



Review Article

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Utility of Sewage Surveillance in Tracking Spread of SARS-CoV-2

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Wastewater Based Epidemiology (WBE) postulates that through the analysis of population pooled wastewater, infectious disease, antimicrobial resistance spread and emergence of new disease outbreak to the community level can be monitored especially in resource constrained settings [1]. Sampling sewage for pathogenic particles is a time-tested method of environment surveillance and is routinely resorted to for understanding circulation of several pathogens-wild and vaccine-derived polio, rota virus, hepatitis E and typhoid-in the community. Environmental surveillance of polio in wastewater has been utilised since the 1980s for analysing the sewage samples in order to assess polio circulating within populations [2].

Nearly 16-73% of patients with COVID-19 have diarrhoea in addition to respiratory symptoms [3]. SARS-CoV-2 virus is excreted in faeces and urine of symptomatic and asymptomatic patients and hence can be found in sewage drainage of the area with such patients. In reports from Australia and Netherlands, the virus has been detected in sewage 2-13 days before anyone in the area was symptomatic [4,5]. Hence it may prove to be an effective surveillance tool to predict and track the evolution of spread in community where no previous cases have been found. No definitive end point of excretion of virus in sewage in an area with previously known COVID-19 positive patients has been reported as yet. Sewage surveillance can be carried out independent of testing in humans and will be able to pick up early signs even when people in the community do not show symptoms [6].

The shedding of SARS-CoV-2 has been studied in stools of a cluster of 9 cases and was found to be 107 RNA copies/g faeces one week after symptom onset and decreased to 103 RNA copies/g three weeks after symptom onset. In stool samples with high RNA copies, viable SARS-CoV-2 was detected. Although it is unlikely that wastewater will become an important transmission pathway for coronaviruses like SARS-CoV-2, increasing circulation of the virus in the population will increase the virus load into the sewer systems of our cities [7].

Assuming typical stool sizes of 200 g, diluted into an average volume of 1.36×10^9 L, and a population of 2.3×10^6 individuals each producing one stool per day, and we further assuming that there is no loss of viral RNA in sewer lines and

that excreted viruses are fully suspended in sewage, then we expect the viral titre in faeces to be about 3000 times higher than that in sampled raw sewage, or about 30,000 particles per ml [3].

Estimates of viral load in stool from positive patients are still a matter of uncertainty, but at least one recent study suggests levels as high as 600,000 viral genomes per mL of faecal material [3]. Another study reported a maximum observed value close to 30,000,000 viral particles per mL in a single faecal sample [8].

The SARS-CoV-2 load to municipal wastewater is estimated to be bracketed by the lower and upper bounds estimate of 56.6 million to 11.3 billion viral genomes per infected person per day. This mass load translates into concentrations of 0.15 to 141.5 million viral genomes per litre of wastewater generated in North America and Europe. This is based on the presence of reported 600,000 to 30,000,000 viral genomes of SARS-CoV-2 per mL of faecal material, and assuming a faecal load of 100-400 g faeces/day/person with a density of 1.06 g/ml [9]. Depending on local conditions, detection in community wastewater of one symptomatic/asymptomatic infected case per 100 to 2,000,000 non-infected people is theoretically feasible, with some practical successes now being reported from around the world. Computer simulations for past, present and emerging epidemic hotspots (e.g., Wuhan, Milan, Madrid, New York City, Teheran, Seattle, Detroit and New Orleans) identified temperature, average in-sewer travel time and per-capita water use as key variables of concentration of viral genomes in sewage. Assuming a detection limit of 10 coro-

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navirus RNA genomes per mL sewage, and further assuming absence of additional stormwater, commercial, and industrial flow inputs to the sewer system, successful detection of SARS-CoV-2 by qRT PCR in fully homogenized wastewater will require at worst as much as 0.88% of the population in a monitored sewershed to be infected (1 in 114 individuals) and at best, as few as 0.00005% (1 infected case in about 2 million non-infected individuals). This implies that the practical limit of detection of SARS-CoV-2 in community wastewater is well within the useful range and potentially superior to the alternative approach of randomly testing of 100 to 2 million people to establish presence or absence of symptomatic or asymptomatic cases in a population of interest [10].

In Australia, SARS-CoV-2 RNA was concentrated from wastewater and viral RNA copies were enumerated using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) resulting in two positive detections within a sixday period from the same wastewater treatment plant (WWTP). The estimated RNA copy numbers observed in the wastewater were then used to estimate the number of infected individuals in the catchment via Monte Carlo simulation. Given the uncertainty and variation in the input parameters, the model estimated a median range of 171 to 1090 infected persons in the catchment [4].

In Massachusetts, the scientists found the presence of SARS-CoV-2 at high titres in the period from March 18-25 using RT-qPCR. Viral titres observed were significantly higher than expected based on clinically confirmed cases in Massachusetts as of March 25 [3].

In Netherlands, sewage samples of 7 cities and the airport were tested using RT-PCR against three fragments of the nucleocapsid protein gene (N1-3) and one fragment of the envelope protein gene (E). No SARS-CoV-2 was detected in samples of February 6, three weeks before the first case was reported in the Netherlands on February 27. On March 5, the N1 fragment was detected in sewage of five sites. On March 15/16, the N1 fragment was detected in sewage of six sites, and the N3 and E fragment were detected at 5 and 4 sites respectively. The detection of the virus in sewage, even when the COVID-19 prevalence is low, indicates that sewage surveillance could be a sensitive tool to monitor the circulation of the virus in the population [5].

In recent times, paper analytical devices have emerged as powerful tools for the rapid diagnosis of pathogens and determination of infection transmission [11]. The paper-based device is a small analytical tool with different functional areas printed with a wax printer that integrates all processes (extraction, enrichment, purification, elution, amplification, and visual detection) required for nucleic acid testing into an inexpensive paper material. The whole testing process can be completed through simple folding of a paper-based device in different ways in different steps without a pump or power supply, which overcomes the limitation of PCR and avoids multiple processes. Paper analytical devices enable multiplexed, sensitive assays that rival PCR laboratory assays and provide high-quality, fast precision diagnostics for pathogens. For example, a recent work has demonstrated that the mul-

tiplexed determination of malaria from whole blood using a paper-based device in rural Uganda [12].

A fast "sample-to-answer" analysis method can provide quantitative monitoring of nucleic acids and genetic information through the analysis of sewage, which can be confirmed with a robust electrophoresis and agarose gel image assay, with high reliability for wastewater analysis [13].

Sewage surveillance for SARS-CoV-2 may be useful in the following scenarios:

- 1) Surveillance of areas where no case of COVID 19 has been reported as yet.
- 2) In areas with known transmission, to predict spikes in number of cases before they actually happen.
- 3) Theoretically, this can also be used to monitor the containment zones after the last symptomatic patient has been declared cured. However, the duration of this kind of surveillance remains undefined and may become available soon through reports.

Methodology employed in polio surveillance suggests that grab samples of sewage at peak flow times or at the same time everyday may give best yields of viral genomes [14]. The sewage samples will require centrifugation of large volumes of sewage (250 ml - 1 litre). Such centrifuges can typically be found in the labs of sewage treatment plants. The clear supernatant can be further processed in microbiological laboratories equipped to carry out RT-PCR. Epidemiological surveillance of the catchment area will have to be conducted to detect the number of cases in the catchment of sewage drainage.

In densely populated countries, for example like India or Bangladesh, sewage surveillance may be an efficient and cost-effective way to monitor the spread of disease in urban areas. However, challenges shall be encountered in smaller cities and rural areas where population density is low and the sewage drainage systems are not well developed.

The public health/wastewater management departments, which handle sewage treatment plants, can be involved in centrifugation of grossly contaminated sewage followed by transporting the small volume clear supernatant to microbiological testing sites for RT PCR for SARS-CoV-2. This can be achieved at minimal additional cost. Standardization of RT PCR for sewage sample shall be needed before adequate reliability can be achieved. Finally modelling simulations shall be needed to quantify the force of infection in the population based on the viral genomic load in sewage.

Whether SARS-CoV-2 is viable under environmental conditions that could facilitate faecal-oral transmission is not yet clear. However, recent evidence that the virus spreading easily and sustainably in the community in some affected geographic areas such as China [15].

Enteric transmission of SARS-CoV-2 is possible and exposure to SARS-CoV-2 in wastewater could pose a major public health risk. Environmental surveillance of SARS-CoV-2 could serve as a data source which can indicate the circulation of

the virus in the community. Previously, this method has been successfully applied for preclinical identification of Aichi virus in Netherlands [16].

The possibility of faecal-oral transmission of COVID-19 has various other implications, especially in areas with poor sanitation and diagnostic capacity, such as Africa. Wastewater surveillance, especially in areas with a scarcity of data, might provide information about the status of antibiotic resistance [17].

Sewage surveillance for SARS-CoV-2 requires multidisciplinary teams to work in close co-ordination and may have a role in monitoring circulation of virus in populations with manifold reduction in costs and need for testing kits. Needless to say, all personnel involved need to take precautions and use appropriate protective equipment. Such kind of surveillance is effectively being carried out for poliovirus almost in all countries with guidance and support from WHO.

Sewage surveillance for SARS-CoV-2 may be a cost-effective way of tracking spread of the virus in areas which are currently free of COVID 19 and to predict a spike in cases in areas with the disease. This can be achieved without human testing. Multidisciplinary teams are needed to formulate protocols for correlating data from sewage to epidemiological data.

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