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Tracking COVID-19 via sewage

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Purpose of review

We discuss the potential role of the faecal chain in COVID-19 and highlight recent studies using waste water based epidemiology (WBE) to track Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**.**

Recent findings

WBE has been suggested as an adjunct to improve disease surveillance and aid early detection of circulating disease. SARS-CoV-2, the aetiological agent of COVID-19, is an enveloped virus, and as such, typically not associated with the waste water environment, given high susceptibility to degradation in aqueous conditions. A review of the current literature supports the ability to detect of SARS-CoV-2 in waste water and suggests methods to predict community prevalence based on viral quantification.

Summary

The summary of current practices show that while the isolation of SARS-CoV-2 is possible from waste water, issues remain regarding the efficacy of virial concentration and subsequent quantification and alignment with epidemiological data.

Keywords

SARS-CoV-2, waste water based epidemiology, sewage, faecal chain

Introduction

Since the emergence of novel COVID-19 efforts have primarily focused on non-pharmacological preventative strategies to mitigate disease transmission, in an attempt to alleviate disease burden and support healthcare infrastructures. With societal progression from the initial phases of infection, and easing of public restrictions there is potential for disease resurgence and hence a further reliance on systematic public health measures of testing and contact tracing (1), both expensive and labour intensive. Recognition of missed circulating SARS-CoV-2 upon testing historic respiratory samples (2) reveals lost opportunities for potential public health interventions. Additionally, the role of asymptomatic carriage (3) as a source of contagion increases the potential for undetected disease clusters. Unfortunately, the above combination of factors acts to impede a rapid, real time, cost effective screening programme. Waste water based epidemiology (WBE), which provides normalised population level information on human activity within a defined geographical unit, (4*) has been mentioned as a potential additional tool to enhance COVID-19 surveillance.

As obligate parasites, human viruses do not replicate within the environment and must be introduced into waste water via human bodily fluids. This creates an opportunity to generate surveillance systems at critical sites, capable of early detection of potential viral outbreaks. Additionally, attempts have been made to equate viral RNA copy number with clinical epidemiological data to track infection rates and compare estimates of disease prevalence within a specific geographical boundary (5-7**). The use of WBE is already established for tracking infectious agents including hepatitis A (8) and polio (9) and provides a potential addition to surveillance systems that is scalable, cost effective, flexible with target organism(s) and reproducible in a low resource setting (10).

Coronavirus in stool

As initial case reports of COVID-19 emerged the symptomology centred on respiratory disease, with little reported involvement of the gastrointestinal system (11). However, this rapidly changed as

larger cohorts of patient data was analysed and the disease time course taken into account. A recent systemic review and meta-analysis investigating a total of 4805 patients over 23 published and 6 preprint articles demonstrated the prevalence of gastrointestinal symptoms, including diarrhoea, nausea and vomiting to be approximately 12%, while SARS-CoV-2 RNA shedding in faeces was detected in up to 41% of patients (12). SARS-CoV-2 shares 79% homology to SARS-CoV and 50% homology to MERS-CoV (13), utilising the same cellular receptor, angiotensin-converting enzyme 2 (ACE2) (14). ACE2 is known to be expressed within of the gastrointestinal, respiratory and oral epithelia (15, 16) and support the prospect of SARS-CoV-2 replication within the gut. Several studies have documented the detection of SARS-CoV-2 RNA from faecal samples using quantitative PCR (qPCR) (17-20), including paediatric populations (21) and asymptomatic individuals (22, 23). Of note, faecal viral shedding has been demonstrated to persist longer than respiratory shedding. In a study investigating 98 patients of which 41 (55%) had detectable virus in their stools (qPCR), the mean days from symptom onset to end of viral detection was 27.9 days in stool compared with 16.7 days in respiratory samples (24); one patients' faecal samples remained positive for SARS-CoV-2 RNA for 47 days after symptom onset.

The faecal chain

The application of the faecal chain of infection is primarily reserved for enteric viruses associated with human disease, for example hepatitis A. Enveloped viruses, such as coronavirus, are generally regarded as more susceptible to inactivation and destruction in aqueous environments (25). However, it has been demonstrated that SARS-CoV-2 RNA is detectable in the stools of infected individuals and indeed live viral particles have also been recovered from the faeces of infected patients (26). This, combined with the high sensitivity of molecular methods to detect SARS-CoV-2 genetic material, has revived the premise of the faecal chain for understanding and tracking COVID-19 infections. Viral metagenomics studies of sewage have demonstrated diverse viral communities including the presence of enveloped viruses (27, 28).

Viruses present in the faeces, urine or vomitus of an affected individual enters the sewage system and is transported to a municipal waste water treatment plant (WWTP). Within the WWTP the influent is subject to physical, biological and chemical treatment and the resultant effluent is returned to surface waters (25). Following screening, primary treatment (settling) results in minimal reduction of viral load, less than 1 log (29), where secondary (aerobic biological processes) and tertiary (filtration/disinfection) treatment are critical for reducing viral numbers (30). A survey of 5 WWTP in the USA reported a significant reduction of viral load (RT-PCR) between sewage influent and effluent (1.9-5.0 log reduction), interestingly viral cytopathic effect was still demonstrated within the final effluent (31*). While the above study demonstrates the potential for enteric viruses to persist following sewage treatment, coronaviruses are less likely to survive the treatment process due to their encapsulated structure. This was investigated by Randazzo et al. where none (n=12) of the tertiary effluent samples had detectable SARS-CoV-2 from a survey of 6 waste water treatment plans in Spain (32**) and hence the possibility of transmission of SARS-CoV-2 via waste water remains slim.

However, within the remit of the faecal chain remains the possibility of environmental contamination and generation of aerosols from untreated sewage, within household plumbing, as demonstrated during the SARS-CoV-1 outbreak (33*). A cluster of 321 cases, accounting for 18% of total reported cases in Hong Kong has been linked to the generation of faecal particles within the bathrooms of an apartment block from a single symptomatic individual. Investigation by WHO verified that sewer gas and aerosolised droplets were drawn into apartment bathrooms, due to a combination empty U bends and extractor fans, from the plumbing system containing infected stool passing in the waste pipe (33, 34). This proposed mechanism of transmission was later replicated using a waste water test-rig which demonstrated dispersal of bacteria between rooms on different floors of a building (35), adding further validity to this argument as a method of coronavirus transmission.

Detection of SARS CoV-2 in waste water

As previous explained encapsulated coronavirus are thought to be more readably destroyed in aqueous environments owing to their structural differences compared to enteric viruses, hence methods developed for the concentration of common enteric viruses are unlikely to faithfully recover coronavirus (36). Despite this, studies investigating waste water influent have identified the presence of coronavirus using multi target microarrays (37) and shotgun viral metagenomics (28) and confirmed the presence of coronaviruses within surface waters (38). Given the dilution factors at play within waste water a viral concentration step is needed to recovery coronavirus from stool. Techniques presently employed include absorption-elution, polyethylene glycol precipitation, ultrafiltration, ultracentrifugation, and flocculation and have been reviewed elsewhere (39*).

Tracking SARS-CoV-2 in waste water

Given the potential utility of WBE in tracking local SARS-CoV-2 outbreaks initial reports have emerged from Australia (5**), France (7**), Spain (32**), Italy (40**), The Netherlands (41**) and USA (6**, 42**) providing some evidence and proof of concept. These studies detected and quantified by qPCR the levels of SARS-CoV-2 genetic material in incoming waste water, reporting values ranging from $1.2x10^2$ to $3x10^6$ genome copies/L. No detectable SARS-CoV-2 was found in waste water effluent (5**, 32**). Ahmed et al. applied the Monte Carlo simulation method to estimate the number of infected individuals within the WWTP catchment area, based on viral RNA copy numbers in waste water. However, given the large number of variables, the estimated number of infections ranged by an order of magnitude (171- 1,090) within a 6 day surveillance period, which reportedly correlates with clinical observations (5**). Wurtzer et al. also correlated viral RNA copy number with reported clinical cases and found that viral RNA quantity within waste water influent followed the COVID-19 epidemic curve (7**). Furthermore, they illustrated the potential capacity of WBE to monitor the impact of public health interventions, as the waste water surveillance period

encompassed the initiation of social 'lockdown' within the catchment area and a subsequent decreased in SARS-CoV-2 detection, albeit a crude indirect measurement.

The advantage of WBE as an early warning system has been reported by Randazzo et al. where longitudinal sampling of waste water provided early evidence of circulating coronavirus 12-16 days prior to the reporting of the first clinical case within WWTP catchment area (32**). However, the authors' state their quantitative assessment of viral RNA copy within waste water does not generally correlate with the numbers of clinical infections within the area. In addition to the clinical correlation discrepancies reported, that study used two different viral concentration methods (electronegative membrane and ultrafiltration) and two qPCR assays (N_Sarbeco and NIID_2019_nCOV) with conflicting results reported on replicate samples suggesting further standardisation of WBE is needed, especially at low viral RNA copy numbers, expected in waste waters (5**). Medema et al. also reported the ability to detect SARS-CoV-2 in waste water prior to the clinical reporting systems (41**). The authors evaluated N1, N2, N3 and E gene primer/probe sets with reported clinical cases and found N1 primer/probe was capable to detection of cases at a prevalence of 1.0 case per 100,000 people and N3 at 3.5 cases per 100,000 people, although this was not consistent (41**). Overall they demonstrated N1 to have higher sensitivity for detection of SARS-CoV-2 in waste water, followed by N3, and E gene primer/probe sets, findings which mirror the US FDA reported sensitivity on SARS-CoV-2 RNA primer/probe assays (43).

Wu et al. used qPCR to estimate the number of total SARS-CoV-2 particles/ml of waste water influent from 10 samples from a single WWTP. They hypothesized that ~5% of faecal samples in the WWTP catchment area were positive for SARS-CoV-2 (based on reported viral genomes/g of stool (44**)), a rate much higher than the reported prevalence of COVID-19 for that area (0.026%). The authors suggest their prevalence estimate is conservative given no loss of viral RNA (degradation, processing or extraction) was incorporated into the estimate, but accept that the estimate is based on large number of uncertain assumptions, not least of which is the amount of SARS-CoV-2 RNA/g of

stool present in an infected/shedding individual. Interestingly, this study highlights minimal viral RNA degradation in sample storage at 4^{o}C for one week, and again lower sensitivity of N2 primer/probe set (42**).

Following alignment of clinical epidemiological data with qPCR results (N1 and N2 primer/probe sets), after both point and subsequent composite sampling of waste water in Montana (USA), linear regression was used to predict the correlation between SARS-CoV-2 primer/probe sets with clinical disease onset (6**); 8 and 7 day lags for N1 and N2 gene targets, respectively were shown. Using Sanger sequencing, the authors identified a homogenous SARS-CoV-2 population in the collected waste water samples, and further investigated the ancestral phylogenetic relationship of their SARS-CoV-2 strain using long read Oxford Nanopore sequencing. By comparing with available SARS-CoV-2 genomes, the Authors determined their strain contained 11 nucleotide variants distinct from the original Wuhan strain (6**). The Authors concluded that their predominant SARS-CoV-2 strain to be more closely related to strains circulating in California (USA) and Victoria (Australia) and demonstrate WBE could be used to identify global movements of particular strains and potentially identify advantageous mutations within specific strains to specific geographical locations/communities.

Conclusion

Effective public health surveillance systems with prompt intervention are key to outbreak management. WBE could provide additional information to established practices. We have highlighted its potential role as an early warning system of circulating virus, thereby providing important additional time for health authorities to act. Issues remain regarding viral concentration and gene targets, which impede quantification and subsequent correlation with clinical data. Disease prevalence data currently rely on multiple assumptions: homogenous sample collection, static populations, viral shedding volume in faeces, and viral degradation, and therefore requires further research before its epidemiological application.

Key points

- WBE could be a potential adjunct to current public health measures of SARS-CoV-2 surveillance.
- Further work is needed on the standardisation of viral concentration and amplification methods from waste water.
- WBE could provide an early detection system for circulating SARS-CoV-2 in a defined geographical location.
- WBE can provide information regarding phylogenetic diversity and ancestral origin of circulating SARS-CoV-2.

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