Human Genomics

PERSPECTIVE

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Establishing a European wastewater pathogen monitoring network employing aviation samples: a proof of concept

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Abstract

Pathogens know no borders, and the COVID-19 pandemic highlighted the urgent need for comparable, globally accessible pathogen data. This paper proposes a European wastewater pathogen monitoring network using aircraft and airport samples as a proof of concept for an effective cross-national surveillance system. The study emphasizes the importance of genomic data collection from strategic sites to produce high-value data for disease surveillance and epidemiological analysis. The authors suggest establishing "Super Sites" in key locations, particularly major transportation hubs like airports, to serve as focal points for wastewater-based pathogen surveillance. The European Commission has identified over 20 candidate Super Sites and supports their integration into a Global Wastewater Sentinel System. In October 2023, the European Commission's Joint Research Centre (JRC) and Ginkgo Bioworks conducted an ad hoc exercise, collecting and analyzing wastewater samples from airports and aircraft across Europe. This exercise demonstrated the feasibility of coordinated sampling, centralized processing, and data sharing across different countries. Samples were collected from eight airports over two weeks, employing various methods for different types of wastewater, including samples from terminals and aircraft. Across airports, 96% of wastewater samples tested positive for SARS-CoV-2, with similar viral loads between aircraft and airport sewage, and multiple lineages were identified, including the EG.5 variant, which is consistent with the publicly reported variant data. The results underscore the potential of routine aircraft wastewater monitoring as an early warning system for emerging pathogens. The study also highlights the need for standardized protocols and real-time reporting systems and the importance of addressing ethical considerations in handling passenger data. By creating a network of Super Sites, and integrating cross-national wastewater surveillance data with passenger flight data, the European Union aims to strengthen global public health responses to future pandemics. Establishing this surveillance network is a crucial step towards a pan-European surveillance system for pathogens, providing a non-intrusive complement to existing systems that rely on individual testing. This system will significantly improve early detection capabilities, leading to more rapid and robust responses and ultimately enhancing global health security.

Keywords Wastewater surveillance, Pathogen monitoring, Aviation samples, Public health, Pandemic preparedness, European Commission, Super sites, Epidemiological models

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Background

The problem statement

Pathogens do not respect borders, and COVID-19 has added to the list of pathogens that demonstrate how rapidly infectious diseases can spread globally [22]. During the COVID-19 pandemic, the lack of comparable, globally available pathogen data limited our understanding of the current and future spread of the virus, as well as the effectiveness of interventions within and between nations [8, 21]. The main drivers of this gap were differing strategies for monitoring infection, varying methods of testing/sampling strategies, and limited data sharing. Consequently, countries' capacity to monitor the spread of the virus was highly variable, creating inequities in the response and hampering preparedness and mitigation strategies.

The vision of a global sentinel system

Multiple authors have identified the need for a globally coordinated approach to increased pandemic preparedness [6, 11, 19]. One proposed solution is a coordinated cross-national network of nodes providing pathogen detection and rapid insights into types, levels, and spread to assist in regional, national, and global prevention, detection, and response. These data will help epidemiologists drive crucial early insight and forecasts to enable well-guided responses and focus scarce resources to control the spread of diseases while minimizing disruption to travel and economic activity. Accomplishing this is complex and requires establishing new methods and sites for collection to complement existing surveillance systems, rapid and advanced analytics, and agreements and methods for data sharing. Solving these complexities will result in important benefits, including:

- **Real-time persistent monitoring of emerging pathogens at points of high human mobility**, like airports, seaports, and transit centers, provides real-time data on pathogen presence and spread. This enables health authorities to track infectious agents across regions and implement timely public health measures, such as targeted testing, non-pharmaceutical interventions (NPIs), and vaccination campaigns to contain outbreaks early.
- **Crucial information for decision-making through genomic surveillance by:** (a) identifying novel strains for molecular testing and primer design (e.g., S-gene dropouts), (b) supporting epidemiology to track new variant emergence and circulation duration, (c) conducting risk assessments to determine the spread and severity of pathogens and necessary response actions (e.g., masking and other NPIs, hospital preparation, resource allocation), and (d) providing data for vaccine and medical countermeasure development.
- **Enhance the capacity to conduct comparative analysis and share information across borders** by integrating data from these monitoring nodes into a centralized database. Collaboration can enable countries to learn from each other's experiences and strategies, leading to more effective and harmonized responses regionally and globally. For example, insights into how specific interventions have worked in one country or region could inform policy decisions elsewhere. This approach can help address inequities by providing resource-limited countries with access to crucial data and technological support, thereby bolstering global capacity to respond to infectious disease threats.
- **Cost-effective interventions:** Investing in infrastructure, technology, and coordination is minimal compared to the economic devastation of unchecked epidemics. Trillions of dollars were lost globally due to COVID-19 [10, 25]. Early detection and efficient responses can significantly reduce the financial impact of future outbreaks.

The value of airports in a global sentinel system

Almost all countries have, at some point during the COVID pandemic, introduced border control measures such as restricting inbound travel from specific locations, requiring evidence of a recent negative test or vaccination before departure, or mandating testing of passengers upon arrival [7, 18, 23]. Many of these requirements have been lifted due to global population immunity thanks to infection or vaccination programmes, reduced pathogenicity of circulating variants, and the need to limit further economic and social disruption. Before their removal, border screening programmes provided valuable data on the importation of SARS-CoV-2 [13] and helped map the international prevalence of new variants. With the reduction in large-scale screening of inbound passengers, a few groups have demonstrated the feasibility of monitoring aircraft and airport wastewater as a potentially non-intrusive and pervasive alternative for tracking pathogens entering a country or region, including in the UAE, Australia, France, the UK, and the US [4]. However, most of these research studies were limited in their scale and duration, preventing their use as a long-term surveillance tool.

A few programmes, on the other hand, have focused on demonstrating their feasibility at scale and over a longer period, including the US CDC's Traveler-based Genomic Surveillance (TGS) programme [17, 26] and other similarly modeled programmes [15]. These airport testing programmes have demonstrated the utility of airports as sentinel nodes to detect pathogens and have been shown to enhance the early detection of new pathogens and variants weeks before they spread among communities [3].

Moreover, pathogen monitoring and next-generation pathogen genomic sequencing enable countries to fill gaps in global surveillance by providing data about travelers who may not otherwise be tested because they are not symptomatic on arrival or not seeking health care, and about geographies with limited pathogen monitoring or reporting. These efforts provide additional information about how a pathogen is evolving over time, and can support public health authorities in determining how and when to deploy countermeasures and how those countermeasures are working.

The value of wastewater-based epidemiology at airports and in aircraft

Air travel biosurveillance of individual passengers can be highly valuable but expensive and potentially disruptive to airport operations. On the other hand, environmental surveillance can provide pathogen monitoring without impacting the flow of travelers, without the complexity of dealing with personal information, and at a lower cost. While environmental surveillance is not designed to replace clinical testing, it has been shown to be a helpful complement to fill in data gaps and reduce uncertainty and bias in clinical data. One form of environmental surveillance, aircraft wastewater monitoring, has demonstrated its ability to perform early detection and tracking of SARS-CoV-2 variants, including Omicron. For instance, genomes identified from aircraft lavatory wastewater in the United Kingdom, France, and the Netherlands were consistent with the prevalent Western European sequences at the time [17]. Following the classification of B.1.1.529 as a Variant of Concern (VOC), aircraft wastewater testing played a critical role in identifying Omicron's global spread. This was evidenced by the detection of Omicron in wastewater from a flight arriving in Darwin, Australia, shortly after the variant's initial report [2]. Further, SARS-CoV-2 RNA, suspected to be from the Omicron BA.1 variant, was found in wastewater from flights arriving in Marseille from Ethiopia [12]. Additionally, genome sequencing of wastewater at Frankfurt Airport revealed the presence of Omicron mutations before the first clinical case was reported among arriving passengers, underscoring the preemptive value of this surveillance method [1]. These findings collectively demonstrate that aircraft wastewater monitoring is a valuable additional approach for the timely detection of SARS-CoV-2 variants, providing important insights into their international transmission dynamics.

Implementing a proof of concept

Pathogen surveillance with genomic analysis should be implemented at key strategic sites in a coordinated fashion to enable these insights. This would enable the generation of comparable, high-value data that can be utilized for epidemiologic analysis, including modeling. The first step is identifying sampling sites and demonstrating the feasibility of coordinated sampling, analysis, and data sharing. We propose an approach for identifying optimal sampling sites at international airports and demonstrate the feasibility of coordinated sampling, laboratory analysis, and data sharing through a specific ad-hoc exercise conducted by the European Commission's Joint Research Centre (JRC), in collaboration with Ginkgo Bioworks and individual national public health agencies.

In October 2023, the JRC and Ginkgo partnered to conduct an ad-hoc exercise to collect and analyze wastewater samples from airports and aircraft across the European Union in a centrally coordinated and synchronized manner. The exercise was meant to be a proof of concept to demonstrate the feasibility of cross-European surveillance at scale, i.e., by working closely with national teams on the collection, by centralizing sample processing, and by aggregating data into one single repository and reporting system.

The objectives of the Proof of Concept were:

- **OB1:** Demonstrate the potential and limits of coordination for a Global Sentinel System across sample collection, sample processing, bioinformatics and data analysis, and data reporting.
- **OB2:** Demonstrate the comparability of results obtained through coordination.
- **OB3:** Compare and contrast results from airport WWTP with aircraft-extracted samples.
- **OB4:** Demonstrate the potential for public–private partnerships to deliver cost-effective environmental surveillance solutions for a Global Sentinel System.
- **OB5:** Gather learnings and insights to inform future iterations of an environmental surveillance-based Global Sentinel System

Selection of airports for the proof of concept

In designing the proof of concept of a Global Sentinel Wastewater System at airports, expanded from COVID-19 to a multi-pathogen approach, a number of factors are important to consider in order to assess feasibility, effectiveness, and scalability:

Selection of Pilot Airports: Select a diverse set of airports, including those with high passenger volumes, those in regions with varying health surveillance capabilities, and those serving as major international hubs. In the future, the selection should also consider airports from different continents to ensure global representation.

Infrastructure Setup: Install the necessary equipment for wastewater collection and testing at the selected airports. This included setting up on-site laboratories and establishing rapid transport systems to nearby testing facilities.

Multi-Pathogen Test Development: Develop or procure testing assays that can detect a range of pathogens of interest, including viruses, bacteria, and parasites known to be significant public health threats. These tests should be sensitive, specific, and capable of high-throughput screening.

Standardised Protocols: Create standardised protocols for sample collection, handling, and processing to ensure consistency and reliability of data across different airports.

Data Collection and Analysis: Collect wastewater samples over a defined period and analyze them for the presence of multiple pathogens. This data should be fed into a computational model to simulate the spread and detection of these pathogens.

Integration with Passenger Data: Where possible, integrate findings with anonymized passenger flight data to understand travel patterns and potential spread dynamics.

Real-time Reporting System: Establish a real-time reporting system that alerts public health authorities to the detection of any pathogen, allowing for rapid assessment and response.

Evaluation Metrics: Define clear metrics for evaluation, such as the time to detection, the rate of false positives/negatives, and the cost-effectiveness of the surveillance system.

Stakeholder Engagement: Engage with stakeholders, including airport authorities, public health officials, and international health organizations, to ensure the pilot study's findings are actionable.

Ethical Considerations: Address ethical considerations, including privacy concerns and the use of passenger data, ensuring compliance with international regulations and standards.

Pilot Study Review: At the end of the pilot period, conduct a thorough review of the system's performance, including an assessment of the detection rates for multiple pathogens, the effectiveness of the real-time reporting system, and the impact on public health responses.

Scalability Assessment: Evaluate the scalability of the system based on the pilot study results, considering the resources

Ultimately, based on the selection criteria, feasibility (including logistical considerations and time factors), and resource constraints, six sites were recruited for the proof of concept. These are shown in Fig. 1.

Methods

Sample collection and shipping

The sampling strategy used was simultaneous sampling at airport locations across Europe. Samples were collected from six airports across Europe: Amsterdam, Brussels, Frankfurt, Milan, and two additional de-identified airports in France and Italy (Fig. 1).

Over a two-week period between the 16th and 30th of October 2023, we collected a total of 23 wastewater samples. All collected samples contained 500 ml of wastewater and corresponded to three different modalities: aircraft wastewater, terminal wastewater, and airport treatment plants.

Depending on the sample type, different collection methods were employed (summarized in Table 1):

- **Aircraft wastewater samples** were collected directly from the aircraft's lavatory port using a purpose-built collection device. Collection was carried out during the regular serving of the aircraft. The aircraft wastewater samples were collected from flights originating from China, the United States of America, and the United Arab Emirates (UAE).
- **Terminal wastewater** samples were collected from the sewage system using a specific access point within the

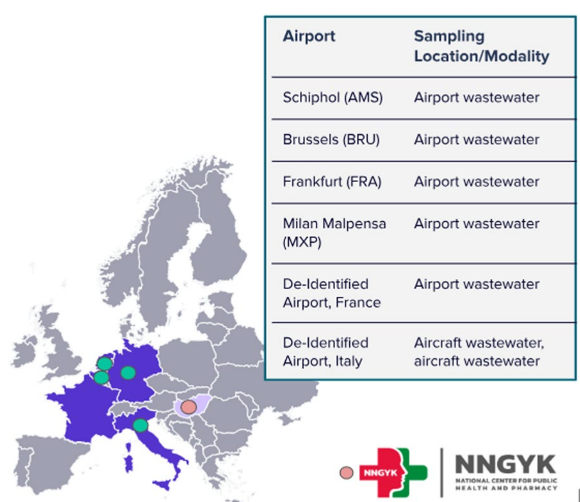

Fig. 1 Map of European airports participating in the ad-hoc exercise

Table 1 Sample collected at each airport during the ad-hoc exercise

Airport	Sample Count			
	Week of 15 Oct	Week of 22 Oct	Week of 30 Oct	Total
AMS	3	3	0	6
BRU	2	1	1	4
FRA	1	1	0	2
MXP	2	2	0	4
Anonymous Airport WWTP	0	0	1	1
Aircraft WW*	3	3	0	6
Total	**11**	**10**	**2**	**23**

Collection period: Oct 16—Oct 30. Samples were collected from airports, triturators, and aircraft

* Aircraft from China, US, UAE

airport terminal. The correct sampling point was determined in collaboration with the airport facility team to yield a sample representative of the entire terminal. An autosampler was installed at each collection point to create a 24-h composite sample.

- **Wastewater treatment plant samples** were collected from the airport wastewater treatment facility and were representative of the entire airport. An autosampler was installed in the plant to create a 24-h composite sample.

Collection and shipping materials

- Sample Form
- Chain of Custody Form
- Sample containers: six Falcon type 50 ml plastic tubes, properly labelled
- Ice (packs) to be immediately placed at -20°C (they must be ready the day of sample collection).
- Ziplock plastic bag for samples
- Polystyrene box.

Collection was performed using the materials provided centrally by the JRC team.

After collection, samples were packaged and shipped at 4° C to the pre-agreed laboratory, i.e., the National Center for Public Health and Pharmacy (NNGYK) of Hungary, where they underwent processing and analysis.

Sample processing

After a concentration of 25 or 50 mL using an in-house flat sheet membrane ultrafiltration method developed at NNGYK [20], samples were tested for SARS-CoV-2 by quantitative reverse transcription–polymerase chain reaction (RT-qPCR) of the N1 gene region. Positive samples underwent amplicon-based whole genome sequencing.

Bioinformatics pipeline and analysis

Our analysis pipeline was developed using Nextflow (v22.04.5), primarily leveraging nf-core/viralrecon with tailored parameter adjustments. In summary, raw sequencing reads were demultiplexed using BCL2fastq (Illumina), filtered for reads with Q30 ≥ 75% quality (fastqc v0.11.9), adaptor-trimmed (fastp v0.23.2), and human reads were removed (Kraken2 v2.1.2). The quality-filtered reads were then aligned to the SARS-CoV-2 reference genome, MN908947.3, using bowtie2 (v2.4.4). Primer sequences were excised (iVar v1.3.1), alignments were sorted and indexed (samtools v1.15.1), and duplicate reads were flagged to ensure high-quality alignments (picard v2.27.4 and samtools v1.15.1). We performed variant calling and annotation with freebayes (v1.3.6), SnpEff (v5.0), and SnpSift (v4.3) and generated a consensus genome based on quality > 100, depth > 10, and alternative observations/depth > 0.5 (bcftools v1.15.1 with INFO/AO / INFO/DP > 0.5 & QUAL > 100 & INFO/DP > 10). Given the complexity of wastewater samples, SARS-CoV-2 lineages were deconvoluted and frequencies estimated per sample using Freyja, while Pangolin (v4.3.1) was used to identify lineages from consensus sequences. Default settings were maintained for most tools except for fastp (–cut_front –cut_tail –trim_poly_x –cut_mean_quality 30 –qualified_quality_phred 30 –unqualified_percent_limit 40 –length_required 50), freebayes (–ploidy 1, –min-alternate-fraction tested at 0.1, 0.3, and 0.4, –min-coverage 10), and bcftools (INFO/AO / INFO/DP > 0.5 & QUAL > 100 & INFO/DP > 10).

Data reporting

Detailed results were reported to participating teams. Moreover, aggregate results were presented at the GLOWACON conference in Frankfurt (15–17th of November, 2023).

Note on privacy

Passenger privacy and anonymity were important considerations in the design of this pilot. Wastewater samples collected from airport terminals and aircraft are anonymous by design since they are representative of a large population, ranging from a few hundred in the case of an aircraft to several thousand from an airport. As it is not possible to connect a sample to a specific individual, the anonymity of the passengers is maintained.

Results

Across all airports, 96% of samples were positive for SARS-CoV-2 based on PCR testing. SARS-CoV-2 copy numbers were similar among samples (within 1 log). Numbers found in aircraft and airport wastewater were similar. With regard to SAR-Cov-2 variants, whole genome sequencing was conducted on 18 samples (86%), and all samples contained at least one SARS-CoV-2 lineage, with multiple SARS-CoV-2 lineages found in 17 samples (95%) (Fig. 2). We found that samples obtained from aircraft wastewater generally contained fewer lineages (~ 2–3) than samples obtained from airport sewage (4–6 lineages per sample). Of identified lineages, EG.5 was the most common lineage found across the airport locations (Fig. 3), which was also consistent with the publicly reported variant data [5]. (Fig. 4)

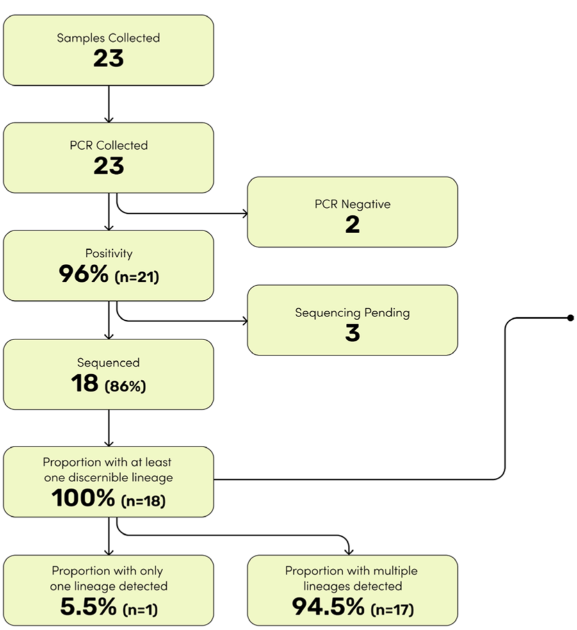

Fig. 2 Analysis Flow Chart. Collection period: Oct 16—Oct 30 NOTE: Three samples were not included in sequencing because they arrived after the cut-off: 2 samples from BRU (10/25 and 10/30) and De-Identified Airport, France (10/30)

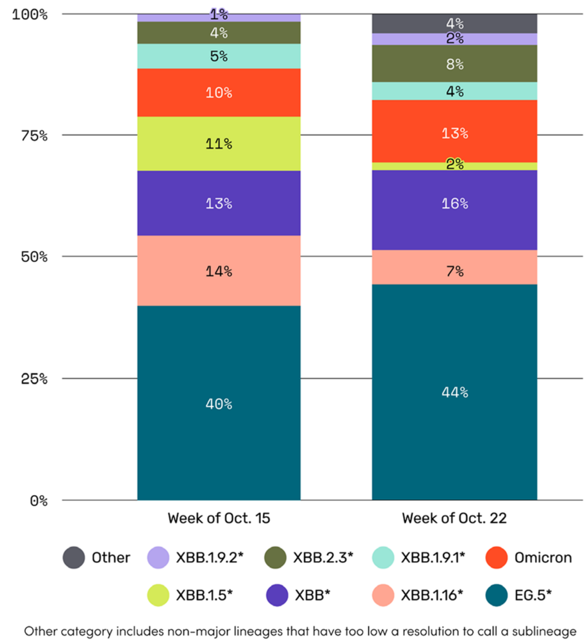

Fig. 3 Deconvoluted Lineage Proportion % of total lineage abundance by collection date. Collection period: Oct 16—Oct 30 NOTE: Three samples were not included in sequencing because they arrived after the cut-off: 2 samples from BRU (10/25 and 10/30) and De-Identified Airport, France (10/30)

Conclusions

As we introduced above, the Proof of Concept was designed with clear objectives that would enable us to evaluate the utility of the project and extract important lessons learned for future capability development.

- **OB1:** With regards to our first objective (*Demonstrate the potential—and limits—of coordination for a Global Sentinel System across sample collection, sample processing, bioinformatics, data analysis, and data reporting)*, the Proof of Concept demonstrated that it is, indeed, possible to coordinate a Sentinel System across multiple travel nodes, employing multiple sampling modalities. However, there were also limits to that coordination; for example, not all nodes contributed the same number of samples. Two nodes provided three samples per week, two nodes contributed two samples (one of which, the last sample from BRU, arrived outside the Week 2 time frame), one airport contributed one sample per week, and the final node provided one sample for the entire period (which also arrived outside the week two-time frame). The limitations on coordination, in all cases,

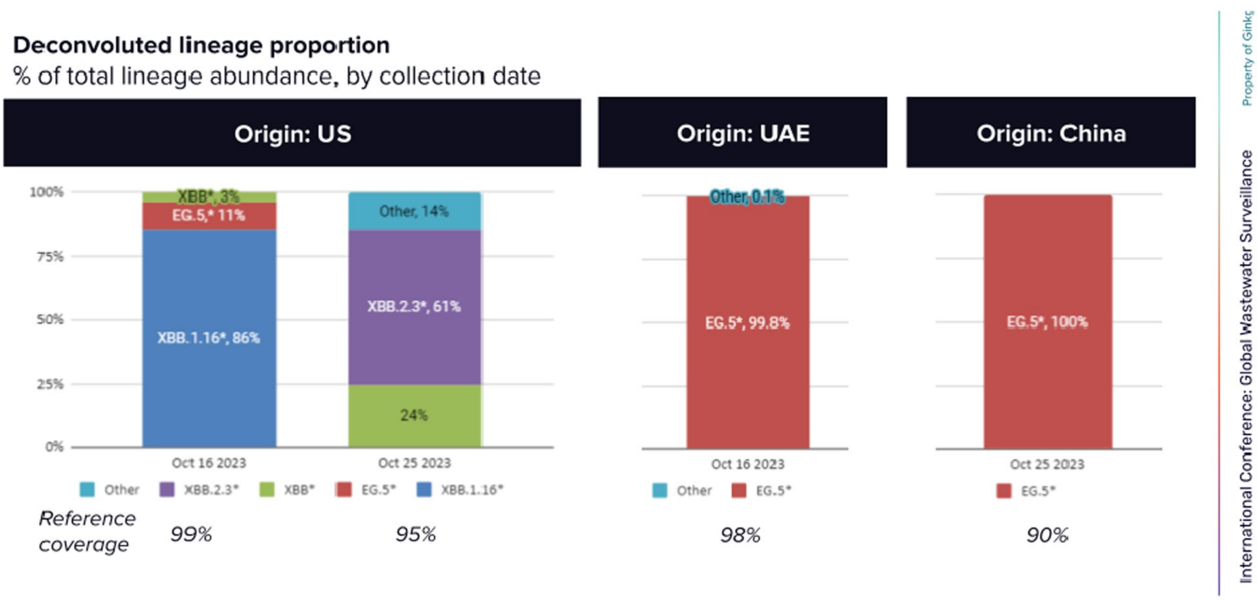

Fig. 4 Results for four aircraft wastewater samples from the US, UAE, and China

were due to problems and issues at the sampling and logistics end of the value chain, highlighting the complexity of operational environmental surveillance and the need for rigorous operations management.

- **OB2:** Our second objective was to *demonstrate the comparability of results obtained through coordination*. By transporting samples to a single laboratory within a specified time frame and applying the sample processing, testing, and bioinformatics techniques, we were able to show that the outputs showed remarkable consistency across countries and nodes. The sample sizes were too small to perform any robust statistical analysis, but the results indicate both spatial and temporal stability. However, given the low sample sizes and the lack of a truly long time series, we were unable to integrate these data with origin/destination information derived from passenger data. Without that ability, we cannot place the observed result stability in a broader epidemiological context.
- **OB3:** Our third objective was to *compare and contrast results from airport WWTP with aircraft-extracted samples*. Here, we demonstrated that there were more lineages associated with samples extracted from airport wastewater systems than from aircraft wastewater. As with all the results, the small sample sizes warrant caution, but such a finding suggests that the epidemiological profile of a passenger load in one aircraft is distinct from that of the "pooled" samples to be found in airport wastewater systems; that in turns suggests that there is indeed value in extracting samples using both modalities in order to fully model pathogen and viral transmission patterns.
- **OB4:** The fourth objective was to *demonstrate the potential for public–private partnerships to deliver cost-effective environmental surveillance solutions for a Global Sentinel System*. The Proof of Concept was undertaken by a range of partners, including public health organizations, academic institutions, and private companies. One of the key observations arising from this project is that all the partners contributed something unique and valuable to the effort, and yet no one partner has the full range of expertise and capabilities to undertake it themselves.
- As we noted above, one of the more challenging areas for any coordinated system is operations management, especially since working in an airport environment is associated with a wide range of complexities and dependencies. Flights are canceled, arrive late, and can be diverted; one type of aircraft may be substituted for another; ground handling crews are operationally stretched at the best of times, and any delay in sample extraction may pose an operational and financial risk to an airline. For these types of programmes to succeed, efficient and effective project management is a prerequisite. Nonetheless, we demonstrated that public–private partnerships are an effective tool to ensure harmonization and rapid implementation; we believe that they can also provide sustainability and scalability and the encouragement of continuous technological innovation. This exercise can serve as a successful example of pub-

lic–private partnerships that can be expanded and aligned with the emergence of a Global Sentinel System.

- **OB5:** Our final objective in implementing this Proof of Concept was to *gather learnings and insights to inform future iterations of an environmental surveillance-based Global Sentinel System*. Even with a relatively short duration (two weeks) and a limited number of samples (n = 23), the EU pilot project demonstrated the utility of airport surveillance to fill gaps and complement existing global surveillance efforts. Current surveillance that depends only on clinical testing tends to be spatially agnostic (we can observe differences among regions, but we do not have much explanation for them) and retrospective (we cannot say much with predictive power about the spreading patterns of individual pathogens and viruses). Adding in mobility data helps build predictive models, while including test data associated with mobility helps calibrate those models to any particular spread scenario.

In the future, computational tools will be used to support the selection of sampling sites, enabling longitudinal coordinated sampling, laboratory analysis, and data analysis. Establishing a regional European network could be a significant step toward creating a global coordination mechanism for monitoring pathogens, with a focus on airports and international passengers as sentinels. It will be important to link these efforts with existing and new surveillance efforts. Collaborations between academia, industry, and government have the potential to turn this vision into reality. This proof of concept demonstrates the feasibility of creating a coordinated system that can be scaled into a global sentinel system.

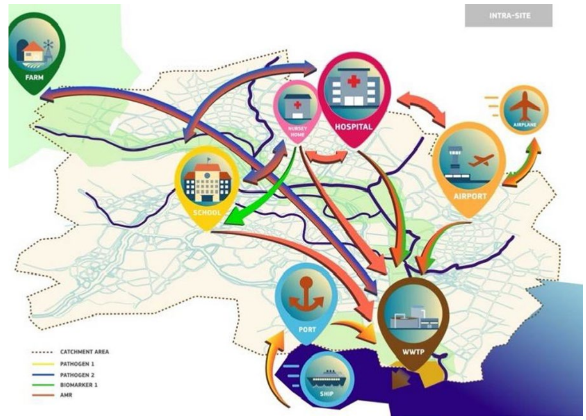

Fig. 5 Illustration of a European Super Site for the comparative wastewater analysis to support public health information

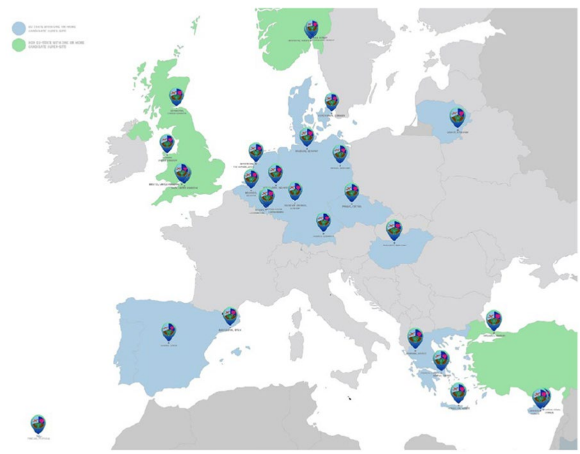

Fig. 6 Overview of Candidate Super Sites for a European System (status 26 July 2024)

Towards a global sentinel system

EU Super sites

Working toward this vision and within the context of developing a Global Wastewater Sentinel System, the European Commission is creating a network of "Super Sites," which are strategically chosen municipalities that serve as focal points for intensive wastewater-based surveillance. These sites are selected for their ability to provide epidemiological insights into the presence and spread of pathogens, including novel forms and antimicrobial resistances, by analyzing wastewater from key transportation hubs, such as airports, train stations, or ports, alongside municipal wastewater (Fig. 5). The European Commission has already identified more than 20 candidate Super Sites across the continent and continues to accept applications[1] (Fig. 6).

Super Sites act as advanced outposts within a Global Sentinel System to provide critical data on pathogen dynamics between travelers and the local population. To facilitate the integration into a European and Global Sentinel System, the European Union has established criteria to classify a location as a Super Site and a process to compare data among Super Sites and the use of wastewater as a pandemic preparedness, detection, and response tool.

[1] European Commission, EU4S-Call for Super-Sites, accessed on the 26 July 2024, https://ec.europa.eu/eusurvey/runner/EU4S_SuperSites

Key features of super sites

- **Strategic Location:** Super Sites are typically located at or near major transportation nodes, which are likely to be entry points for emerging pathogens due to international travel and trade. In the early phase, the focus will be on the aviation sector, but the European Commission anticipates an enlargement to other transportation hubs, such as ports or bus terminals. The same applies to specific facilities, such as hospitals, nursing homes, and or large-scale events, based on the targeted parameters.
- **Global Initiative Participation:** The European Super Sites are designed to be part of a specific effort, the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON), to contribute to a worldwide network aimed at early detection and response to public health threats.
- **Support:** The European Commission provides support to Super Sites, including assistance in liaising with the aviation sector and potentially other transportation hubs, to ensure the successful integration of these sites into the Global Sentinel System.
- **Integration with Aviation Sector:** Super Sites work closely with the aviation sector, with assistance from the European Commission, to ensure that samples from airfields or aircraft are included in the surveillance efforts.

Ethical and legal issues: It is important to balance carefully ethical and legal concerns, including the rights to privacy of passengers and private-sector entities and the interest to protect the general public.

Analytical considerations: The computational approaches of these models incorporate statistical sampling from parametric and non-parametric distributions, epidemiological compartment models to simulate person-to-person spread, and global metapopulation dynamics in which geographic spread explicitly occurs through the inclusion of passenger flows within the air travel network. The models are then used, based on the factors described below, to select which airports would optimize certain features of the network, such as the detection time of a pathogen and coverage of geographies of interest.

Factors that enabled enhanced data comparability between Super Sites.

- **Collaboration:** Each Super Site would collaborate with the European Commission by facilitating sampling and providing samples to a laboratory service for analysis. This collaboration may also extend to national wastewater surveillance programmes for parallel processing.
- **Sampling and Analysis:** Super Sites would regularly sample wastewater from transportation hubs and compare it with general municipal wastewater system samples. This dual-source sampling allows a more nuanced understanding of pathogen prevalence among travelers versus the resident population.
- **Sampling Frequency:** This programme envisages a sampling frequency of twice per month over 24 months, with some flexibility in scheduling to accommodate varying needs and conditions.
- **Service Providers:** The European Commission, through the Health Emergency Preparedness and Response (HERA) and the Joint Research Commission (JRC), contracts with laboratories and service providers to assess the selected Super Sites, ensuring standardised and high-quality analysis without additional costs to the sites. To this end, the European Commission has indeed provided significant resources and has initiated a public procurement procedure. This approach also secures a de facto standardisation and allows the Initiative to connect local, regional, and national surveillance programmes internationally.
- **Metadata Collection:** Super Sites would collect and provide additional information necessary for comprehensive surveillance, such as digital maps of sewer sheds, contacts at transportation hubs, and other relevant data on public health statistics, population dynamics, demography, and mobility.

GLOWACON aviation surveillance programme

The study led to the implementation of the GLOWACON Aviation Surveillance Programme with the ultimate objective of creating an international sentinel system for the early detection, prevention, and real-time monitoring of epidemic threats and outbreaks. The launch took place on March 19th and 20th in Brussels, with the involvement of over 300 key collaborators such as the Bill and Melinda Gates Foundation, the World Health Organization (WHO), the Africa Centres for Disease Control and Prevention (CDC), the United States CDC, amongst others.[2] As part of this effort, several policies and supporting activities were defined and aligned at national, regional, and global level. Table 2 gives an overview of the most prominent ones.

[2] Health Emergency Preparedness and Response Authority, "Launching GLOWACON: A global initiative for wastewater surveillance for public health", 21 March 2024, link.

Table 2 Overview on GLOWACON-induced and -related, policy-relevant impact

Initiative	Description	Impact	Impact category	Reference
WHO Activities	WHO Wastewater and environmental surveillance for one or more pathogens Guidance on prioritization, implementation and integration	Global Guidance on the selection of pathogens for inclusion in	Guidance	https://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/sanitation-safety/wastewater
UNEP Activities	As a Member of GLOWACON, UNEP contributes to the global efforts around wastewater surveillance for environment and public health In close collaboration with the African CDC, the European Commission and GLOWACON, UNEP promotes the rollout of WES in Africa and seeks to build the necessary capacities	Reinforcing infrastructure and know-how on WES in Africa. Linking WES to the implementation of the SDGs	Capacity Building	https://www.unep.org/topics/ocean-seas-and-coasts/ecosystem-degradation-pollution/wastewater/wastewater-surveillance
GLOWACON Regional Conferences and Funders' Forum	The Regional Conferences in Asia and Africa address regional perspectives on wastewater and environmental surveillance for epidemics and pandemics, explore bioinformatics, modeling, and data sharing, discuss priorities, existing capacities, and regional needs. The respective Funders Forum seeks to facilitate the co-development of collaborative projects	Regional and global coordination	Capacity building	https://wastewater-observatory.jrc.ec.europa.eu/#/content/glowacon-events
EU4Health – Joint Action EU WISH	The EU-WISH Joint Action seeks to improve national public health wastewater surveillance capacities by strengthening knowledge exchange and sharing best practices based on scientific evidence.	Co-funded Project for the institutionalisation of WES in the EU	Capacity building	https://www.eu-wish.eu/
EU4Health Framework contracts to measure pathogens and pollutants in untreated wastewater	32 Mio € Framework Contract of the European Health and Digital Executive Agency (HaDEA)	Instrument for the operation of the European Sentinel System as part of the GLOWACON network	Public Procurement	https://hadea.ec.europa.eu/news/eu4health-call-tenders-framework-contracts-measure-pathogens-and-pollutants-untreated-wastewater-2024-06-24_en
European Urban Wastewater Treatment Directive 2024/3019	EU Member States must coordinate public health and wastewater authorities to monitor health parameters, allocate roles, determine sampling, and communicate results, especially during health emergencies, with required antimicrobial resistance monitoring for large areas	Common framework for the management of urban wastewaters in the European Union. Article 17 establish WES a part the directives implementation strategy in all 27 EU Member States	Law	https://eur-lex.europa.eu/eli/dir/2024/3019/oj/eng

Table 2 (continued)

Initiative	Description	Impact	Impact category	Reference
The EU Wastewater Observatory for Public Health	The EU Wastewater Observatory for Public Health, set up by the European Commission, facilitates the sharing of wastewater monitoring data through a digital platform, a sentinel system for transportation hubs, and a developing academy, all aimed at enhancing global pandemic preparedness	Science-to-policy influence on European policies in relationship to water and health	Knowledge management	https://wastewater-observatory.jrc.ec.europa.eu/

As part of GLOWACON, public health agencies, governments, and other stakeholders are carefully defining the frameworks to handle passenger data, privacy concerns, national interest, and data ownership. The preliminary viewpoint is that:

- Passenger data will not be needed for this exercise, given that wastewater testing is an anonymous sample by construct. Hence, there will be no need nor desire to track individual passenger information on a specific flight. Moreover, flight data will only be used to understand the origin of a specific signal and conduct epidemiological analysis on the global circulation of pathogens.
- As the countries are the entities sharing data into the GLOWACON framework, they would fully control what data is released and when. This means that, although committing to sharing data with the full network, countries will have the right to review the data first and trigger the necessary countermeasures as quickly as possible.
- Data ownership would clearly rest with the countries, although data transfer agreements will be put in place in order to allow the seamless flow of information within the network.

From super sites to designing an optimized global sentinel system using advanced computational modeling

The European Super Site network is being supported by the European Commission, but how would it align with a truly Global Sentinel System? Several groups have developed data-driven approaches to designing a Global Sentinel System. Using slightly different models, these groups were able to define a list of priority airports that maximizes the chances of detecting a new pathogen early. Three models worthy of note are: the GLEAM model, led by Prof. Alessandro Vespignani at Northeastern University [24], the model created by Prof. Shihui Jin and colleagues from the National University of Singapore [9], and Ginkgo Bioworks' Epidemiological Modeling group model [14]. Factors that have been considered that are common to these models include:

- **Early Detection**: The probability of detecting SARS-CoV-2 or other pathogens through aircraft wastewater surveillance increases exponentially in the early phase of a pandemic.
- **Global Network Effectiveness:** The likelihood of early detection is much higher if major airports in Asia, Europe, and North America collaborate in wastewater surveillance.
- **Influencing Factors** contributing to early detection include a sampling from a high proportion of inbound flights, a small population size at the outbreak's epicenter relative to travel volume, and a large number of outbound travelers from the outbreak's epicenter.
- **Modeling and Probability Estimates:** Probability models should be used to estimate the likelihood of virus detection from aircraft wastewater at various airports, considering different sampling strategies and hypothetical scenarios.
- **Strategic Airport Selection:** Airports with more frequent and direct flights from the epicenter have a higher chance of early detection.
- **Sampling Proportion:** Even partial sampling of inbound planes could be effective for surveillance.
- **Country context:** Some models, such as Ginkgo's, adopt a risk-based approach to sampling design, taking a variety of country contextual factors into account, including: the risk of disease emergence/spillover for pathogens of interest, a country's capacity for infectious disease surveillance and reporting, media bias in favor of incumbent governments, which prior research has found to be associated with infectious disease underreporting [16], and the presence of BSL3 and BLS4 facilities.

A comparison of their findings is shown in Table 3.

It is important to note that sampling optimization is not a static problem. Once a set of Super Sites has been established, each node within the network will have a finite capacity for sample collection and processing. The specific capacity limit will be specific to each site, and can be increased or temporarily "surged" with additional resources (including human resources, financing, equipment, and laboratory systems). Nonetheless, within a fixed capacity level, sampling and surveillance resources can be optimized both within each node and across the network in a variety of ways, including sampling high-risk flights traveling from geographies experiencing spillover events or localized epidemics of pathogens with pandemic potential or adjusting flight selection within each node to construct a representative sample of geographies of interest. The specific optimization plan will be dictated by surveillance needs and public health priorities across participating countries and adjusted dynamically using computational models. This approach can help derive the greatest value for money and public health benefits.

These findings indicate that routine aircraft wastewater monitoring could feasibly serve as an approach for the early identification and tracking of emerging pathogens, particularly when implemented through a

Table 3 Illustrative list of airports being part of an ideal wastewater surveillance network (overlapping airports are bolded)

Region	GLEAM (Northeastern University)	National University of Singapore	Ginkgo Bioworks
Europe	**LHR (UK)** **CDG (Paris)**	AMS (Netherlands) **CDG (France)** FRA (Germany) **LHR (UK)** MAD (Spain)	AMS (Netherlands) BCN (Spain) **CDG (France)** DUS (Germany) FRA (Germany) **LHR (UK)** LIS (Portugal) MAD (Spain) VKO (Russia)
Americas	JFK (USA) GRU (Brazil) MIA (USA) YYZ (Canada)	ATL (US) JFK (US) LAX (US) ORD (US)	CUN (Mexico) DFW (USA) LAX (USA) YYZ (Canada)
Sub-Saharan Africa	ADD (Ethiopia) JNB (South Africa)		
Middle East and North Africa (MENA)	ALG (Algeria) **DXB (UAE)** DOH (Qatar) **IST (Turkey)** JED (Saudi Arabia)	DOH (Qatar) **DXB (UAE)** **IST (Turkey)**	AYT (Turkey) **DXB (UAE)** **IST (Turkey)** JED (Saudi Arabia) KWI (Kuwait)
Asia	**ICN (South Korea)** BKK (Thailand) DEL (India) **SIN (Singapore)** HKG (Hong Kong) KUL (Malaysia)	BKK (Thailand) HKG (China) **ICN (South Korea)** NRT (Japan) PEK (China) PVG (China) **SIN (Singapore)** TPE (China)	**ICN (South Korea)** **SIN (Singapore)**
Oceania	SYD (Australia)		

global surveillance network of major airports. Such a system could significantly enhance global public health responses to future pandemics by providing early warnings and facilitating rapid containment measures.

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Author contribution
Robert Morfino wrote the main manuscript text. Bernd Manfred Gawlik, Simona Tavazzi, Andrew Engeli, and Jasmine Grimsley contributed to portions of the manuscript. Amy Schierhorn provided data and tables. Andrew Franklin, Marta Vargha, Nita K. Mahdav and Mitchell Wolfe reviewed the manuscript and provided edits.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Conflict of interest
The authors declare no competing interests.

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