

Wastewater is a robust proxy for monitoring circulating SARS-CoV-2 variants

Genomic surveillance is important for pandemic preparedness. By deep sequencing of wastewater samples, we monitored the genetic dynamics of circulating SARS-CoV-2 virus variants in Austria from December 2020 to February 2022. We show how wastewater-based epidemiology enables robust quantification of viral spatiotemporal dynamics as well as the detection of emergent new variants.

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The problem

Pathogenomic surveillance during a pandemic is critical for monitoring developments in pathogen biology, from which suitable mitigation strategies can be anticipated. However, limitations of classic surveillance strategies include potential sampling biases and high operating costs. Such limitations do not pertain to the surveillance of pathogens discharged into the sewage downstream of a municipality, offering valuable complementary insights into infection epidemiology on a population level¹. Such approaches, for which the term wastewater-based epidemiology (WBE) has been coined, has experienced a strong impetus during the COVID-19 pandemic². In the meantime, WBE is widely used to detect viruses and track their quantitative trends in the catchment areas³. So far, the suitability of wastewater for gaining insights into the genetic assembly of circulating virus populations has not been systematically validated against case-based epidemiological data on a national scale⁴.

The solution

We collected wastewater samples from up to 94 Austrian wastewater treatment plants on a weekly basis from December 2020 to February 2022 and deep sequenced the transported SARS-CoV-2 RNA. We developed and applied bioinformatic tools to deduce from these data the spatiotemporal dynamics of SARS-CoV-2 variants across the country. Further, we delineated the reproductive advantage of variants of concern (VoCs) and explored the possibility of discerning novel emerging variants (Fig. 1). Comparison with comprehensive epidemiological surveillance data of more than 300,000 genotyped cases from patients resident in monitored catchments allowed us to validate the reliability of our approach, corroborating the idea that wastewater-based epidemiology can draw an accurate map of the whereabouts of virus variants for a whole country in a quantitative manner.

Our analysis enabled us to deduce from wastewater alone the diversity of pre-Alpha variants with distinct geographic patterns. The first VoCs, namely Alpha, became entrenched first in the eastern part of Austria and took around three months to dominate the whole country. By contrast, the takeover of subsequent VoCs, namely Delta and the Omicrons, was much more abrupt and emerged in the entire country almost simultaneously. Our data enabled the dissection of further

differences between the different VoC waves. For example, upon the introduction of Alpha and the Omicron subvariants BA.1 and BA.2, a pronounced selective sweep – that is, reduced genetic diversity due to the expansion of a relatively homogenous clade – was observed. This contrasted with the introduction of Delta, which lacked a pervasive selective sweep. Finally, we confirmed the growth advantage of Alpha and Omicron variants relative to their respective predecessors.

Future directions

Our long-term surveillance of SARS-CoV-2 in Austria demonstrated that WBE alone yields a time-resolved map of the genetic dynamics during a pandemic. Yet one task of pathogenomic surveillance is to link genetic pathogen information with clinical manifestation and the immunological status of patients. WBE is limited in that regard since the available data are anonymized to start with. Nonetheless, WBE provides invaluable population-level guidance on epidemiological developments, which complements case-based surveillance and provides information for optimal resource allocation. This notion can also be transferred to a global perspective. WBE provides a tool to shed light on blind spots of pathogen surveillance in places and communities with poor healthcare accessibility. If carefully set up and used in respectful and coequal terms, WBE of infectious diseases could make an important contribution to global safety.

To this end, several challenges must be overcome. Current WBE methods need to be expanded to other pathogens beyond SARS-CoV-2 and validated with case-based epidemiological data. Furthermore, current methods must be adapted and optimized to be applicable in locations without a centralized sewer infrastructure⁵. Finally, international sharing of wastewater-based pathogen sequencing data will be needed to unleash the full potential of WBE for global pathogen surveillance.

We are confident that our study will support initiatives already working in these directions, as well as encouraging intensified efforts to exploit such population-level surveillance approaches in the global fight against infectious diseases.

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EXPERT OPINION

|| This report represents a large dataset over a significant period of time, both of which are strengths. The time period studied captured the rise of Alpha, Delta and Omicron variants in Austria, and given the interest in genetic

sequencing of wastewater the work is quite timely. A significant strength of the work is the cross-validation of the wastewater surveillance with clinical surveillance (>300,000 genotyped cases).” **David Larsen, Syracuse University, New York, NY, USA**

FIGURE

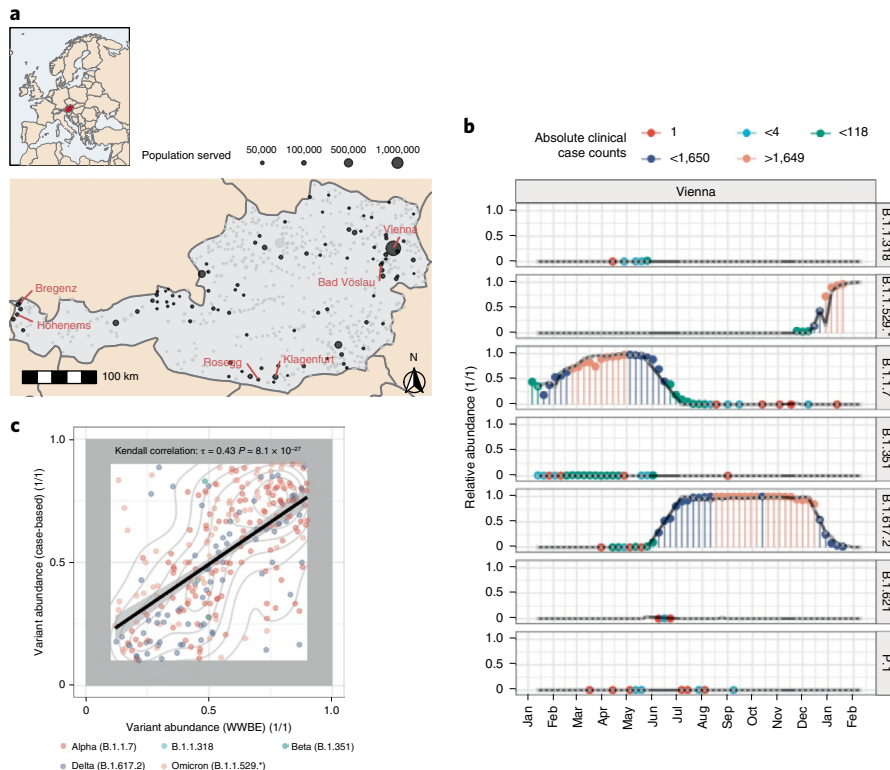


Fig. 1 | Wastewater-based epidemiology applied to SARS-CoV-2. **a**, Locations of Austrian wastewater water treatment plants (WWTPs, gray) and those included in the surveillance program (black). **b**, Comparison of relative variant frequencies between case-based surveillance (dots) and wastewater-based surveillance (black lines) for an example WWTP serving Vienna, Austria. Colors of dots indicate absolute number of detected cases in the catchment. **c**, Rank correlation between relative variant frequencies deduced from case-based and wastewater-based surveillance across all detected variants and all surveyed WWTPs. © 2022, Amman, F. et al.

BEHIND THE PAPER

In mid-2020, we started to sequence SARS-CoV-2 in wastewater samples out of scientific curiosity. We were surprised to reliably detect a respiratory virus in wastewater to an extent that allowed us to track single mutations. Yet at that time SARS-CoV-2 evolution was still slow and viral variants of epidemiological consequence had not been identified. This changed in winter 2020 with the emergence of the

then-called B.1.1.7 variant (now Alpha). Wastewater-based epidemiology became an added layer of pandemic surveillance in Austria and other countries. This led us to develop and refine the bioinformatic tools described in our study. The study exemplifies the power of collaborative teamwork across disciplines, opening exciting new avenues for national pandemic management and global pathogen surveillance. **A.B.**

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FROM THE EDITOR

|| The combination of a clever computational algorithm and extensive data collection from wastewater sampling allows robust quantification of SARS-CoV-2 variants as well as the detection of emerging variants. A high correlation between results from wastewater analysis and epidemiologic data from single-individual testing suggests that wastewater-based epidemiology is a promising strategy, especially for regions with limited individual testing capacities.” **Editorial Team, Nature Biotechnology**