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Editorial

Pathogen Detection and Identification in Wastewater

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1. Introduction

The COVID-19 pandemic has renewed research needs for the detection and monitoring of various pathogens in urban wastewater systems including sewerage systems and wastewater treatment or recycling plants [1]. Not only have the detection methods evolved from culture-based to molecular-based technology over the years, but also the purposes and applications have expanded from water quality monitoring to wastewater-based epidemiology (WBE) [2–5]. To ensure the effectiveness of wastewater treatment and the protection of public health, much research has been completed to enhance wastewater sampling, pathogen recovery and concentration, detection sensitivity and specificity, data analysis, results interpretation, and mathematical modelling [6]. These recent developments allow the more accurate and timely identification of the occurrence of pathogenic bacteria, fungi, and viruses in wastewater. Meanwhile, it has been a great challenge to detect new pathogens, such as SARS-CoV-2, at very low levels in wastewater. Significant research is still needed to improve the detection and identification capability for any emerging pathogens. Genome sequencing and other molecular-based methods are promising in providing comprehensive information including the identification of pathogen variants, resistance genes, and the design of PCR probes.

The challenges of public health and safety impose an ever-increasing importance on the fate and spread of pathogens in wastewater systems. WBE has been developed and widely applied as an important supplementary tool for pathogen surveillance through wastewater analysis. It is widely recognized for its unique advantages, in comparison to conventional epidemiological surveillance, of low-cost, nearly real-time detection, and early warning capacity [7]. It thus finds fit-for-purpose use in tracking COVID-19 outbreaks for low-middle income regions and countries, although it is not limited to such regions or countries. The inherent limitations and associated uncertainties of the WBE approach are partly due to the pathogen detection methodologies [8,9]. Thus, it is essential to improve current pathogen detection in wastewater for the higher accuracy of WBE back-calculation of infections. Eventually, WBE can be designed as a useful tool in managing wastewater treatment and the interventions of pandemic through process optimization and public health intervention and policymaking [10]. Looking into the future, wastewater can be used to monitor and track a range of pathogens of significance to population health.

This Special Issue "*Pathogen Detection and Identification in Wastewater*" has captured the above-mentioned new opportunities and challenges for advancing the detection of pathogens and its application, focusing on methodological advancements and innovations, and their applications for WBE. Many manuscripts were submitted for consideration for this Special Issue, and all of them were subject to the rigorous review process instituted by the journal, *Water*. In total, 11 papers were finally accepted for publication and inclusion in this Special Issue (eight articles and three reviews). These papers were the contributions of researchers from Australia, Canda, China, Finland, India, Italy, Japan, Mexico, Sweden, and

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USA. This Editorial will briefly summarize the articles published within this Special Issue, with the main purpose of guiding and encouraging readers in exploring the collection of studies.

2. An Overview of Published Articles

The first review article (contribution 1) by Zhang et al., as the feature paper of this Special Issue, provided a comprehensive overview of pathogens in wastewater and the range of available molecular detection methods, including nucleic acid targeting methods, immunology-based methods, biosensors, and paper-based detection. It also discussed the recent developments in wastewater sampling, DNA/RNA extraction, detection and quantification of pathogens, and the profiling of multiple pathogens. It is thus a starting point for novice research students or researchers to efficiently obtain an overview of the information and grasp some key background knowledge of pathogen detection and identification in wastewater.

Contribution 2 by Tiwari et al. provided a systematic review on the tracing of COVID-19 viral variants in wastewater. Following the PRISMA guideline, it identified and summarized 80 studies for the variants detected, detection methods, and the geographical distribution of studies. It is valuable in understanding the use and effectiveness of WBE for different SARS-CoV-2 variants.

Contribution 3 by Guo et al. is another review on how temperature and water types (i.e., wastewater, freshwater, and seawater) affect the decay of coronaviruses in terms of both infectivity and RNA. The article stated that the sensitivity of the WBE back-estimation of enveloped SARS-CoV-2 was higher than non-enveloped enteric viruses, which were less degradable in wastewater. Also, wastewater dilution by stormwater inflow or seawater infiltration has implications for the decay of coronaviruses and thus the transmission risk and WBE data interpretation.

Four papers of this Special Issue have focused on the development and evaluation of pathogen detection methods in wastewater.

Sajid et al. (contribution 4) demonstrated the use and effectiveness of membrane-inlet mass spectrometry (MIMS) as a low-impact method to differentiate between the pathogenic and non-pathogenic bacterial strains, through analyzing the volatile compounds produced by bacteria.

Hasing et al. (contribution 5) developed and optimized a method for SARS-CoV-2 detection in wastewater using moderate-speed centrifuged solids. This method showed advantages by having a similar sensitivity when compared to the ultrafiltration reference method but with lower PCR inhibition, lower costs, fewer processing steps, and a shorter turnaround time.

Pellegrinelli et al. (contribution 6) reported on an inter-laboratory proficiency test to evaluate the performance of different pre-analytical (three viral concentration methods in combination with three RNA extraction protocols) and analytical methods (two primer/probe sets in three master mixes) for identifying the presence of SARS-CoV-2 in untreated municipal wastewaters samples. The results indicated that the PEG-8000 precipitation in combination with real-time RT-PCR targeting the N gene was the best performing workflow in detecting SARS-CoV-2 in raw wastewater.

Zhang et al. (contribution 7) studied the impact of sewer biofilms on the dynamics of SARS-CoV-2 RNA concentration in naturally contaminated real wastewater using lab-scale reactors. A significant reduction in viral concentration in the water phase and its accumulation in biofilms were revealed. The findings indicated the complex role of sewer compartments and the implications for the interpretation of WBE data.

Four articles in this Special Issue have reported on the surveillance of SARS-CoV-2, its variants, and human adenovirus (HAdVs) through wastewater analysis in different countries. They highlighted the diverse applications (case studies), effectiveness, and challenges of WBE as a supplementary epidemiological surveillance tool.

Sosa-Hernández et al. (contribution 8) reported on the first long-term WBE study of COVID-19 in Mexico and demonstrated its value in providing information regarding community infections in low–middle-income populations. It indicated the importance of strategically placing WBE control centers in target communities.

Arora et al. (contribution 9) investigated the SARS-CoV-2 detection at each treatment stage of 14 wastewater treatment plants (WWTPs) in Northern India. It reported that aerobic biological wastewater treatment can be effective in removing SARS-CoV-2 viruses due to the evidence of no-detection in secondary- or tertiary-treated samples.

Nag et al. (contribution 10) reported a case study on monitoring SARS-CoV-2 variants from 11 distinct wastewater treatment plants across Jaipur (India). Wastewater surveillance for variant characterization was shown to be a reliable and practical method for tracking the diversity of SARS-CoV-2 strains in the community that is considerably faster than clinical genomic surveillance.

Maniah et al. (contribution 11) detected human adenoviruses in three wastewater treatment plants in Saudi Arabia. Sequencing showed that the F species of HAdVs, especially serotype 41, dominated in wastewater. Seasonal variations were found to be negligible, with relative humidity being a significant factor, rather than wind speed.

3. Conclusions

This compilation of articles devoted to the pathogen detection and identification in wastewater encompasses a diverse range of research, with a topical focus on its extensive use in WBE. It is a topic of enduring importance and research interest, as reflected by the different existing methodologies, their optimization, and the emergence of novel methods. The COVID-19 pandemic provided researchers with ample opportunities in conducting research on viral detection in wastewater. The profound knowledge and experiences generated over this time will have long-lasting and widespread positive effects for this research field in the future.

The challenges of current pathogen detection in wastewater lie in the fact that there are highly varying performances and very low comparability of different wastewater surveillance studies, largely due to the highly diverse sampling and analytical methods being used to detect pathogen concentrations in wastewater. Large variations in reproducibility, sensitivity, and efficiency of different analytical protocols lead to high analytical uncertainties. Nevertheless, future research should aim to improve the reproducibility and comparability by standardizing wastewater sampling, analysis, and data reporting protocols.

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Conflicts of Interest: The authors declare no conflicts of interest.

List of Contributions:

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