Chapter 5
Wastewater-Based Epidemiology for Early Detection of Viral Outbreaks

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Abstract  The immense global burden of infectious disease outbreaks and the need to establish prediction and prevention systems have been recognized by the World Health Organization (WHO), the National Institutes of Health (NIH), the United States Agency of International Development (USAID), the Bill and Melinda Gates Foundation, and the international scientific community. Despite multiple efforts, this infectious burden is still increasing. For example, it has been reported that between 1.5 and 12 million people die each year from waterborne diseases and diarrheal diseases are listed within the top 15 leading causes of death worldwide. Rapid population growth, climate change, natural disasters, immigration, globalization, and the corresponding sanitation and waste management challenges are expected to intensify the problem in the years to come.

5.1  Introduction

The immense global burden of infectious disease outbreaks and the need to establish prediction and prevention systems have been recognized by the World Health Organization (WHO), the National Institutes of Health (NIH), the United States Agency of International Development (USAID), the Bill and Melinda Gates Foundation, and the international scientific community. Despite multiple efforts, this infectious burden is still increasing. For example, it has been reported that between 1.5 and 12 million people die each year from waterborne diseases [1, 2] and diarrheal diseases are listed within the top 15 leading causes of death worldwide [3]. Rapid population growth, climate change, natural disasters, immigration, globalization, and the corresponding sanitation and waste management challenges are expected to intensify the problem in the years to come.

Most infectious disease outbreaks in the United States have been related to microbial agents [4–7]. In the vast majority of cases, the infectious agents have not

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been identified. However, the Environmental Protection Agency (EPA) suggests that most outbreaks of unidentified etiology are caused by viruses [8]. Viruses have been cited as potentially the most important and hazardous pathogens found in wastewater [9] and are included in the EPA contaminant candidate list. Viruses can lead to serious health outcomes, especially for children, the elderly, and immunocompromised individuals, and are of great concern because of their low infectious dose, ability to mutate, inability to be treated by antibiotics, resistance to disinfection, small size that facilitates environmental transport, and high survivability in water and solids.

Infectious outbreaks can cause uncontrollable negative effects especially in dense urban areas. Traditional disease detection and management systems are based on diagnostic analyses of clinical samples. However, these systems fail to detect early warnings of public health threats at a wide population level and fail to predict outbreaks in a timely manner. Classic epidemiology observes disease outbreaks based on clinical symptoms and infection status but does not have the ability to predict “critical locations” and “critical moments” for viral disease onset. Recent research efforts in developing optimized detection systems focus on rapid methods for analyzing blood samples, but this approach assumes that patients are examined at a clinical setting after the outbreak has been established and recognized.

The central premise of the proposed approach is that community wastewater represents a snapshot of the status of public health. Wastewater analysis is equivalent to obtaining and analyzing a community-based urine and fecal sample. Monitoring temporal changes in virus concentration and diversity excreted in community wastewater, in combination with monitoring metabolites and biomarkers for population adjustments, allows early detection of outbreaks (critical moments for the onset of an outbreak). In addition, carefully designed spatial sampling will allow detection of locations where an outbreak may begin to develop and spread (critical locations for the onset of an outbreak) (Fig. 5.1).

![Fig. 5.1 Photomicrograph of adenovirus particles (left) and influenza virus particles (right). Adenovirus image from Dr. G. William Gary, Jr./CDC and influenza image from National Institute of Allergy and Infectious Diseases](image-url)
5.2 Background

Similar detection systems have been used for the investigation of illicit drugs in various locations around the world [10–12]. The approach was first theorized in 2001 [10] and first implemented and reported for several illicit drugs in 2005 where the method was termed sewage epidemiology [11]. The methodology considers raw untreated wastewater as a reservoir of human excretion products; among these products are the parent compounds and metabolites of illicit drugs. If these excretion products are stable in wastewater as they travel through the sewage system, then the measured concentration from a wastewater treatment plant (WWTP) could correspond to the amount excreted by the serviced population. Table 5.1 presents a summary of prior studies utilizing the wastewater-based epidemiology methods to assess levels of various substances in a population.

Any substance that is excreted by humans and is stable (or has known kinetic pathways) in wastewater can be back-calculated into an initial source concentration. An important step in the application of wastewater-based epidemiology is the estimation of the contributing population and its sampled wastewater. Both census and biomarker data can be used in this approach to estimate the number of individuals that contribute to the wastewater sample.

5.3 Occurrence of Viruses in Wastewater

Waterborne viruses comprise a significant component of wastewater microbiota and are known to be responsible for disease outbreaks. A critical characteristic of viruses is that they do not grow outside the host cells. Therefore, viral concentrations in the wastewater stream will represent the concentrations excreted by the corresponding human population. Table 5.2 summarizes studies that detected waterborne and non-waterborne viruses in wastewater and human excrement.

| Table 5.1 Summary of substances investigated via wastewater-based epidemiology |
|---|---|---|
| Substance | Country | References |
| Alcohol | Norway | [13] |
| Amphetamines | Australia, Belgium, Italy, Spain, South Korea, the United Kingdom, the United States | [14] |
| Cocaine | Australia, Belgium, Germany, Ireland, Italy, Spain, the United Kingdom, the United States | [14] |
| Counterfeit medicine | The Netherlands | [15] |
| Opiates | Germany, Italy, Spain, South Korea | [16] |
| Tobacco | Italy | [17] |
5.3.1 Waterborne Viruses

There are several groups of commonly detected and studied waterborne viruses, including adenoviruses, astroviruses, enteroviruses, hepatitis A and E viruses, noroviruses, and rotaviruses. Adenoviruses are known to cause gastroenteritis and respiratory disease [18] and have been linked to outbreaks of disease [19, 20].
Adenoviruses are a commonly studied group of viruses in water. They are commonly detected in raw wastewater [21–36] and have been cited as among the most significantly abundant human viruses in wastewater [24, 27, 28, 33, 37]. Adenoviruses have also been detected in human excrement of infected persons, including both feces and urine [38–47]. Studies have found the concentration of adenovirus in the stool of infected persons to range from 10^2 to 10^{11} copies per gram with an average concentration in the range from 10^5 to 10^6 copies per gram of stool [39, 41, 42, 46] as quantified by qPCR.

Astroviruses are a group of RNA viruses that have been linked to outbreaks of gastroenteritis [19, 48]. They have been cited as one of the more important viruses associated with gastroenteritis [49], but they have not been as commonly studied in wastewater compared to other groups of human enteric viruses. Nonetheless, they have been detected using standard PCR in wastewater in prior studies [24, 29, 50, 51]. They have also been detected in clinical samples of human excrement of infected people [43, 44, 47, 52–54], making them a viable candidate for wastewater epidemiology. While qPCR has been used as a detection method for astroviruses in human feces [44, 47, 54], and for quantification purposes in wastewater [51], no cited studies have reported quantitative values for astroviruses in human excrement.

Enteroviruses comprise several types of human enteric viruses, including polioviruses, coxsackieviruses, and echoviruses [55, 56]. Enteroviruses can cause an array of afflictions depending on type, including common cold, meningitis, and poliomyelitis [57], and have been linked to outbreaks of these diseases [19]. Enteroviruses have been detected via PCR in raw wastewater by numerous studies [25, 26, 28, 29, 31, 33, 34, 58, 59], as well as detected in human feces [43, 53, 60–63]. qPCR has not as yet been extensively employed to quantify enteroviruses in stool samples, though one study determined the enterovirus load to be in the range of 1.4*10^4 to 6.6*10^9 copies per gram of stool [60].

Two species of hepatitis viruses, hepatitis A virus and hepatitis E virus, are considered to be waterborne viruses. Hepatitis is a liver disease that can cause numerous afflictions, including fever, nausea, and jaundice [64]. Hepatitis A virus has been linked to disease outbreaks [65], and it has been suggested that even low levels of viral water pollution can produce infection [66]. Hepatitis A virus is often detected via PCR in raw wastewater [29, 30, 58, 67, 68] and several studies have also detected the virus in human stool samples [69–72]. Like enteroviruses, there has not been significant investigation into the quantification of hepatitis A virus in stool, though one study reported values in the range of 3.6*10^5 to 5.6*10^9 copies per gram of stool [70].

Hepatitis E virus, meanwhile, has only recently begun to become a pathogen of interest compared to other waterborne human viruses [73]. Like hepatitis A, hepatitis E virus can cause liver disease with many of the same symptoms; in fact, hepatitis E is not clinically distinguishable from other types of viral hepatitis infection [74]. While not investigated to the extent of other human enteric viruses, hepatitis E virus has been detected via PCR in raw wastewater [21, 34, 75]. There have also been studies that have detected hepatitis E virus in human stool samples [76–78]. One such study also used
RT-qPCR to quantify the concentration of hepatitis E virus in stool and reported values in the range of $10^1$ to $10^6$ copies per μL of stool [77].

Noroviruses, also known as Norwalk-like viruses, are a genus of viruses within the *Caliciviridae* family. They are one of the more significant gastroenteritis-causing viral agents, considered to be a leading cause of the disease [79–81], and are commonly associated with disease outbreaks [19, 82, 83]. Noroviruses are one of the more commonly investigated and detected viruses in wastewater [24–26, 28–30, 32, 33, 36, 59, 60, 84, 85]. A number of studies have also investigated the presence of noroviruses in human feces [43, 47, 53, 54, 79, 86–88]. One such cited study reported quantification values for norovirus in stool following qPCR, in the range of $9.7 \times 10^5$ to $1.1 \times 10^{12}$ copies per gram, with a mean value of approximately $10^{11}$ copies per gram [87].

Rotaviruses are another primary cause of gastroenteritis with symptoms including diarrhea, vomiting, and fever, in accordance with other enteric viruses [89]. They are commonly detected via PCR in raw wastewater [29–31, 36, 50, 58, 59, 90–92] and are commonly investigated and detected in human feces [43–45, 47, 53, 93–95]. Like other waterborne viruses, though, only a handful of studies on rotaviruses have used qPCR as a detection tool, and none reported quantification values in terms of the number of copies.

In addition to the commonly investigated waterborne viruses described above, there are other human viruses that are commonly detected in wastewater and human stool but not as frequently studied, such as Aichi virus, polyomaviruses, salivirus, sapovirus, and torque teno virus. Aichi virus is a member of the *Picornaviridae* family, the same family as enteroviruses, and is believed to cause gastroenteritis [96]. Salivirus, another member of the *Picornaviridae* family, is also associated with gastroenteritis, as well as acute flaccid paralysis [97]. Sapovirus, like norovirus, is a member of the *Caliciviridae* family and like its relative is a common cause of gastroenteritis [98]. Polyomaviruses are associated with a variety of diseases in humans, including nephropathy, progressive multifocal leukoencephalopathy, and Mercel cell carcinoma [99]. Torque teno virus is commonly detected in humans, but the clinical consequences of infection are unclear [100]. These viruses are included in Table 5.2.

### 5.3.2 Non-waterborne Viruses

Non-waterborne viruses have also been detected in wastewater or human excrement (included in Table 5.2). While it is logical to investigate the applicability of waterborne viruses to wastewater-based epidemiology, it is also important to note the potential for other categories of viruses to fit into this methodology.

There exists a category of water-related viruses that are transmitted via insects (like mosquitoes) that breed in water, such as Zika virus, West Nile virus, Rift Valley fever virus, yellow fever virus, dengue virus, and chikungunya virus, in addition to confirmed waterborne viruses. These viruses also fall into the category of zoonotic
viruses, which are viruses that can be transmitted between humans and animals. Other zoonotic viruses include avian influenza virus, SARS (Severe Acute Respiratory Syndrome) coronavirus, Menangle virus, Tioman virus, Hendra virus, Australian bat lyssavirus, Nipah virus, and hantavirus. Specific animal species of concern that are vectors for these zoonotic viruses include avian species, bats, rodents, and mosquitos. While these zoonotic viruses are not classified as waterborne, they are associated with potential waterborne transmission, such as exposure to aerosolized wastewater, which can occur when wastewater undergoes turbulence, such as in flush toilets, converging sewer pipes, and aeration basins [101, 102] as well as irrigation and land application systems.

It has been shown that coronaviruses have been detected in wastewater [103] and SARS coronaviruses have been detected in stool and urine samples. Furthermore, detection in both human stool and urine [104–106] as well as wastewater [107] has been reported for influenza. Detection in urine has been reported for the mosquito-associated Zika virus [108], West Nile virus [109, 110], dengue virus [111, 112], and yellow fever virus [113]. These observations indicate that the concept of wastewater-based epidemiology could be applied to a wide range of viruses beyond the confirmed waterborne viruses.

5.4 Variations of Viruses in Wastewater

The quantity of human enteric viruses in wastewater has been shown to have seasonal variation, indicating that infection resulting from these viruses is more prevalent at certain times of the year. A study conducted in Japan by Katayama et al. (2008) found that norovirus concentrations in wastewater were highest during the months of November through April [26], while enterovirus and adenovirus concentrations were largely consistent throughout the year. A 9-year study in Milwaukee, Wisconsin, by Sedmak et al. (2005) found that concentrations of reoviruses, enteroviruses, and adenoviruses were highest during the months of July through December. This study also analyzed clinical specimens of enterovirus isolates and found the incidence of clinical enterovirus infection corresponded to the concentration of these viruses in wastewater during the same time periods [31]. Another study in Beijing, China, by Li et al. (2011) found that rotavirus concentrations were highest during the months of November through March [90] and that these findings also corresponded with clinical rotavirus data reported in China [128].

Additionally, variation in viral concentration in wastewater can occur on a smaller timescale. For example, tourist locations could experience higher wastewater loads, and consequently higher viral concentrations, on weekends where there is an influx of population. For example, Xagoraraki’s research group conducted a study which observed an increase in adenovirus concentration in wastewater following the July 4th holiday in Traverse City, Michigan, a popular vacation destination [27]. Likewise, urban centers may experience higher loads during the day on
weekdays, while people are at work. Accounting for these population changes would be vital for understanding when viral outbreaks occur.

Wastewater has been used in the past as a tool to investigate viruses for other purposes as well, such as spatial surveillance and evaluation of immunization efficacy. Two particular studies were able to use wastewater to observe the spatial variation of particular viral strains; Bofil-Mas et al. observed that particular strains of polyomavirus were endemic to specific regions, while Clemente-Cesares et al. detected Hepatitis E virus in areas previously considered non-endemic for the virus [129, 130]. Lago et al. (2003) investigated the efficacy of a poliovirus (a type of enterovirus) immunization campaign in Havana, Cuba, by quantifying concentrations of the virus in wastewater [61]. Poliovirus was detected in 100% of wastewater samples prior to the start of the immunization campaign and dropped to a 0% detection rate in wastewater 15 weeks after the campaign, indicating the usefulness of wastewater surveillance. A study by Carducci et al. (2006) investigated the relationship between wastewater samples and clinical samples and found that the same viral strains could sometimes be detected between the two sets of samples [131].

5.5 Proposed Methodology

Waterborne and non-waterborne viruses have been detected in wastewater, variations of concentrations in time have been observed, and virus presence in wastewater has on occasion been correlated with occurrence of clinical disease. However, wastewater-based epidemiology methods have not yet been applied to assess and predict viral disease outbreaks in a systematic way. Wastewater-based epidemiology has the potential to predict “critical locations” and “critical moments” for viral disease onset. Designing spatial and temporal sampling appropriate to the area of concern, as well as modeling the fate of viruses, is critical for the effectiveness of the proposed method. This methodology is summarized in Fig. 5.2. In the following sections, critical factors for implementation are discussed.

5.5.1 Sampling in Urban and Rural Locations

The most critical parameter for the effective application of wastewater-based epidemiology is the selection of a surveillance program, including spatial and temporal sampling. Considerations must be made in the differences between urban and rural wastewater systems. Urban sewage systems offer a convenient confluence of wastewater in the serviced population, as all wastewater will ultimately flow to a WWTP, providing a sampling point representing the entire community. Additionally, localized sampling can be performed in specific neighborhoods where access points are available. By surveying both the combined wastewater at the treatment plant and the localized samples from neighborhoods, viral outbreaks can be traced to a more
specific location and the urban areas of concern can be identified. Xagoraraki’s research group is currently conducting a National Science Foundation-funded study of this nature in the city of Detroit, sampling at several interceptors at the Detroit wastewater treatment plant, as well as sampling from sewer lines in residential areas throughout the city.

More rural or underdeveloped areas that do not have sewage collection systems pose sampling problems. In these areas, wastewater is often disposed in open space, latrines, or septic tanks. As a result, for wastewater-based epidemiology sampling to be effectively applied to these areas, disposal, fate, and transport of wastewater in the environment must be taken into account. Watershed modeling would therefore become an integral component of the wastewater-based epidemiology methodology for rural locations. In a study performed by Xagoraraki’s research group, preliminary investigation into the wastewater epidemiology methodology was conducted [132]. Samples were collected from a wastewater treatment plant and surrounding surface waters in Kampala, Uganda. Three sampling events were conducted in 2-week intervals. Four human viruses (adenovirus, enterovirus, hepatitis A virus, and rotavirus) were quantified at each sampling location via qPCR. Concentrations of each virus at each location from each sampling event were compared to one another to determine if significant differences could be observed from one sampling event to the next. Results indicated that statistically significant differences in viral concentration were observed for the measured viruses at several sampling locations.

Fig. 5.2 Summary of the proposed wastewater-based epidemiology methodology
The selection of the sampling times and locations is of paramount importance to the methodology, regardless of whether sampling takes place in urban or rural areas. Sampling should be based upon expected critical pathways of viral transport and transmission. These critical pathways include environmental reservoirs for viruses and the timing and locations where viruses are most easily transported and transmitted between humans and the environment. By determining sampling times and locations based upon critical pathways, “critical locations,” and “critical moments,” areas and times most impactful to the spread of viral disease would be most readily and effectively identified.

5.5.2 Quantification of Viruses

Quantitative data of viruses of concern, such as those obtained with qPCR, are critical for the proposed methodology, as peaks in viral concentrations will indicate potential onset of disease outbreaks. While detection in human excrement or raw wastewater has not been reported for all viruses, it is possible that they have simply not been investigated in this context, as detection of viruses via conventional methods (cell culture, PCR, qPCR) is specific to the virus being investigated. Thus, while qPCR is important to detect and quantify common waterborne viruses, next-generation sequencing and metagenomic methods could also be performed to screen for the presence of other viruses. If genomic sequences of viruses of concern are found, then quantification with qPCR can follow.

Metagenomic methods have been applied to investigate viruses in wastewater and have been found to produce more conservative results of viral detection compared to conventional methods; viruses detected with metagenomic methods are typically also detected with conventional methods, whereas viruses detected via qPCR may not be detected with metagenomic methods. These metagenomic methods, however, can detect the presence of viruses not commonly quantified using qPCR [37, 133–136]. Xagoraraki’s research group’s studies have used metagenomic methods to identify human viruses of potential concern in wastewater. The first of these studies, conducted with samples from both Michigan and France, detected a comparatively high number of metagenomic hits for human herpesviruses and also detected human parvovirus and human polyomavirus in wastewater effluents [37]. Their other study, conducted in Uganda, detected human astroviruses, papillomaviruses, as well as a BLAST (Basic Local Alignment Search Tool) hit for Ebola virus [132]. While more research is still required to attain more robust genomic information and comparison databases, metagenomic methods can still be a useful tool for the identification of potential viruses that can then be monitored with qPCR methods. Table 5.3 presents a summary of studies that have used metagenomic methods to detect human viruses in wastewater and human excrement.
5.5.3 Population Normalization

Population normalization is also a critical factor for the application of wastewater-based epidemiology. Proper quantification of biomarkers in wastewater would allow for an appropriate estimation of serviced population via statistical modeling, which would provide context to measured viral concentrations and ensure that differences in viral concentration could not be attributed to changes in population. When observed viral concentrations are significantly high relative to the estimated population, a viral outbreak could be indicated.

Quantification of biomarkers (substances naturally excreted by humans) in wastewater can be used as a method of estimating population in an area. Governmental census information has been found to underestimate the population of a community compared to estimation using biomarkers [140], and certain substances detected in wastewater have been shown to correlate with census data [141]. Several substances have been proposed and investigated as population biomarkers (Table 5.4), including creatinine [142], cholesterol, coprostanol [143], nicotine [144], cortisol, androstenedione, and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) [145]. Nutrients such as nitrogen, phosphorus, and oxygen [12], as well as ammonium [146], have also been proposed as population biomarkers, but these may more adequately reflect human activity and industry footprint rather than population [145, 147, 148].

Table 5.3 Summary of studies using metagenomic methods to detect viral sequences in wastewater and human excrement

| Detected in | Virus                                                                 | References |
|------------|----------------------------------------------------------------------|------------|
| Wastewater | Adenovirus, enterovirus, polyomavirus, papillomavirus                  | [135]      |
|            | Adenovirus, Aichi virus, coronavirus, herpesvirus, torque teno virus   | [137]      |
|            | Adenovirus, Aichi virus, astrovirus, coronavirus, enterovirus, herpesvirus, papillomavirus, parechovirus, parvovirus, rotavirus, salivirus, sapovirus, torque teno virus | [133]      |
|            | Adenovirus, Aichi virus, astrovirus, norovirus, papillomavirus, parechovirus, polyomavirus, salivirus, sapovirus | [136]      |
|            | Adenovirus, herpesvirus, parvovirus, polyomavirus                      | [37]       |
|            | Adenovirus, astrovirus, Ebola virus, enterovirus, papillomavirus, rotavirus, torque teno virus | [132]      |
| Human excrement | Adenovirus, astrovirus, enterovirus, norovirus, parvovirus, rotavirus | [138]      |
|            | Adenovirus, Aichi virus, enterovirus, parechovirus, rotavirus          | [139]      |

Note: The following sequences have been confirmed via PCR for the listed study. Bibby and Peccia [133] adenovirus, enterovirus, parechovirus [131], [136], adenovirus, polyomavirus, salivirus [134], adenovirus [37], [132], adenovirus, enterovirus, rotavirus [130]
### Table 5.4 Summary of biomarkers proposed for population adjustment

| Biomarker      | Description                                      | Excreted in | References |
|----------------|--------------------------------------------------|-------------|------------|
| 5-HIAA         | Metabolite of serotonin                         | Urine       | [145]      |
| Ammonium       | Form of ammonia found in water                   | Urine       | [146]      |
| Androstenedione| Sex hormone precursor                            | Urine       | [149]      |
| Atenolol       | Drug (beta blocker) used to treat hypertension   | Urine       | [140]      |
| Cholesterol    | Lipid molecule, key component of cell membranes  | Feces       | [143]      |
| Coprostanol    | Metabolite of cholesterol                        | Feces       | [143]      |
| Cortisol       | Steroid hormone produced by adrenal glands       | Urine       | [150]      |
| Cotinine       | Metabolite of nicotine                           | Urine       | [145]      |
| Creatinine     | Metabolite of creatine phosphate in muscle       | Urine       | [142]      |
| Nicotine       | Stimulant found in tobacco                       | Urine       | [144]      |
| Nutrients (N, P, BOD) | Water-quality parameters                       | n/a         | [12]       |

### Table 5.5 Summary of reported shedding rates for viruses

| Virus           | Range of shedding rate, copies/g stool | References |
|-----------------|----------------------------------------|------------|
| Adenoviruses    | 1.0×10^2 to 1.0×10^11                  | [39, 41, 42, 46] |
| Enteroviruses   | 1.4×10^4 to 6.6×10^9                   | [60]       |
| Hepatitis A virus | 3.6×10^3 to 1.0×10^11               | [70]       |
| Hepatitis E virus | 1.0×10^7 to 1.0×10^6                | [77]       |
| Noroviruses     | 1.1×10^8 to 1.1×10^12                  | [87]       |
| Sapoviruses     | 1.3×10^8 to 2.5×10^11                  | [98, 118]  |

#### 5.5.4 Estimation of Shedding Rates

The shedding rate (the rate with which viruses are released from the body in excre-ment) for each waterborne virus group encompasses a wide range, from 10^2 copies per gram at minimum to 10^12 copies per gram at maximum. This variability is summarized for selected viruses in Table 5.5. For example, mean concentration values of adenoviruses in excrement ranged from 10^4 to 10^6 depending on the study and whether the virus is excreted in stool or urine, indicating a wide data variance [39, 41]. Many factors can impact the shedding rate of viruses in excrement, including viremia (the presence of the virus in the bloodstream) [40, 87, 151]. The duration of the presentation of a particular disease can also impact the shedding rate [105, 121].

#### 5.5.5 Transport of Viruses in the Environment

Waterborne viruses survive well in water, but all viruses are susceptible to natural degradation determined by factors such as temperature, exposure to UV light, and the microbial community [152, 153]. The kinetic decay rate of a virus would thereby
be primarily dependent not only on the characteristics of the individual virus but also environmental conditions within the sewage system, which could vary from location to location. Moreover, the fate of viruses may be different between wastewater systems in urban areas which typically use enclosed underground sewer pipes and rural areas which may utilize septic tanks, catchments, and the open environment. Viruses can also adsorb to or be enveloped by particulate matter in wastewater which would lead to confounding factors in measurement of these viruses.

5.5.6 Correlation with Public Health Records and Unidentified Clinical Data

Comparison with clinical data is another key component of these methods. Correlations between measured viral concentrations in wastewater and reported clinical cases of disease could be established, strengthening the proposed methodology. The establishment of these correlations can serve as a validation for a prediction model that accounts for the factors discussed above, providing evidence for the notion that changes of viral concentrations in wastewater will indicate changes in viral disease cases in humans. Moreover, should preventative public health measures be implemented after the identification of an outbreak, the tracking of clinical data could provide a quantifiable indicator of the efficacy of these preventative measures.

5.6 Conclusions

Infectious viral outbreaks can cause uncontrollable negative effects especially in densely populated areas. Early detection is critical for effective management and prevention of outbreaks. Recent research efforts in developing optimized detection systems often focus on rapid methods for analyzing blood or excrement samples; however, these approaches require that individuals are examined in clinical settings, typically after an outbreak has been established. Wastewater-based epidemiology is a promising methodology for early detection of viral outbreaks at a population level. Analyzing wastewater is equivalent to obtaining and analyzing a community excrement sample. In the determination of whether an outbreak is imminent or already in progress, quantifying viral concentration in raw wastewater is a crucial first step in this process. Waterborne viruses appear to be prime candidates, as they are detectable and quantifiable in both wastewater and human excrement. Non-waterborne viruses have been shown to be detected in human excrement, and some have been reported to be detected in wastewater. Wastewater-based epidemiology therefore has the potential to expand beyond waterborne viruses.

Routine monitoring for temporal changes in virus concentration and diversity in community wastewater, in combination with monitoring metabolites and biomarkers for population adjustments, allows early detection of outbreaks (critical moments
for the onset of an outbreak. In addition, carefully designed spatial sampling of wastewater will allow detection of locations where an outbreak may begin to develop and spread (critical locations for the onset of an outbreak). Considerations in sampling locations must be taken with regard to the area of investigation, as urban and rural areas may have differences in the respective wastewater systems that can affect viral transport in the water environment. Moreover, to obtain an accurate estimation of disease cases in a population, other factors must be considered such as viral shedding rates, environmental transport and degradation rates, and correlation with reported clinical disease data. Ultimately, there is great opportunity for the use of wastewater-based epidemiology to investigate viral outbreaks within a community. Comprehensive application of the various factors discussed above is crucial for the full potential of this methodology to be realized. Further research could clarify many of these issues and allow for the full development and application of this new epidemiological technique for studying, identifying, and predicting viral outbreaks.

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