

Epidemiological surveillance and environmental hygiene, SARS-CoV-2 infection in the community, urban wastewater control in Cyprus, and water reuse

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ABSTRACT

Background: Nowadays public health faces many challenges. Epidemiological surveillance and environmental hygiene and infection in community from SARS-CoV-2 along with its control in municipal wastewaters and reuse of water are amongst them. Epidemiology and environmental microbiology considers wastewater release of great importance. The purpose of this study is the detection and classification of COVID-19 infection in community wastewater and their removal by efficient functional processes.

Methods and materials: A descriptive review of the published literature over the last 15-years in Greek and English was carried out via Medline, Scopus, and Google Scholar databases with the perspective of creating a research protocol. The study material consists of recent articles on the subject using keywords. Thus, the most effective techniques, wastewater-based epidemiology, and quantitative microbial risk assessment, for virus surveillance in wastewater are further analyzed.

Results and contexts: The process and management of wastewater at a global level, is a high risk and a great challenge due to the huge amount of biomedical waste and wastewater that ends up in wastewater treatment plants. It may be an obstacle to human health, especially in the ongoing pandemic situation that must be evaluated in the operations of the plant in the environment along with the presence of other dangerous pathogenic microbes.

Conclusions: It is vital the relationship between humanity and environment be reconsidered and more sustainable behavioural choices be encouraged. Scientific evidence pinpoints that the onset of new viral pathogens with a high epidemic-pandemic potential is often the result, complex, interactions among animals, individuals, and environment.

Keywords: SARS-CoV-2 COVID-19, environmental microbiology, wastewater, public health, reuse water, monitoring epidemiological surveillance

INTRODUCTION

Nowadays public health faces many challenges. Environmental hygiene and infection in community from SARS-CoV-2 (COVID-19) along with its control in municipal wastewaters and reuse of water are amongst them. Environmental microbiology considers wastewater release of great importance. In this context, the interest of the scientific community has been attracted towards potential interactions of SARS-CoV-2 with environmental compartments.

The emerging COVID-19 pandemic has strongly modified people's lifestyle, making urgent to reconsider the humans-environment relationships and stimulating towards more

sustainable choices in our daily behavior. Scientific evidence pinpoints that the onset of new viral pathogens with a high epidemic-pandemic potential is often the result of complex interactions among animals, individuals, and environment. According to this framework, the interest of the scientific community has focused on the possible interactions of SARS-CoV-2 in the different environments [1, 2].

Scientists tend to study the epidemiology and persistence of SARS-CoV-2 in water bodies, the potential implications of lockdown measures on environmental quality status [3]. In some countries, these wastewater treatment facilities are insufficient due to inadequate infrastructure and therefore raw waste containing live pathogens may enter water systems, endangering the environment [4].

Water microbiologists argue is that wastewater does not appear to be a major way of transmitting SARS-CoV-2 [5]. The genetic material of SARS-CoV-2 is detectable in the faeces of a large part of COVID-19 cases and therefore in urban wastewater. This fact was confirmed early during the spread of the COVID-19 pandemic and led to several studies that suggested monitoring its impact on wastewater. It is estimated that landfilled waste is globally increased more than 3,500 thousand tons in the first pandemic year, only due to facemasks disposal. Nevertheless, there is no more recent data, which also mentions the expected increase in waste going to landfills due to the pandemic.

It is evident that several countries will have an impermeable problem in waste management, with high consequent risk derived from possible pollution due to leachate, and possibility of virus spread. Detection and monitoring of the SARS-CoV-2 virus in wastewater is challenging due to dilution, thus requiring concentration of viral particles in water samples for accurate quantification [5, 6].

The emerging threat posed by COVID-19 pandemic has strongly modified our lifestyle, making urgent to re-consider the humans-environment relationships and stimulating towards more sustainable choices in our daily behavior [5]. It is vital the relationship between humanity and environment be reconsidered and more sustainable behavioural choices be encouraged scientific evidence pinpoints that the onset of new viral pathogens with a high epidemic-pandemic potential is often the result of complex interactions among animals, individuals, and environment.

Anyone who encounters contaminated materials during that period is likely to become infected due their long survival time. SARS-CoV-2 is a spherical particle with spike proteins on its surface and single-stranded RNA within the lipid enclosure. SARS-CoV-2 can be transmitted through the air through aerosols due to its small size.

With 656 million cases and the death toll exceeding 6.6 million in mid-September 2022, the COVID-19 pandemic is certainly one of the greatest threats that humanity has faced in modern history.

The detection and monitoring of the SARS-CoV-2 virus in wastewater is a challenge due to dilution, thus requiring the concentration of viral particles in water samples for accurate quantification [6]. Nevertheless, a study from 2013 reported the detection of coronaviruses in wastewater, and studies of the SARS-CoV-2 outbreak in 2004 showed that virus RNA being detected in 100% of untreated and 30% of disinfected wastewater samples collected from a hospital in Beijing, China.

The presence of SARS-CoV-2 has already been reported in wastewater in Australia, France, the Netherlands, and the USA [5, 7], confirming that it can be concentrated from and detected in varied wastewater environments. It was identified three main approaches for the detection and monitoring of SARS-CoV-2 in wastewater such as qualitative, quantitative molecular and in vitro measurements with plate formation units (PFU) [8].

Each of these approaches have different capabilities for detection different speeds of detection, and different levels of sensitivity, which require certain virus concentrations to be present in wastewater. Many of the most recent studies focusing on detection of SARS-CoV-2 in wastewater have

employed detection of viral RNA and targeted gene analysis via quantitative RT-qPCR.

There is a lot of scientific evidence about the impact of the pandemic on the environment. Positive and negative aspects of the pandemic for the environment are mentioned. All the different environmental elements are involved in the pandemic [9].

The importance of public health labour inspection services for carrying out their inspections and epidemiological surveillance preventively and the identification of biological risks in the context of occupational safety with policy and management is linked to job satisfaction, burnout in the workplace [10-14].

The purpose of this study is the detection and classification of COVID-19 infection in community, the epidemiological surveillance, and control in municipal wastewater in Cyprus, and water reuse along with feedback in order a research protocol to be created.

METHODS AND MATERIALS

A descriptive review of the published literature over the last 15-years in Greek and English was carried out via Medline, Scopus, and Google Scholar databases. The study material consists of recent articles on the subject using keywords such as “SARS-cov-2 COVID-19”, “Environmental Microbiology”, “Wastewater”, “Public Health”, “Reuse Water”, “Monitoring Epidemiological Surveillance”. Thus, the most effective techniques, wastewater-based epidemiology (WBE), and quantitative microbial risk assessment, for virus surveillance in wastewater are further analysed.

RISK MANAGEMENT

Assisting Public Health Authorities

Recommendations against the spread of SARS-CoV-2 or, conversely, the relaxation of protection measures in large populations, with minimal cost in human lives and economy, require reliable methods of large-scale early warning. Medical data are suitable for providing early warning signs because only cases of infected patients are reported when detected. Furthermore, the clinical trials practically targeting small parts of the population are not sufficient to demonstrate the true magnitude of the pandemic as there are probably many more asymptomatic patients [5]. **Figure 1** presents the association and correlations from all the factors effective in global environment global public health.

Wastewater-Based Epidemiology

WBE has been used in the past to monitor infectious diseases such as polio, hepatitis A and norovirus. Viruses are detectable in sewage samples days before symptoms appear. Researchers can test these samples for specific viral genetic signatures without having to perform extended sequencing.

It is recommended WBE be a promising method for rapid and inexpensive tracking of viruses in the general population; thus, allowing effectively the population targeting for clinical diagnostic testing and subsequent prompt advancement of mass protective measures or vaccination campaigns.

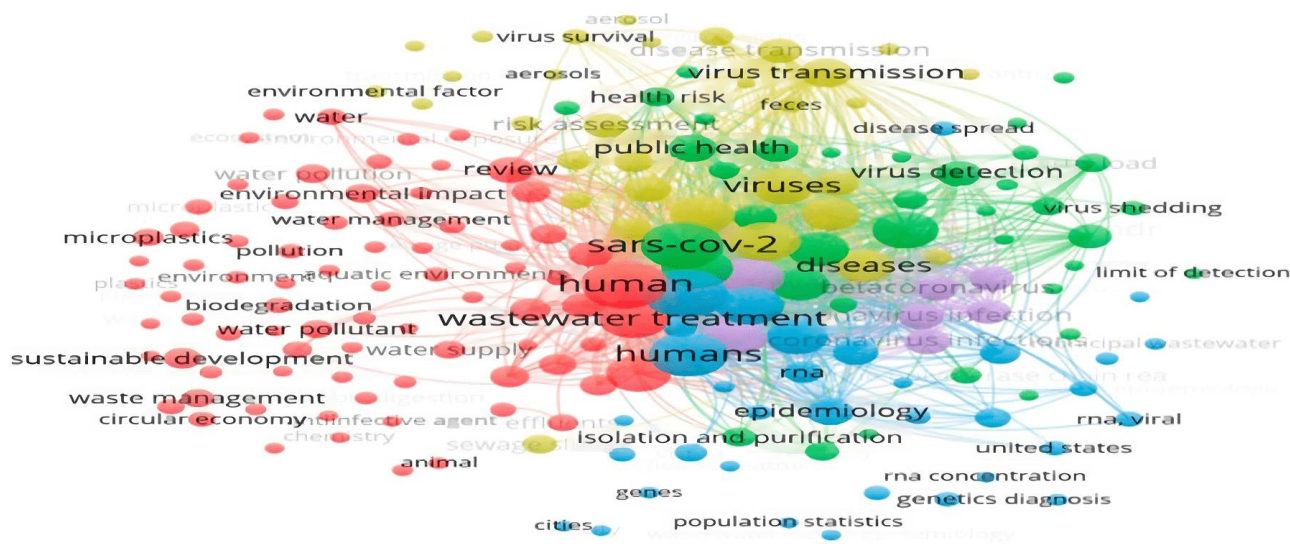


Figure 1. Global environment global public health (Ji et al., 2021)

By analysing infectious disease biomarkers in wastewater taken from wastewater collection points, the transmission of infectious diseases in certain areas can be comprehensively monitored in near real time [8, 15]. New challenges related to COVID-19 continue to arise even after the vaccines are released. Scientists and public health officials have sounded the alarm about new variants of the SARS-CoV-2 coronavirus that have emerged in the United Kingdom (B.1.1.7), South Africa (B.1.351), Brazil (P.1), California (B.1.429), and New York (B.1.526). Each variant is characterized by a set of specific, known mutations in its genomic sequence, some of which can increase transmissibility, virulence, or the viruses' ability to evade immune responses. To fully understand these changes and respond accordingly, researchers need to reliably identify where the variants are, when they appear, and how prevalent they become over the time.

WASTEWATER SURVEILLANCE

Wastewater Surveillance With Droplet Digital PCR

Analysis of SARS-CoV-2 in sewer networks provides information about COVID-19 infection in the community since symptomatic and asymptotically infected with SARS-CoV-2 people excrete viral RNA in sewage systems. WBE for the detection of SARS-CoV-2 has been applied in several countries. As a result, positive correlation has been found between the number of COVID-19 cases and the concentration of the SARS-CoV-2 gene in wastewater. Therefore, the basis for the epidemiological role of wastewater testing is formed [7].

Wastewater surveillance using droplet digital PCR (ddPCR) is a comprehensive, cost-effective, highly sensitive way to detect specific variants of the SARS-CoV-2 coronavirus in communities, allowing scientists and public health officials to monitor the spread of viruses and act [1].

ddPCR provides the sensitivity and accuracy required to reliably detect the presence of specific variants in wastewater samples, even at extremely low concentrations. The use of ddPCR for WBE allows scientists to detect the virus six days before clinical trials at the sensitivity of one infected person in 10,000, which is extremely important because only 32% of infected people seek medical help [1].

ddPCR was used to monitor SARS-CoV-2 virus elimination in weekly wastewater samples from nine wastewater treatment plants for several months. Elevated SARS-CoV-2 RNA levels in wastewater predicted increases in the numbers of confirmed cases, highlighting the ability of wastewater surveillance to detect outbreaks before they occur. Wastewater testing can also be employed by the transport industry to monitor SARS-CoV-2 transmission among aircraft and cruise liner passengers. Wastewater testing can also be employed by the transport industry to monitor SARS-CoV-2 transmission among aircraft and cruise liner passengers.

Another study used ddPCR to analyze wastewater from large transport vessels with their own sanitation systems with implications for contact tracing of disembarking passengers if SARS-CoV-2 is detected in wastewater. SARS-CoV-2 RNA was detected in aircraft and cruise ship wastewater, concluding that wastewater testing has the potential to aid clinical testing and contact tracing for disembarking passengers [5].

While global education providers return to in-person from distance-learning education, administrators must be ready to support a safe learning environment. Campus WBE is a crucial component of a complete COVID-19 prevention plan. Academic laboratories are assessing new technology of leverage wastewater testing to prevent campus COVID-19 outbreaks [11].

Guidance on wastewater surveillance testing methods and laboratory workflow has been published by CDC to detect SARS-CoV-2 across the USA. Specifically, ddPCR has been recommended as the detection method for RNA measurement of SARS-CoV-2 in wastewater [3].

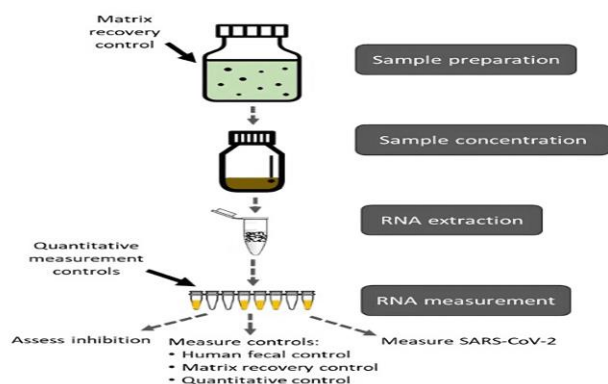
Policy and CDC Wastewater Guidelines

ddPCR technology is a comprehensive, cost-effective, highly sensitive way to detect globally specific variants of the SARS-CoV-2 coronavirus in various communities through raw sewage.

Each variant is characterized by a set of specific, known mutations in its genomic sequence, some of which can increase transmissibility, infectivity, or the ability of viruses to avoid immune responses. Researchers must determine where those variants occur, and how widespread they might become over time [2].

Table 1. Health care wastewaters infection in community SARS-CoV-2 epidemiological surveillance, & control in municipal wastewaters in Cyprus

Type or level of facility	Level of activity	Location (rural, urban, & semi-urban areas)	Volume (l)/weight (kg)
1-22	1-9	RA, UA, & SU	
1. Hospitals			
2. University hospital			
3. General hospital			
4. District hospital			
5. Other health-care facilities			
6. Emergency medical care services			
7. Health-care centres & dispensaries			
8. Obstetric and maternity clinics			
9. Outpatient clinics			
10. Dialysis centres			
11. Long-term health care establishments & hospices			
12. Transfusion centres			
13. Military medical services			
14. Prison hospitals or clinics			
15. Related laboratories & research centres			
16. Medical & biomedical laboratories			
17. Biotechnology laboratories & institutions			
18. Medical research centers			
19. Mortuary and autopsy centers			
20. Animal research & testing			
21. Blood banks & collection services			
22. Nursing homes for the elderly			

**Figure 2.** Laboratory examinations of wastewaters RNA measures SARS-CoV-2 (Adamopoulos et al., 2022)

Main Factors for SARS-CoV-2 Variants in Wastewater

Theoretically, scientists could detect different variants of SARS-CoV-2 by sequencing viral genomes individually. However, genomic sequencing of individual cases is expensive and sporadic, with the results being published days, weeks or even months after the tests are carried out.

Additionally, many individuals are asymptomatic or do not have easy access to testing services. A more effective way to identify variants of SARS-CoV-2 is wastewater surveillance consisting of an established epidemiological strategy that has been used in the past to monitor infectious diseases. Even in asymptomatic patients, SARS-CoV-2 is detected in their feces while viruses are detectable in wastewater samples days before symptoms appear. Researchers can test these samples for specific viral genetic signatures without performing extensive sequencing. DdPCR may be the exact tool for examining laboratory wastewater sampling. It has the sensitivity and accuracy necessary to detect the presence of specific variants in sewage samples, even at extremely low concentrations. Compared to common alternative technologies, it has simpler data analysis.

Bio-Rad digital analysis design is available online, so researchers can also create their own custom tests to detect any SARS-CoV-2 algorithm. The scientific community is now able to continue its efforts to control the COVID-19 pandemic. **Table 1** presents the wastewater management associated with hazards surveillance and policy.

Hazards Related to Wastewater Reuse for Irrigation

The risks associated with the reuse of wastewater for irrigation include health risks, environmental and agricultural risks. Raw sewage may contain human intestinal pathogens, viruses, bacteria, protozoa that may resist to wastewater treatments. It is proved that workers in wastewater treatment plants may be infected as shown by antibodies against hepatitis A and/or hepatitis B detected in a significantly high proportion of workers, although contrasted results have been found in another study. Moreover, increased temperature and stagnant water may favor the development of human non-intestinal water-based pathogens. **Figure 2** shows laboratory tests of wastewater RNA measuring SARS-CoV-2.

Urban sewage contains various pathogens. It is essential the effectiveness of cleaning treatments and disinfection practices that were often insufficient to reduce the viral load below the risk level be ensured for the reuse of that wastewater. Laboratory analysis of recycled water should target viral markers. The results of environmental monitoring, carried out in a wastewater treatment plant, showed the presence of adenovirus DNA in 100% of the samples collected. There was no significant correlation on firming their inadequacy in the virological risk assessment.

ENVIRONMENTAL & PUBLIC HEALTH ISSUES

It is a fact that there is not enough data available from exposure to urban wastewater and the effects on citizens' health. The volume of wastewater produced worldwide is very high, even from infrastructure wastewater.

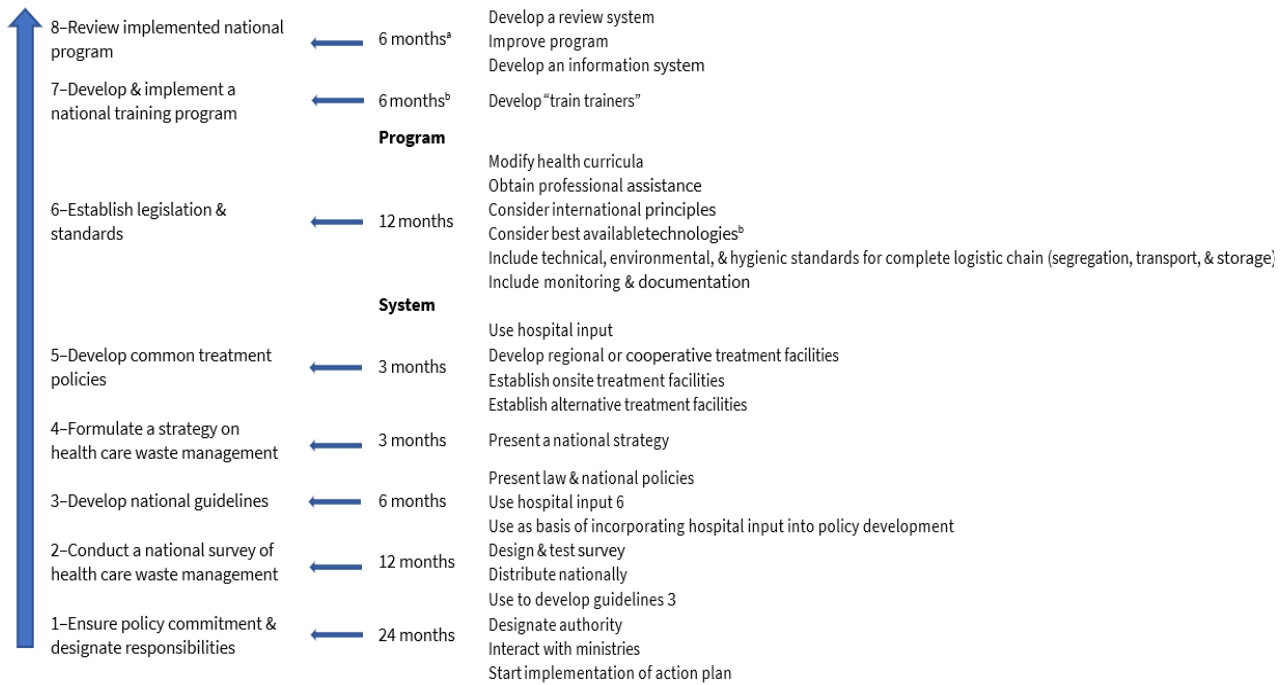


Figure 3. Action plan & steps for systems tools elements to organize epidemiological surveillance (Adamopoulos et al., 2022; WHO, 2014)

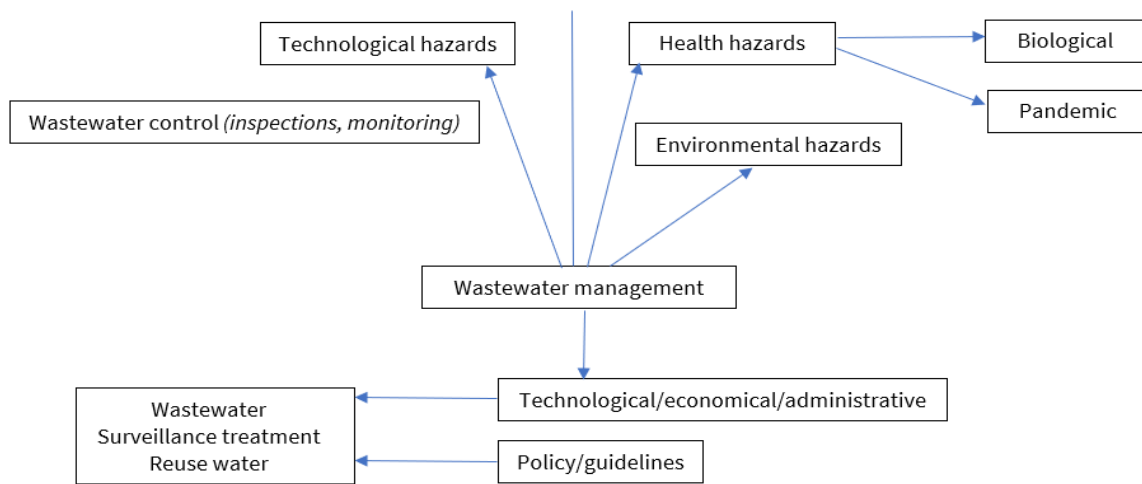


Figure 4. Wastewater management associated with hazards surveillance & policy (Adamopoulos, et al., 2022)

The world’s population is at risk from environmental exposure or public health exposure to limited waste management in the field of public health. Better assessment of the risks and impacts of exposure would allow for improvements in wastewater management and in the design of appropriate protective measures. Initial evaluations of epidemiology tools are difficult to control, methodological complications and uncertainties regarding the assessment of both exposure and health outcomes.

It is necessary pollution be monitored for recording and assessment tools provide recognition of the key role and management of the administrative control of environmental wastewater. Risk context of surveillance allows all the hazards to be recognized and investigated. Guidelines and holistic policy of public health services that determine contamination are important factors in providing control measures, improving efficiency and preventive measures. Individuals and public

health workers are exposed to various health risks regarding wastewater.

Figure 3 shows the flow sequence diagram of the action plan and the steps for the elements of systems tools for the organization of epidemiological surveillance.

Also, in **Figure 4** the check list tools for control measures in health care wastewaters infection in community SARS-CoV-2 epidemiological surveillance, and control in municipal wastewaters in Cyprus are presented.

CONCLUSIONS

It is vital the relationship between humanity and environment be reconsidered and more sustainable behavioural choices be encouraged. Scientific evidence pinpoints that the onset of new viral pathogens with a high

epidemic-pandemic potential is often the result of complex interactions among animals, individuals, and environment.

Existing techniques for detecting and inactivating pathogens in urban wastewater should be available, and thorough examinations of all possible transmission pathways associated with the sewage system should be carried out. Thus, the most effective techniques, WBE, and quantitative microbial risk assessment, for the surveillance of viruses in wastewater, are systematically analysed.

Other emerging impacts related to the COVID-19 pandemic, such as water-related impacts, need to be explored in depth. This includes strategies to identify these impacts and technologies to mitigate them and prevent further impacts on aquatic ecosystems, but also on human health. The most significant potential impacts of the COVID-19 pandemic on the path of wastewater to surface waters, as well as technologies can serve to address the most important threats and challenges.

Finally, current knowledge gaps and possible directions for further research and development have been identified. While the COVID-19 pandemic is a rapidly evolving situation, gathering existing knowledge about wastewater pathways in relation to environmental impacts and related technologies will certainly help mitigate the issue.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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