

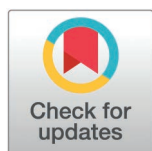
RESEARCH ARTICLE

Multimodal genomic surveillance for respiratory pathogens at four U.S. international airports: A comparison of air, wastewater, clinical, and national surveillance data

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Data availability statement: All raw sequencing data are available in the NCBI Sequence Read Archive (SRA) under the CDC Traveler-Based Genomic Surveillance Program

Abstract

Early detection of outbreaks and emerging pathogens is critical for public health and global biosecurity. Airports, as major international travel hubs with dense, enclosed populations, are high-risk settings for disease transmission and potential pathogen introduction. The U.S. Centers for Disease Control and Prevention, in collaboration with Ginkgo Biosecurity and the University of Wisconsin–Madison, implemented air monitoring for pathogen surveillance in congregate areas at four U.S. international airports. From October 2023 to August 2024, SARS-CoV-2 was detected by PCR in 98.3% of air samples and influenza A in 17.2%. Influenza A positivity in air samples correlated with aviation wastewater ($r=0.48$), traveler nasal swab positivity ($r=0.73$), and national clinical surveillance ($r=0.86$), whereas SARS-CoV-2 measurements did not correlate significantly across these modalities. Targeted amplicon sequencing of SARS-CoV-2 from air samples identified contemporaneous lineages also detected in wastewater collected from the same airports. Targeted enrichment sequencing detected 30 viral species and recovered high-quality genomes for SARS-CoV-2, influenza, bocavirus, and seasonal coronaviruses. Together, these findings demonstrate that air sampling can complement aviation wastewater surveillance at ports of entry, although performance and concordance vary by pathogen and sample type.

Introduction

Epidemic and pandemic preparedness relies heavily on robust and adaptive surveillance systems capable of early pathogen detection. Global travel hubs, particularly

BioProject PRJNA989177. The datasets underlying the analyses presented in this manuscript are provided as [Supporting information](#) files S1–S6. Code used to generate the figures is available at <https://github.com/concentric-byginkgo/TGS-AirMonitoring-Supplemental>. Requests for data not available through these public sources may be directed to CDC through the Travelers' Health contact page (<https://wwwnc.cdc.gov/travel/page/contact>) or CDC-INFO (800-CDC-INFO), which serve as non-author institutional points of contact for this surveillance activity. Data will be maintained by CDC in accordance with applicable federal records-retention and public-health data-governance requirements.

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Competing interests: The authors have read the journal's policy and have the following competing interests: D.G., B.B.S., A.P.R., V.J.M., C.W.P., X.Q., and T.W.S.A. were employed by Ginkgo Bioworks at the time of the study and hold or received equity in Ginkgo Bioworks. D.H.O. and S.L.O. are managing partners of Pathogenuity LLC, a consultancy that advises on environmental monitoring issues. D.H.O. and S.L.O. therefore have a financial interest in a company operating in a related field. This does not alter our adherence to PLOS ONE policies on sharing data and materials. There are no patents, products in development or marketed products associated with this research to declare.

international airports, are critical interfaces for the introduction and spread of emerging and re-emerging pathogens due to their dense, transient populations and high interconnectedness [1–4]. Detecting pathogens and identifying new variants in airports is especially important, as illustrated during the COVID-19 pandemic, which underscored the urgent need for near real-time genomic surveillance strategies [5]. While testing of nasal swabs from arriving travelers provides one modality for monitoring, the process can be labor-intensive and relies on traveler willingness to participate.

Environmental surveillance has emerged as a non-invasive approach to characterize pathogens circulating in communities. Wastewater surveillance, including community and aviation wastewater, has demonstrated utility in tracking both the prevalence and genetic diversity of viruses such as SARS-CoV-2 [6–8]. Air monitoring serves as a complementary public health surveillance tool, enabling the collection of pathogenic genetic material shed by individuals. This approach is particularly effective for capturing respiratory pathogens from diverse populations, including asymptomatic or pre-symptomatic individuals who might not otherwise engage with clinical testing or contribute to accessible wastewater systems. Studies have shown the effectiveness of air sampling in multiple congregate settings. In hospitals, airborne pathogen detection has correlated strongly with clinical data [9], and airborne pathogen genetic material has also been detected in other communal spaces such as schools [10–12]. As a scalable and cost-effective surveillance option, non-invasive air monitoring is particularly relevant for high-traffic environments, such as international airports, that serve as gateways into communities.

While PCR-based detection confirms the presence of pathogen genetic material, genomic sequencing is essential for identifying specific lineages, tracking variant evolution, and informing the development of diagnostics and therapeutics [13–16]. However, obtaining high-quality genomic data from air samples presents challenges due to low biomass and nucleic acid degradation, especially for RNA viruses [17]. Advanced sequencing methodologies are therefore crucial. Targeted amplicon sequencing offers high sensitivity but is limited to a narrow range of pathogens, while metagenomic approaches provide an agnostic view but often lack sensitivity for low-abundance targets. Targeted enrichment sequencing represents a promising strategy to improve both breadth and sensitivity by enriching viral nucleic acids prior to sequencing [9,18].

In this study, air monitoring surveillance was implemented in congregate areas of four major U.S. international airports to assess the feasibility of detection of SARS-CoV-2 and Influenza A virus genetic material in an international airport setting; evaluate the effectiveness of sequencing methods for viral genome characterization from these air samples; and determine the complementary value of air monitoring within existing pathogen surveillance frameworks. This study explores the development of air monitoring as a proactive biosecurity system, to enhance our capacity for early threat identification at the critical intersection of global travel and community health.

Methods

ThermoFisher AerosolSense active air samplers were placed in congregate areas adjacent to customs and immigration inspection areas and within proximity of several hundred arriving international passengers at San Francisco International Airport, California (SFO); Newark Liberty International Airport, New Jersey (EWR); Washington Dulles International Airport, Virginia (IAD); and Dallas/Fort Worth International Airport, Texas (DFW). Samplers were placed on tables 28–30 inches above the ground. The ThermoFisher AerosolSense has a built-in pump, that operates at 200 liters per minute, and a proprietary sample collection cartridge. Each ThermoFisher AerosolSense unit collected samples for 10 hours per day. ThermoFisher AerosolSense cartridges containing polyurethane foam substrates were removed from each air sampling machine after approximately 20 hours of use Monday through Thursday and after 30 hours of use Friday through Sunday.

Samples were collected from October 18, 2023–August 27, 2024, at SFO, EWR, and IAD, and from February 7–August 14, 2024, at DFW. At SFO, a second sampler was added on June 3, 2024 (denoted by the orange star in [Fig 1](#)), and both instruments operated until study end. Cartridges were packaged and shipped at room temperature to UW-Madison for extraction and qRT-PCR analyses. All results were linked to airport and collection date via unique barcodes. Each substrate was submerged in 500 μ L of 1X phosphate-buffered saline (PBS) and vortexed. RNA was extracted from 300 μ L of eluate using a Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega). Molecular detection of SARS-CoV-2, influenza A, and RNase P is described in [S1 Text](#). From October 18, 2023–March 12, 2024, qRT-PCR reactions were performed on a LightCycler 480 System (Roche) using a TaqMan Fast Virus 1-Step Kit (ThermoFisher) with CDC-modified assays targeting the SARS-CoV-2 nucleocapsid gene (N1, N2) and the influenza A M gene, in duplicate [19]. Pathogen results were categorized as positive if both Ct values were <40, inconclusive if only one Ct <40, and negative if neither Ct <40. This categorization applied to both SARS-CoV-2 and influenza A assays. On March 12, 2024, the thermocycler was changed to accommodate a multiplex assay (denoted by the red star in [Fig 1](#)). For each cartridge, a single multiplex assay measuring the SARS-CoV-2 N1 and influenza A M targets were performed. The human RNase P gene served as an internal control; samples negative for RNase P were excluded from analysis.

For targeted SARS-CoV-2 sequencing, purified total nucleic acid (TNA) was amplified by multiplex RT-PCR (NEB Luna-Script Multiplex One-Step RT-PCR) using the ARTIC primer set (IDT ARTIC V5.3.2 NCOV-2019 Panel) [20]. Amplicons were pooled, and libraries prepared with a modified Illumina DNA Prep protocol. Libraries were sequenced on an Illumina NovaSeq 6000, targeting ~1.5 million paired-end 150 bp reads per sample.

For targeted enrichment sequencing, samples were selected to represent temporal and geographic diversity across airports and did not overlap with samples used for SARS-CoV-2 amplicon sequencing. (EWR $n = 32$, IAD $n = 32$, SFO $n = 34$) The input to library prep was either total nucleic acid (TNA) or clean and concentrated TNA (Zymo Research, Irvine, CA). Extracts were prepared for enrichment using the Illumina RNA for Enrichment Sample Preparation Kit with the Respiratory Virus Oligo Panel, following the manufacturer's protocol (Illumina, San Diego, CA). Libraries were validated for size and adapter dimer removal on an Agilent TapeStation 4200 D1000 High Sensitivity assay, quantified and normalized using the dsDNA High Sensitivity Assay on a Qubit 3.0 (Life Technologies, Carlsbad, CA), denatured/diluted, and pooled per manufacturer's instructions. Pooled libraries were sequenced on the Illumina NovaSeq 6000 (v1.5 chemistry) to a target depth of ~7 million paired-end 150 bp reads per sample. Bioinformatics pipelines and analysis details are provided in [S1 Text](#).

For aviation wastewater and traveler nasal swabs, data were generated from samples collected and processed as part of the [Traveler-based Genomic Surveillance \(TGS\) program](#) from October 18th, 2023–August 27th, 2024. Aviation wastewater was collected from arriving international aircraft at IAD ($N = 330$), and SFO wastewater was collected from triturators ($N = 161$). Triturators are collection points where waste from multiple flights is discharged and homogenized before being combined with airport terminal wastewater. Travelers volunteered to self-collect anterior nasal swabs on arrival at IAD ($N = 16,004$) and SFO ($N = 30,182$). Wastewater samples were concentrated with Nanotrap Microbiome Particles and

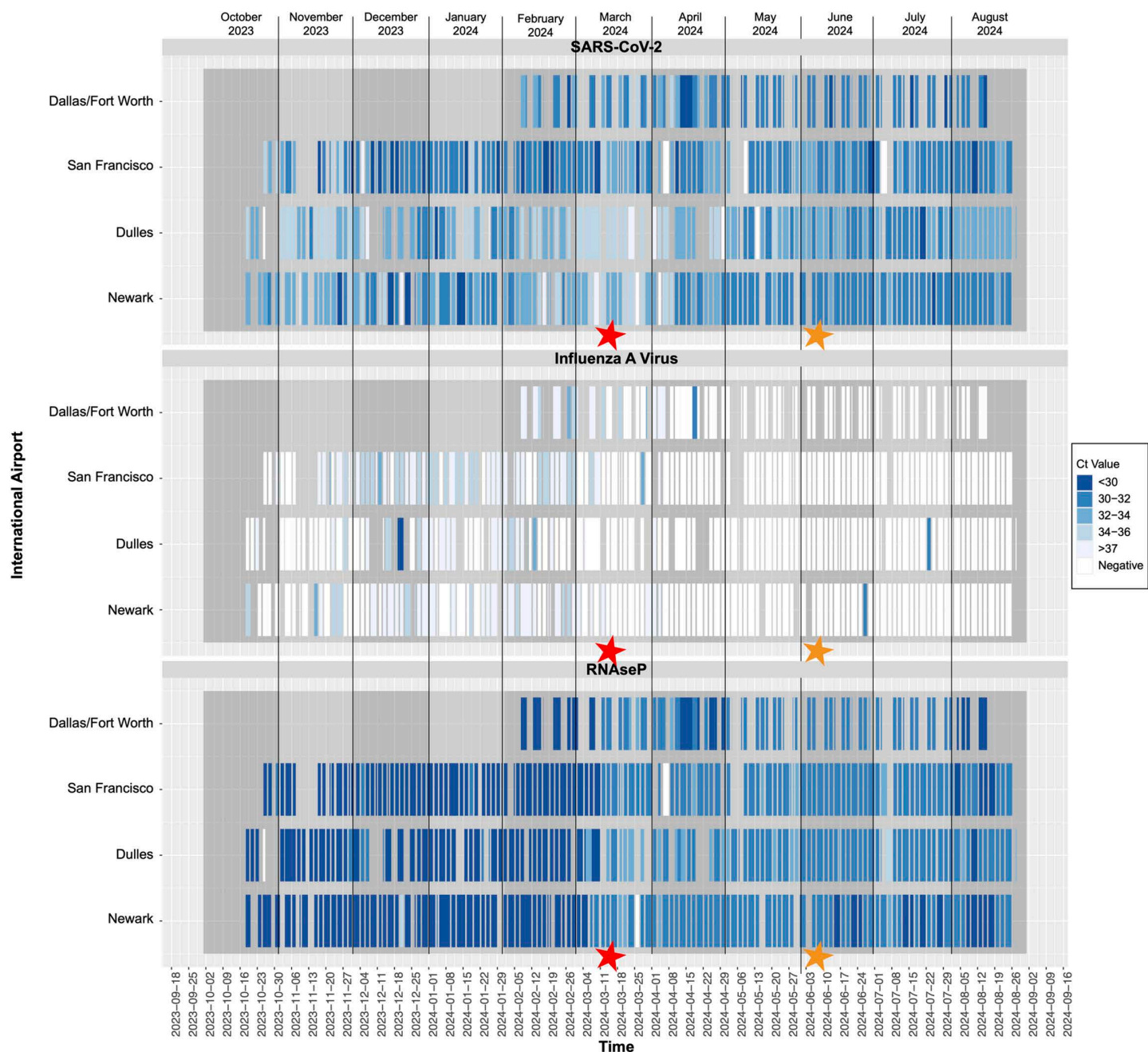


Fig 1. PCR detection from airport air samples. Positive qRT-PCR detections and Ct values of Influenza A, SARS-CoV-2, and RNase P controls captured by ambient air monitoring surveillance from congregate areas in 4 U.S. international airports, October 2023–August 2024. The red star indicates a change of assay; quantitative results before and after this point cannot be directly compared. The orange star indicates when a second sampler was added to the San Francisco airport.

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extracted using a MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher). Extracts were tested by digital PCR for SARS-CoV-2 and influenza A/B (Qiagen), and SARS-CoV-2 sequencing was performed as described above. The underlying aviation wastewater PCR data are provided in [S6 Data](#).

Upon laboratory receipt, nasal swab samples were heat-inactivated at 70°C for 30 minutes, and total nucleic acids extracted using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Thermo Fisher). Extracts were tested by qPCR using the TrueMark SARS-CoV-2/Flu A/Flu B/RSV Select Panel on a QuantStudio 5 qPCR instrument (Thermo Fisher). SARS-CoV-2 sequencing was performed as described above. The underlying traveler nasal swab PCR data are provided in [S5 Data](#).

Pearson correlation analyses were performed to evaluate associations between airport air surveillance results and comparator surveillance modalities (aviation wastewater, traveler nasal swab surveillance, and national clinical surveillance data). Correlation coefficients (r) and corresponding p -values were calculated for each comparison.

This activity was reviewed by CDC, deemed not human subjects research, and conducted in accordance with applicable federal law and CDC policy (45 CFR part 46; 21 CFR part 56).

Results

During October 18, 2023–August 27, 2024, 463 air monitoring samples were collected and tested by qRT-PCR from four international airports ([Fig 1](#)). The RNase P control was positive for 460 of 463 samples (99.4%). Among these 460 RNaseP+ samples, SARS-CoV-2 was detected in 452 (98.3%). Of the remaining eight samples, five were negative and three were inconclusive. When stratified by airport, positive SARS-CoV-2 detections were: SFO: 137 (97.9%), EWR: 124 (98.4%), IAD: 118 (97.5%), and DFW: 73 (100%) ([Fig 1](#); [S1 Data](#)).

Among the 460 RNaseP+ samples, 79 were positive for influenza A (17.2%), while 327 were negative and 54 were inconclusive. By airport, influenza A detections were: SFO: 36 (25.7%), EWR: 20 (15.9%), IAD: 10 (8.3%), and DFW: 13 (17.8%). Positive detections of influenza A occurred weekly between January 1 and March 18, 2024, then occurred sporadically through April 22, 2024, after which no further positives were observed ([Fig 1](#)).

To compare SARS-CoV-2 and influenza A trends across modalities, data from two airports (SFO and IAD) with overlapping aviation wastewater, airport air, and traveler nasal swab collections were analyzed. Publicly available U.S. clinical respiratory specimen data (<https://www.cdc.gov/nrevss/php/dashboard/index.html>) were also included for comparison. Airport air and aviation wastewater had higher weekly positivity rates for SARS-CoV-2 and influenza A than traveler nasal swabs and national clinical samples, reflecting expected differences between population-level (air, wastewater) and individual-level (nasal swab) sampling ([Fig 2A](#)). SARS-CoV-2 PCR values from air sampling did not correlate significantly with aviation wastewater ([Fig 2B](#); $r = -0.02$, $p = 0.88$), traveler nasal swab positivity ([Fig 2C](#); $r = -0.17$, $p = 0.24$), or national surveillance positivity ([Fig 2D](#); $r = 0.22$, $p = 0.13$). Influenza A positivity from air sampling correlated significantly with aviation wastewater ([Fig 2E](#); $r = 0.48$, $p = 0.002$), traveler nasal swabs ([Fig 2F](#); $r = 0.73$, $p < 0.001$), and national surveillance ([Fig 2G](#); $r = 0.86$, $p < 0.001$) ([Table 1](#); [S2](#) and [S3 Data](#)).

Of the 460 RNaseP+ air samples, 338 were tested for SARS-CoV-2 sequences; 216 (64%) yielded high-quality genomes ($\geq 70\%$ genome coverage), and 167 (77%) of those contained multiple lineages including BA.2.86, JN.1, JN.1.16, JN.1.67, KP.1, KP.2, KP.3, KP.3.1.1, and XDV. SARS-CoV-2 variant trends in air and aviation wastewater samples were similar at both airports ([Fig 3A](#), [3B](#)). Between January 29 and August 26, 2024, both sample types showed a decline in Omicron JN.1 and an increase in KP.2/KP.3, consistent with contemporaneous national trends [21]. KP.x variants were detected in aviation wastewater earlier than air samples (42 days earlier for KP.2, 41 days earlier for KP.3).

Targeted enrichment sequencing with hybrid capture enrichment for respiratory virus genomes was performed on a total of 98 air samples, split into two sequencing runs ([Fig 4](#)), using samples that did not overlap with those used for SARS-CoV-2 amplicon sequencing. In the first run ($N = 52$), a mean of 0.04% (95% CI [0.01%, 0.06%]) of total reads per sample mapped to virus targets. After incorporating a TNA concentration step prior to library prep, the second run ($N = 46$) achieved a mean of 11.8% (95% CI: 7.04–16.5%) of reads mapping to viral genomes. Across all samples, reads from 30 viral species were detected ([S4 Data](#)). The most frequent were SARS-CoV-2 (100% of samples; mean RPKM 32.6, 95% CI: 3.6–61.5), seasonal coronavirus HCoV-229E (99% of samples; mean RPKM 7.6, 95% CI: 0–15.9), and influenza

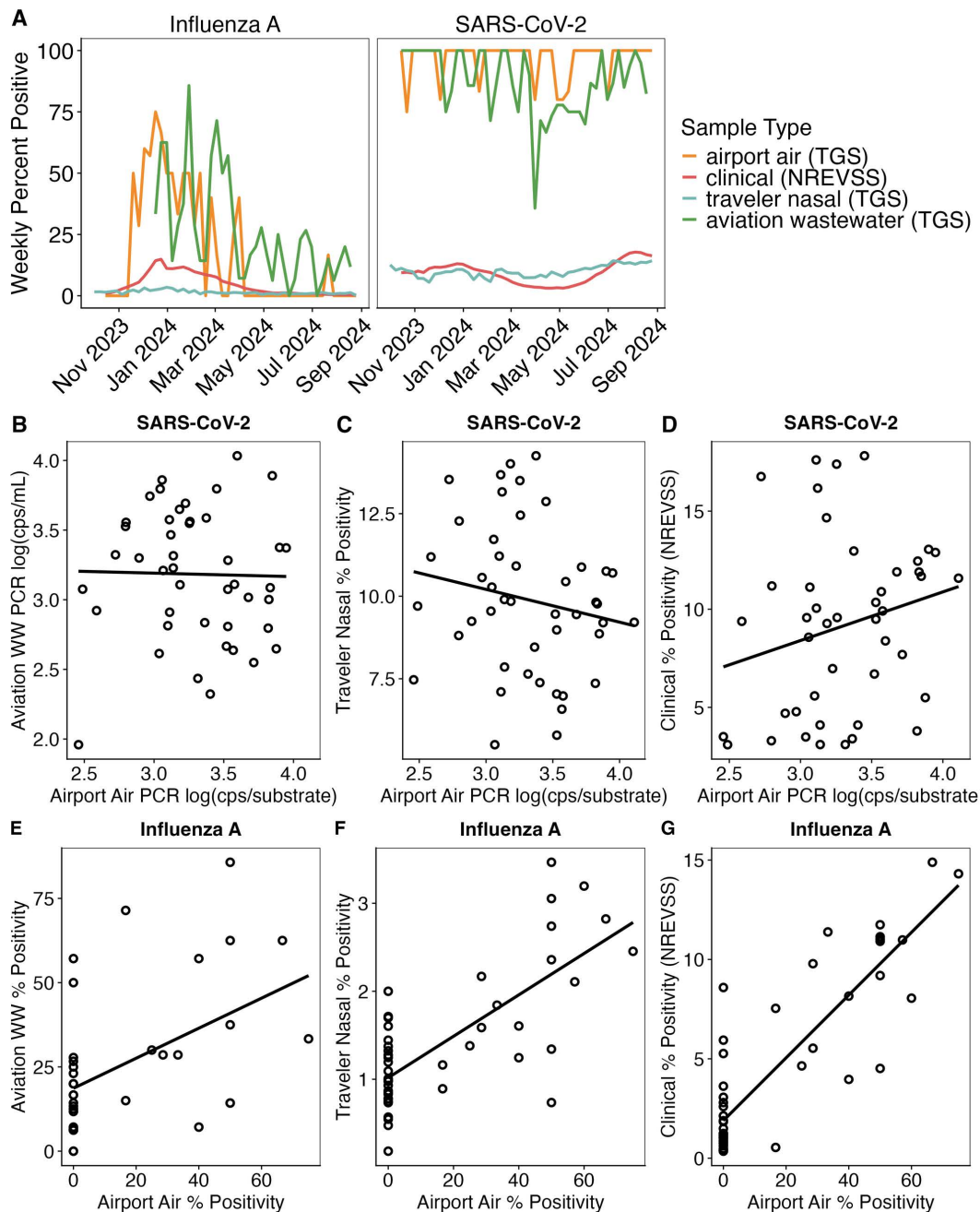


Fig 2. Respiratory virus positivity trends for airport air, aviation wastewater, and traveler nasal swab surveillance. As part of the CDC TGS program, two international airports (SFO and IAD) had overlapping collections for aviation wastewater, airport air, and individual traveler nasal swabs from October 18, 2023–August 27, 2024. Data from these two airports were used to compare virus positivity trends across surveillance modalities. Publicly available data from USA respiratory virus clinical testing (CDC NREVSS) was also included for comparison to national trends. A) Percent positivity for influenza A and SARS-CoV-2 PCR detection compared across surveillance modalities. B–D) Correlation between airport air PCR values for SARS-CoV-2 and (B) aviation wastewater PCR values, (C) traveler nasal percent PCR positivity, and (D) NREVSS clinical percent positivity. Because percent positivity was near 100% for SARS-CoV-2 in airport air and aviation wastewater samples, PCR values (copies/mL wastewater or copies/substrate for air) were analyzed instead of percent positivity. E–G) Correlation between airport air weekly percent positivity for influenza A and (E) aviation wastewater percent positivity, (F) traveler nasal swab percent positivity, and (G) NREVSS clinical percent positivity.

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Table 1. Pearson correlation coefficients (r) and associated p-values comparing airport air surveillance results with aviation wastewater, traveler nasal swab surveillance, and national clinical surveillance data for SARS-CoV-2 and influenza A.

Pathogen	Comparator Surveillance Modality	Correlation Coefficient (r)	p-value
SARS-CoV-2	Aviation wastewater	-0.02	0.88
SARS-CoV-2	Traveler nasal swab positivity	-0.17	0.24
SARS-CoV-2	National clinical surveillance (NREVSS)	0.22	0.13
Influenza A	Aviation wastewater	0.48	0.002
Influenza A	Traveler nasal swab positivity	0.73	<0.001
Influenza A	National clinical surveillance (NREVSS)	0.86	<0.001

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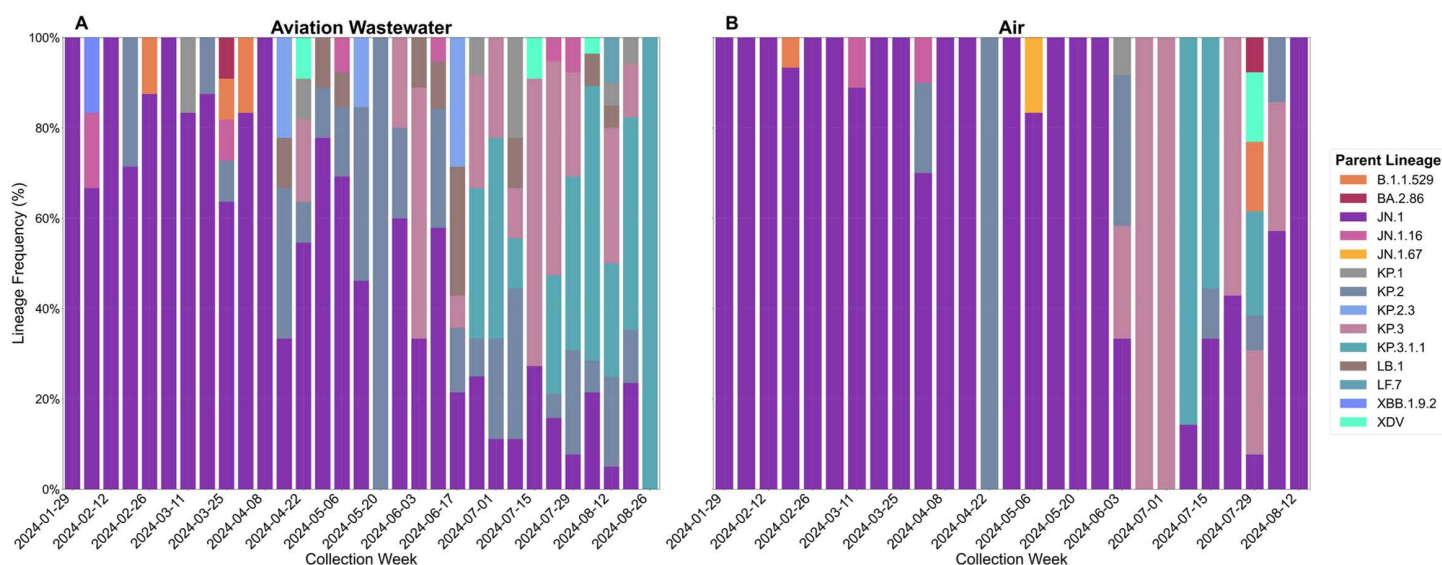


Fig 3. SARS-CoV-2 genomes from wastewater and air samples at two airports (SFO and IAD), samples collected from Jan 29, 2024 to Aug 26, 2024. Genomic sequencing data from A. aviation wastewater (SFO-triturator, IAD-aircraft) and B. Air samples. Aviation wastewater includes both triturator from SFO, which is the aggregate of aircraft lavatory waste, and aircraft wastewater from IAD, which is direct from single aircraft lavatory waste.

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A (80% of samples; mean RPKM 2.2, 95% CI: 1.0-3.4), and influenza B (34%), RSV A (54%), and RSV B (71%) were also detected. For influenza A, 100% (32/32) of PCR-positive samples had sequencing reads mapping to the genome. An additional 44 samples with influenza A reads were PCR-inconclusive (n = 17) or negative (n = 27). For samples with reads mapping to influenza A, the median RPKM for PCR positives was 10-fold higher than for PCR negatives (RPKM 1.0 versus 0.1).

In addition to revealing viral diversity, enrichment sequencing yielded high-quality genomes ($\geq 70\%$ reference coverage at $5 \times$ depth) in 2% (1/52) of samples from the first run and 28% (13/46) of samples from the optimized second run. Overall, 24 high-quality genomes were recovered from 98 samples, including SARS-CoV-2 (n = 6), influenza A (n = 9 segments), WU polyomavirus (n = 5), seasonal coronaviruses (n = 3), and bocavirus (n = 1).

Discussion

The findings of this project support air sampling as a viable option for pathogen surveillance in airports. The data suggest that i) air sampling can serve as a layered or alternative surveillance option, ii) targeted genomic sequencing from air samples provides the resolution needed to identify important variants, and iii) targeted enrichment sequencing enables multipathogen

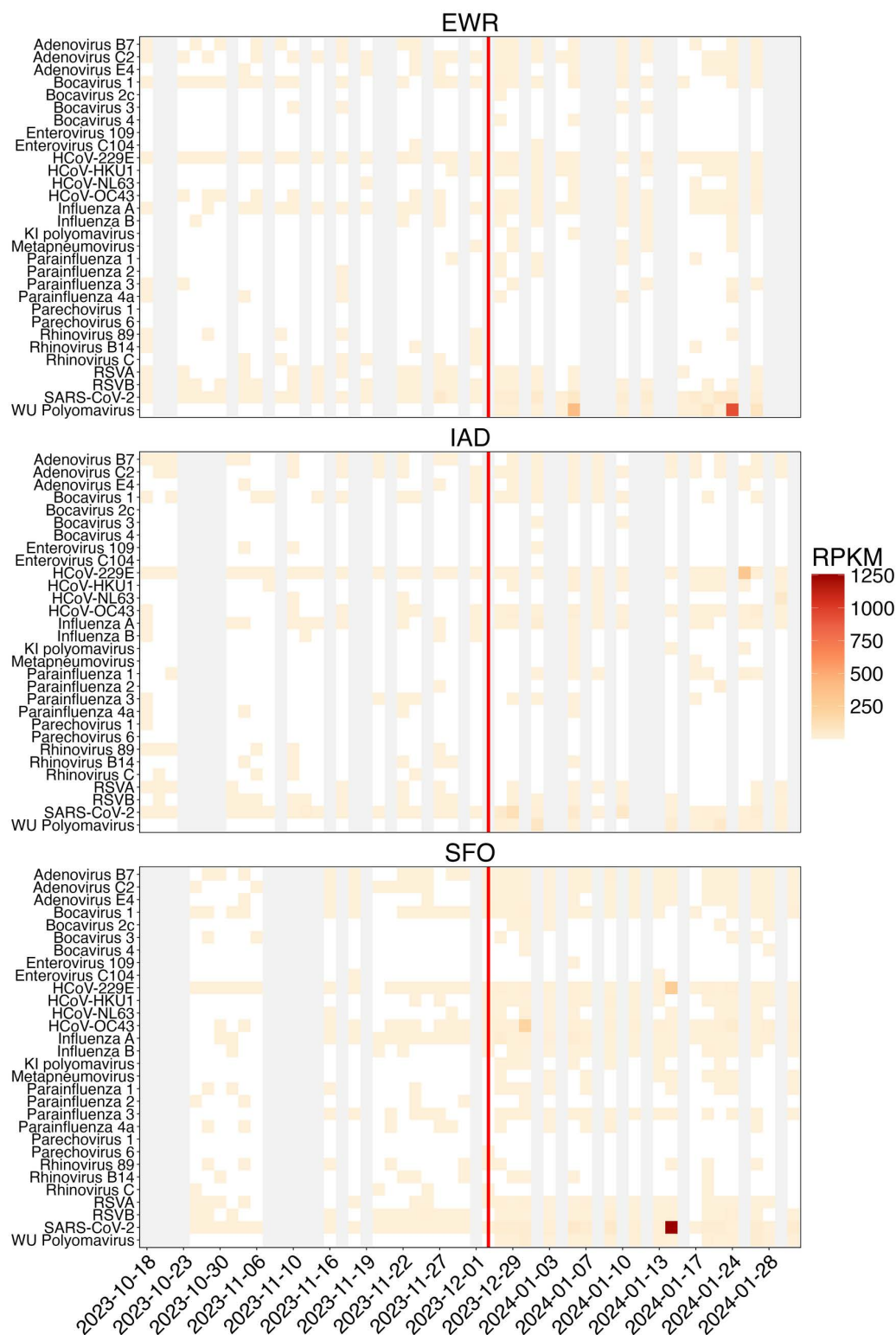


Fig 4. Detection of viral genomes from air samples by targeted enrichment sequencing. Nucleic acid extracted from air filters (N=98) collected from three airports (EWR, IAD, SFO) was sequenced using the Illumina Respiratory Viral Oligo hybrid-capture panel for respiratory viruses. Samples to

the right of the red line indicate when an RNA concentration step was added to the sequencing library prep protocol. The heatmap shows the normalized abundance of reads mapping to each viral target (RPKM=reads per kilobase per million reads).

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detection from air. To our knowledge, this is the first study to compare respiratory viruses detected in air samples with aviation wastewater and nasal swabs collected through traveler-based surveillance at the same airports, as well as with national clinical respiratory data. Influenza A demonstrated significant concordance across the comparator modalities, whereas SARS-CoV-2 did not. These findings indicate that air and wastewater monitoring can provide complementary information within a multilayered surveillance approach, although their relative performance and concordance vary by pathogen and sample type.

Airport air surveillance could support several public health functions, including enhancing situational awareness regarding respiratory pathogen activity among international travelers, prioritizing confirmatory testing and genomic sequencing efforts, identifying unusual increases in pathogen detections that warrant further investigation, and informing the allocation of public health resources. As a non-invasive surveillance approach, air monitoring complements clinical surveillance by capturing signals from asymptomatic or mildly symptomatic individuals who might otherwise go undetected. By providing insights into pathogen dynamics in high-risk environments, air monitoring may support earlier recognition of emerging threats and facilitate timely public health action. In the context of international travel, airport air surveillance may provide an additional layer of biosecurity by complementing existing traveler-based surveillance approaches and facilitating earlier recognition of imported pathogens or emerging variants. Beyond airport settings, air monitoring has been applied in schools (12) and proposed for long-term care facilities and workplace settings and may be particularly valuable where wastewater surveillance is impractical, such as in maritime environments where access to wastewater is limited [22]. In airport environments, air monitoring offers near real-time environmental surveillance with the potential for rapid operational turnaround depending on sampling and laboratory workflows. It serves as a crucial interface between emerging threats and community spread and can eliminate the dependence on participant recruitment that is required with nasal swab testing. In this project SARS-CoV-2 RNA was detected in nearly all samples (98.3%) compared with 17.2% for influenza A. Influenza A detections were concentrated in the winter months and mirrored national clinical data trends from the 2023–2024 influenza season. Influenza A showed stronger temporal concordance across modalities than SARS-CoV-2, potentially because influenza circulated more seasonally and at lower background prevalence. The near ubiquitous detection of SARS-CoV-2 underscores the value of genomic sequencing for tracking new variant introductions of highly prevalent pathogens. Sequencing in our study showed JN.1 as the predominant lineage, followed by increases in KP.2 and KP.3 sublineages, consistent with national surveillance data [21]. In this study, KP.2 and KP.3 lineages were detected substantially earlier in aviation wastewater than in air samples. However early detection was found to be dependent on the sample type when comparing aircraft (IAD) and triturator (SFO) wastewater individually against air samples (S1 Fig). While some KP.x signals appeared earlier in air than triturator wastewater at specific time points, a controlled comparison across all modalities at the same airport and time would be required to determine whether this reflects true early detection or variation related to low-abundance airborne material.

Airport air and aviation wastewater surveillance showed different positivity rates for influenza and SARS-CoV-2, with variable concordance relative to nasal swab surveillance. Differences across modalities likely reflect both biological and operational factors, including pathogen-specific shedding patterns, environmental stability, and differences in the populations and specimen types represented by each surveillance approach. For respiratory viruses, the utility of wastewater surveillance can be difficult to predict because stool and urine shedding are poorly characterized for many pathogens. Even for well-studied viruses such as SARS-CoV-2 and influenza, respiratory and fecal shedding can vary by patient age and disease severity. For example, more severe SARS-CoV-2 illness has been associated with prolonged respiratory shedding, whereas stool shedding duration appears less strongly influenced by symptom severity [23,24]. Fecal shedding of influenza A virus is highly heterogeneous, but may correlate with patient age and presence of gastrointestinal symptoms [25,26].

Genomic sequencing of air sampling has been shown as a highly effective method for pathogen surveillance [11,12,27,28], but methodological considerations remain. Targeted amplicon methods, commonly applied to SARS-CoV-2, have excellent sensitivity, which is valuable for identifying circulating lineages, but interrogate only a narrow set of pathogens. Untargeted metagenomic sequencing captures a broader diversity of pathogens; but the low amount of starting genomic material in air samples presents a technical challenge. Here, we addressed this challenge with targeted hybrid-capture enrichment sequencing, enabling detection of a broad panel of pathogens despite the limitations of input material. Our results show that targeted enrichment sequencing increased mapped reads and improved recovery of high-quality genomes from air samples. This approach also detected more influenza A material than PCR, though this may reflect limitations of the specific PCR assay (suggested by the number of inconclusive results) as well as the low sequencing thresholds applied in our study (any read counted as detection).

Recent work from Barbian et al similarly demonstrated that aggregated indoor air surveillance correlated with wastewater and clinical respiratory virus surveillance, further supporting the growing role of air monitoring as a complementary public health surveillance tool [28]. Unlike community-based surveillance networks, our study evaluated air surveillance at international ports of entry, where rapid detection of imported pathogens and emerging variants may have distinct biosecurity implications.

Our study identified several limitations. First, variations in viral genome reference coverage, due to differing numbers of reads and depth of coverage, may have impacted lineage assignment, particularly in low-biomass air samples. Second, operational constraints meant sampling duration and cartridge change timing were not fully standardized. However, we believe these modest variations did not materially alter the overall findings. Finally, minor temporal uncertainty in signal attribution may have been introduced by the precise placement and removal times of the cartridges. Additional studies are needed to better characterize the spatial and temporal sampling characteristics of airport air surveillance systems, including the influence of sampler placement, airflow patterns, passenger movement, and sampling duration on pathogen detection. Whether strategic placement of samplers near gates, customs areas, or other airport locations could improve attribution of detected pathogens to specific traveler populations or incoming flights remains an area for future investigation. A parallel focus should be on improving nucleic acid recovery and sequencing efficiency to enhance detection of less abundant pathogens. In addition, defining clear detection limits would improve interpretation of genomic findings, particularly in samples yielding low nucleic acid concentrations or incomplete genome recovery.

More broadly, air monitoring technologies currently face technical and operational limitations that require further research and development. Before these systems are widely deployed for biosurveillance, their limits of detection, quantification, and optimal sampling time for pathogens of interest must be rigorously characterized under realistic environmental conditions.

In addition, a better understanding of how airflow dynamics, building design, and population density influence sampling efficiency and detection probability would substantially enhance the capability and reliability of these technologies. Beyond technical considerations, the operational readiness of air monitoring for routine public health use depends on policy, ethical, and implementation factors. These include data interpretability for decision-making, coordination with airport authorities and industry partners, governance around data use, and clear triggers for public health response. As with other environmental surveillance systems, air monitoring is most effective when integrated into established surveillance frameworks rather than deployed as a standalone tool.

Unlike airplane wastewater and traveler nasal swab sampling, which are directly linked to arriving international passengers, air sampling in airport terminals lacks a clear demographic or epidemiological boundary. Air collected in congregate terminal spaces reflects a mixed population that can include airport staff, domestic travelers, and members of the surrounding community, introducing uncertainty regarding attribution to inbound international travel. This limitation reduces the specificity of air sampling for monitoring pathogen importation and must be considered when interpreting variant origin or timing signals relative to other aviation-based surveillance modalities. However, air sampling can provide a complementary layer within a

multi-modality framework. Its primary value lies in continuous detection of respiratory pathogens within high-risk indoor environments, capturing signals from individuals who may not contribute to wastewater or participate in voluntary nasal sampling. While air monitoring did not demonstrate earlier lineage detection compared to all aviation wastewater types in this study, it may offer situational awareness, confirmation of ongoing transmission, and persistence signals during outbreak onset or decline, particularly when other data streams are delayed, unavailable, or operationally constrained.

Air detection of viral pathogens within airports and other international points of entry may represent an important component of layered surveillance systems for future pandemic preparedness. Continuous or intermittent air sampling, coupled with rapid detection technologies, could provide near real-time insights into circulating pathogens and complement existing surveillance approaches. Detection of respiratory pathogens through air monitoring may inform targeted mitigation strategies, including enhanced ventilation [29], air purification [30], public health messaging, and allocation of public health resources. Our study highlights air sampling as a feasible, non-invasive approach for pathogen surveillance in airport settings. Integrating airport air sampling into existing surveillance frameworks may strengthen the ability to identify emerging threats at ports of entry and support more resilient surveillance systems to address future public health challenges.

Supporting information

S1 Fig. SARS-CoV-2 genomes from SFO wastewater (triturator) and SFO air samples and IAD wastewater (aircraft) and IAD air samples, samples collected from Jan 29, 2024 to Aug 26, 2024. Genomic sequencing data from A. Triturator wastewater (SFO) B. Air samples (SFO) C. Aircraft wastewater samples (IAD) and Air samples (IAD). Triturator is the aggregate of aircraft lavatory waste and aircraft wastewater is direct from single aircraft lavatory waste. (TIF)

S1 Data. Air PCR data. Weekly and sample-level PCR results from airport air monitoring surveillance. (CSV)

S2 Data. Airport surveillance dataset. Combined airport surveillance dataset used for analyses presented in the manuscript. (XLSX)

S3 Data. Clinical surveillance data. National clinical respiratory virus surveillance data obtained from NREVSS and used for comparison analyses. (CSV)

S4 Data. Targeted enrichment sequencing data. Viral sequencing results generated from targeted enrichment sequencing of airport air samples. (CSV)

S5 Data. Traveler nasal swab PCR data. PCR results from traveler-based nasal swab surveillance conducted at participating airports. (CSV)

S6 Data. Aviation wastewater PCR data. PCR results from aviation wastewater surveillance samples collected at participating airports. (CSV)

S1 Text. Supplemental methods. Detailed laboratory methods, molecular assays, sequencing procedures, bioinformatic pipelines, and analytical methods used in this study. (DOCX)

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