78–2.27), while the current advanced treatment efficiency needs to be improved according to our compliance examination. In conclusion, the present study demonstrated the importance of environmental surveillance and risk evaluation concerning waterborne viruses. These findings offer a valuable framework for bridging the existing gap in reclaimed water usage standards and can serve as a foundational resource for authorities to formulate health-focused management recommendations, thereby safeguarding public health.

CRediT authorship contribution statement

Jia-Xin Ma: Methodology, Formal Analysis, Investigation, Writing - Original Draft, Visualization. **Xu Wang:** Conceptualization, Formal Analysis, Writing - Review & Editing, Funding Acquisition, Project Administration, Supervision, Resources. **Yi-Rong Pan:** Formal analysis, Visualization. **Zhao-Yue Wang:** Formal Analysis, Software. **Xuesong Guo:** Supervision, Writing - Review & Editing. **Junxin Liu:** Writing - Review & Editing. **Nan-Qi Ren:** Funding Acquisition, Writing - Review & Editing. **David Butler:** Supervision, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2023.100328.

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