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Article

The Lavatory Lens: Tracking the Global Movement of Pathogens via Aircraft Wastewater

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Abstract: Modern commercial air travel connects disparate human populations. The global airline industry transporting up to 4.5 billion passengers annually in the years leading up to the COVID-19 pandemic. While such connections are convenient for commerce and tourism, air travel networks can also be efficient distributors of infectious diseases such as influenza, SARS-CoV-1, hemorrhagic fevers, and more recently SARS-CoV-2 and mpox. During the COVID-19 pandemic, public health agencies used multi-layered control strategies including pre-departure testing and vaccination requirements, masking, post-arrival testing, and quarantine to manage the risk of COVID-19 transmission associated with air travel. Simultaneously, the surveillance of aircraft wastewater developed as a promising new data source to screen for SARS-CoV-2 infections, including newly emergent lineages, among international air travelers. Herein, we review the potential of aircraft wastewater for public health surveillance. The known flight itinerary and precisely defined passenger population along with the resulting concentrated waste stream and convenient sampling during routine ground handling make aircraft wastewater a strategic opportunity for unintrusive surveillance of the global circulation of human pathogens. We estimate in the case of a fecal- or urine-shed pathogen, surveying 10% of all global long-haul flight passengers would require sampling from 3,500 and 1,250 flights per week, respectively. In the case of the United States, achieving 10% coverage of all international arrivals would require sampling from 925 and 322 flights per week for each shedding path, respectively. Aircraft wastewater surveillance could also be integrated with network and infectious disease models to target traditional public health control measures during emerging epidemics. Given the demonstrated potential for public good and the tremendous economic costs of epidemics, governments should consider international collaboration to create a global aircraft wastewater surveillance system.

Keywords: air travel; infectious disease; sentinel surveillance; wastewater surveillance; aircraft

Air Travel and Infectious Diseases

Commercial air travel enables the rapid transport of people and goods within and between continents. In 2019, the global airline industry boarded 4.5 billion passengers – a 127% increase in volume from 2004.[1] While the COVID-19 pandemic significantly reduced air travel volumes in late 2019 and 2020, passenger numbers had recovered to 3.8 billion by 2022, showing an 18% annual growth in 2021 and a staggering 51% growth in 2022.[1,2] Accompanying the hundreds of human travelers on intercontinental flights are human pathogens capable of initiating or propagating infectious disease outbreaks among human populations worldwide.[3,4] The importance of air travel for infectious disease epidemiology was recognized as early as the 1930s and historical examples of outbreaks spread by international air travel are plentiful.[5] International air travel has been associated

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with the global spread of many infectious disease such as influenza[6,7], SARS-CoV-1[8], hemorrhagic fevers[9], multi-drug resistant bacteria[10,11], and SARS-CoV-2[12] to name a few. In some locations, exporting endemic diseases, such as the plague, via international air travel is possible[13], or importation could introduce or reintroduce vector-borne illnesses such as malaria[14,15] or dengue.[16] Transmission of various infectious diseases has even been observed to occur during commercial passenger flights, which could disperse diseases beyond just the origin and destination of a single traveler.[17–20] Even after the heightened awareness and increased control measures in response to COVID-19, international air travelers have been associated with the importation of mpox in the United States (US)[21,22] and mpox and vaccine-derived polio in the United Kingdom.[23,24]

Controlling the Spread of Infectious Diseases via Air Travel

Given the scale of transcontinental travel and trade, border closure and travel restriction strategies alone are increasingly unlikely to fully contain the spread of outbreaks.[25,26] In many jurisdictions, individual passenger screening has been used in lieu of travel restrictions and quarantine. Modeling of an Ebola outbreak suggested that the most efficient screening approach would be to screen air travelers departing from the single major airport within each of the affected countries.[27] But, individual passenger screening programs during the SARS pandemic (2003), influenza pandemic (2009), and most recently the SARS-CoV-2 pandemic (2020-2021) were limited in their ability to identify every infected passenger.[28–31] On the other hand, multi-layered control strategies including pre-departure and post-arrival testing combined with quarantine have been effective in reducing subsequent COVID-19 transmission[32] and positivity[33] among passengers. These successes support a comprehensive multi-layered approach to mitigate the risk of infectious disease dispersion via air travel.

To better inform control measures, a number of mathematical network models have been developed to study the spread of epidemic disease by integrating air travel network and infectious disease modeling.[34] These models are useful to predict the global spread of infectious diseases as a function of their epidemiological features and test various intervention strategies such as passenger screening.[35] Stochastic network models built using International Air Transport Association (IATA) traffic data indicate that disease is spread via specific high-volume routes with certain connections between nodes playing a dominant role.[36–38] Modeling analysis of pandemic influenza H1N1 found that the world’s top 50 airports (by total number of passengers transited) greatly accelerated global spread and suggested that control strategies should be prioritized at these airports.[39] An analysis of the European air travel network found that the critical nodes for disease transmission were characterized by high centrality within the network and co-location with densely populated areas.[40] These results suggest that a resource-efficient and non-intrusive sentinel surveillance system deployed at specific air travel nodes could be a sustainable approach for tracking the global circulation of infectious diseases. Herein, we consider the potential of aircraft lavatory wastewater from long-haul flights (duration > 6 h) as a strategic opportunity to survey the global circulation of human pathogens to inform additional layers of well-established public health risk mitigation activity.

Human Pathogens and Aircraft Lavatory Wastewater

A diverse array of pathogens have been detected in municipal wastewater, including respiratory viruses[41–43], antibiotic-resistant bacteria (ARB)[44,45], and enteric pathogens.[46,47] Consequently, the measurement of pathogens shed from infected humans in municipal wastewater has demonstrated strong potential for the surveillance of a variety of infectious diseases.[48] When it comes to the surveillance of aircraft lavatory wastewater for pathogens shed by passengers, one critical aspect that differs from municipal systems is the human toileting behavior onboard aircraft, which is not well documented. Passengers boarding long-haul flights while infected with pathogens are likely to shed those microbes in various body fluids. For example, persons infected with SARS-CoV-2, whether symptomatic or not, shed the virus and/or its RNA in their stool, urine, sputum, and saliva, all of which are collected in municipal wastewater.[49] Given infected persons are shedding pathogens in their body fluids, the usefulness of aircraft wastewater surveillance is next constrained by how likely passengers are to deposit those fluids into the onboard toilets. Only a single study has investigated onboard lavatory usage, specifically defecation, among air travelers.[50] Using a 5-point Likert scale (never to always), Jones *et al*. reported that 13% of passengers on short-haul flights (<6 h) and 36% of passengers on long-haul flights are likely to use the onboard lavatory for defecation.[50] This is consistent with the assumption that the probability of defecation increases with increasing flight duration.[51] Incorporating these results into a Monte Carlo simulation, Jones *et al*. estimated that when testing aircraft wastewater the average probability of detecting a single passenger infected with SARS-CoV-2 from short-haul flights was 8% while for long-haul flights it was 14%.[50] Aside from this self-reported survey of defecation behavior during flights, there is no published data for other relevant shedding routes such as urination, spitting, gargling with mouthwash or disposal of tissues from nose blowing into the toilets on aircraft. These behaviors could all be relevant pathways for the deposition of pathogens into lavatory wastewater, which could then be detected by testing. Robust datasets characterizing such behavior would be useful to better assess the potential of aircraft wastewater surveillance.

Aircraft Lavatory Systems, Maintenance

After being deposited into toilets in the lavatories, biological wastes are sequestered via the specialized plumbing systems onboard aircraft. The wastewater produced during flight consists of gray water from the sinks in the lavatories and galleys, and wastewater from the toilet in the lavatories. For international flights, the gray water is typically discharged during flight via heated drain masts. An exception to this is the Boeing 787, which collects and stores gray water in the onboard waste tanks. Wastewater from the lavatory toilets is suctioned into the onboard waste storage tanks at velocities greater than 300 miles/h via a vacuum system. These vacuum toilets use very little potable water with flush volumes ranging from 200 to 300 mL per flush compared to 3 to 4 L for typical household toilet flushing.[52] The number of waste storage tanks and total waste storage volume varies by aircraft model and configuration. For example, the Boeing 747 and 777 provide approximately 1,000 and 1,400 L of total wastewater storage, respectively, while the 787 provides around 1,600 L of storage to accommodate the gray water that is kept onboard. Airbus aircraft provide waste storage via 350 L tanks with A330s equipped with two to three tanks (700 to 1,050 L) and the A380 with six tanks (2,100 L). The entire plumbing system is insulated to prevent freezing during flight.

All the toilet wastewater produced during flight is stored in the waste tanks until the aircraft lands. The waste storage tanks are emptied after every flight as part of routine ramp operations. The ground crew accesses the waste tank drain line and rinse line via the waste service panel located on the underbelly of the fuselage just forward of the tail section. Wastewater is drained from the storage tanks into a lavatory service truck using a large diameter hose (sometimes with vacuum applied). After the draining is complete, disinfectant and deodorizer (commonly referred to as "blue juice" and sold under a variety of commercial product names) is pumped at high pressure into each of the waste tanks via a rinsing nozzle. After rinsing, the tanks are sometimes primed with a small volume of disinfectant in preparation to receive wastewater during the next flight segment. The wastewater collected in lavatory service trucks is discharged to the municipal wastewater collection system via an on-site collection point called a triturator. During ground handling, the entire waste tank servicing typically takes three to seven minutes to complete.[53] All ground crew members performing lavatory servicing must wear proper personal protective equipment including face shields and gloves to mitigate the risks of exposure to hazardous materials.[54]

Potential of Aircraft Lavatory Wastewater for Infectious Disease Surveillance

Given the contribution of international air travel to the global dispersion of infectious diseases, the reasonable likelihood of passengers to deposit pathogens into the onboard toilets, and the unique characteristics of aircraft wastewater systems, public health surveillance of aircraft wastewater affords a compelling opportunity to track the global fluxes of pathogens. A global aircraft wastewater surveillance system could add a non-intrusive "always on" layer in the multi-layered control strategies that have been found to be most effective for mitigating infectious disease risks associated with air travel. Data produced by the aircraft wastewater system could also inform the expenditure of the limited resources available for traditional public health control strategies in response to emerging threats. Unlike municipal wastewater systems, aircraft are literally closed systems during flight. Each person that could have contributed biological materials to the wastewater sample is within the aircraft and on the flight manifest. Also, unlike municipal wastewater systems, due to the low flush volume and minimal inputs of other liquids, aircraft wastewater is a highly concentrated waste stream, which can be advantageous for measuring less abundant pathogens. Since the waste storage tanks are cleaned and serviced between each flight, in the absence of cross-contamination, the detection of a pathogen in wastewater derived from a particular flight can be linked to a passenger moving from the point of origin to the point of arrival. While the origin of the flight may not be equivalent to the travel origin of all, or even the majority, of the infected passengers, the information still indicates the movement of the pathogen between two nodes in the air travel network.

Using aircraft lavatory wastewater to achieve a representative sample of circulating pathogens is a different use case than has been primarily examined in light of COVID-19. The previously mentioned study by Jones *et al*. considered the usefulness of aircraft wastewater sampling to "capture" infected individuals and found that this likelihood was too low to prevent disease importation.[50] However, even individual passenger screening with polymerase chain reaction (PCR)-based diagnostic testing was not successful at preventing COVID-19 among arriving passengers.[55] Preventing disease importation is not the only application of aircraft wastewater surveillance. In addition to a "capture and prevent" paradigm, aircraft wastewater is also a compelling opportunity to survey global fluxes of pathogens via passengers on long-haul transcontinental flights. Given that up to one in three passengers may defecate on such flights and that a much larger percentage are likely to urinate, the biological materials in aircraft wastewater are very likely to provide a reasonable sample that is fit for the purpose of assessing circulating pathogens. Several previous studies of human pathogens in aircraft wastewater have established the analytical feasibility of the approach.

Previous Studies of Pathogens in Aircraft Wastewater as Proof of Concept

Since aircraft wastewater is discharged into the municipal wastewater collection system, it has been suggested as a potential biosecurity threat in the event of treatment failure or a potential indicator of emerging biosecurity hazards.[56] One of the earliest investigations used a novel concentration method to recover enteroviruses from aircraft wastewater samples to consider the possibility of importing poliovirus to the United States.[57] The authors found that 7 of 16 aircraft wastewater samples were positive by cell culture and PCR for various echo- and coxsackieviruses.[57] Another early investigation of aircraft wastewater was concerned with workplace exposures associated with waste tank servicing and maintenance operations.[54] Burton and McCleery found that fecal indicator bacteria (FIB) and *Salmonella* spp. did not grow in the presence of disinfectants, but were able to isolate *Morganella morganii*, *Proteus penneri* and *Providencia rettgeri* from lavatory wastewater despite application of disinfectants.[54]

The use of aircraft wastewater for public health surveillance was first suggested in 2015. Peterson *et al*. (2015) used meta-genomic analysis of aircraft wastewater from long-distance flights to assess global antimicrobial resistance and enteric pathogen patterns.[58] The researchers sequenced toilet waste from 18 international flights to Copenhagen, Denmark using shotgun sequencing and observed differences in the abundance of antimicrobial resistance genes and enteric pathogens based on flight origin. However, the researchers also raised the question of how representative the samples are of the country of origin. Despite this limitation, this study was the first to suggest the potential value of aircraft wastewater as a tool in the global fight against infectious diseases and antimicrobial resistance. A subsequent study used shotgun sequencing, quantitative PCR (qPCR), and culture techniques to investigate the presence of ARBs and antibiotic resistance genes (ARGs) in aircraft wastewater.[59] Here, samples from lavatory service trucks that contained wastewater from multiple flights at five airports in Germany were found to contain a high diversity and abundance of ARGs with certain ARGs enriched and distinct compared to municipal wastewater from the city of the respective airport. *E. coli* isolates from the aircraft wastewater demonstrated resistance to a greater number of third-generation antibiotics compared to eight clinical isolates from Germany. These findings suggest that aircraft wastewater may be an important reservoir for antibiotic resistance, potentially contributing to the spread of antibiotic-resistant bacteria and associated genes between countries. In 2019, Hjelsmo *et al*. conducted a study to investigate the potential use of metagenomic analysis of viruses from aircraft wastewater for the surveillance of enteric and respiratory viruses.[60] The researchers collected toilet waste from 19 international flights arriving at Copenhagen, Denmark and analyzed the samples using virus capture probes and high-throughput sequencing. Enteric and respiratory pathogens were detected in the samples. The study also revealed that the virus community composition, including species richness and abundance, exhibited geographic associations, with regional and city-specific associations observed for some viruses. Together these studies were the first to suggest and demonstrate the potential of using aircraft wastewater for global public health surveillance of a variety of enteric and respiratory pathogens, and antimicrobial resistance.

During the onset of COVID-19 pandemic, Ahmed et al. (2020) investigated the potential use of commercial passenger aircraft and cruise ship wastewater as a surveillance tool for assessing the presence of COVID-19 among travelers.[61] The study included three aircraft wastewater samples from international flights arriving in Australia, which were then tested for SARS-CoV-2 RNA using five different reverse transcription (RT)-qPCR assays and RT-digital (d)PCR. The aircraft samples tested positive for SARS-CoV-2 RNA by two RT-qPCR assays at low concentrations (~40 to 300 gene copies (GC)/100 mL). Seeding experiments were conducted to assess the effects of aircraft wastewater disinfectant on a surrogate coronavirus (murine hepatitis virus), and no significant differences were observed in the Cq value at 24 h, with a slight increase observed after 48 h. Despite the positive detection of SARS-CoV-2 RNA in the aircraft wastewater, quarantine isolation for 14 days and nasopharyngeal swab testing did not identify infected passengers, suggesting that the positive results could be due to persistent shedding by convalescent persons or carryover from the lavatory service truck. To avoid possible carryover from other flights due to mixing in the vacuum truck, Qantas (the flag carrier of Australia) designed a new sample extraction system allowing direct sampling from the plane waste tank before wastewater enters the lavatory service truck. In a follow up study published in 2022, Ahmed *et al*. analyzed wastewater samples from 37 long-haul repatriation flights to assess the potential of wastewater surveillance in predicting incident COVID-19 infections during the mandatory 14-day quarantine period.[55] All passengers tested negative by nasal and oropharyngeal swabs prior to boarding the repatriation flights. During quarantine, 112 COVID-19 cases were identified by diagnostic testing, and aircraft wastewater demonstrated 84% accuracy for predicting COVID-19 incident cases. Using the new sample extraction system, waste tank rinsate testing was conducted on 28 post-wash samples, with 2 of 28 samples testing positive for SARS-CoV-2 RNA (below limit of quantification). Interestingly, when there was only a single case of COVID-19 among passengers during quarantine, aircraft wastewater was positive in 6 of 8 instances, suggesting up to a 75% probability that an individual diagnosed with COVID-19 deposited SARS-CoV-2 RNA in the toilet. Albastaki *et al*. analyzed commercial aircraft wastewater samples from 198 commercial passenger flights arriving at Dubai International Airport.[62] Buckets were used to collect samples from the lavatory service panel on the aircraft fuselage. Wastewater collected from flights arriving to Dubai from 59 destinations representing all six continents demonstrated a 13.6% positivity rate for SARS-CoV-2 RNA.

In a later publication, Ahmed *et al*. reported on the detection of the Omicron (B.1.1.529) variant of SARS-CoV-2 in aircraft wastewater.[63] The study involved the analysis of an aircraft wastewater sample from a flight arriving in Darwin, Australia from Johannesburg, South Africa on November 25, 2021, just prior to the WHO's designation of Omicron as a variant of concern. During quarantine after arrival, one passenger onboard the flight was found to be infected with the Omicron variant by sequencing of a nasopharyngeal swab on November 29, 2021. The initial putative detection of the Omicron variant in the aircraft wastewater sample by RT-qPCR was confirmed by subsequent amplicon-based sequencing (ARTIC V3), which revealed a consensus genome clustering with the B.1.1.529 BA.1 sub-lineage. Consistent with the previous publications, the findings of these studies suggests that wastewater surveillance could be a valuable tool for monitoring passengers for COVID-19 and emerging variants of concern, especially in tandem with diagnostic passenger testing and quarantine measures.

In a small-scale study, Le Targa and colleagues investigated the efficacy of aircraft wastewater testing for detecting COVID-19 cases among travelers and identifying viral genotypes.[64] In December 2021, wastewater from two flights arriving in Marseille, France from Addis Ababa, Ethiopia was tested using RT-PCR and screened for variants of concern. The wastewater screening tests were conducted between the time of aircraft landing and customs clearance and later tiled amplicons (ARTIC V3) were sequenced by MiSeq Illumina. All 56 passengers on the second flight were tested using antigenic tests of nasal swabs with subsequent sequencing by NovaSeq. SARS-CoV-2 RNA suspected of being from the Omicron BA.1 variant was detected in the aircraft's wastewater and SARS-CoV-2 RNA was detected in 20% of passengers and the Omicron BA.1 variant was later identified by sequencing. The study demonstrated the potentially low efficacy of diagnostic screening of individuals for COVID-19 case identification among arriving passengers even when a vaccine pass and negative test was required before boarding. The authors proposed the use of rapid testing of aircraft wastewater immediately upon landing as a screening tool for initiating nasopharyngeal testing and strict quarantine until final diagnostic results for individual passengers are available.

In the UK in March 2022, Farkas *et al*. studied the feasibility of wastewater as a potential tool for international public health surveillance by measuring SARS-CoV-2 RNA in aircraft and airport terminal wastewater samples collected at three major international airports.[65] Viruses were concentrated by polyethylene glycol (PEG) precipitation, and RT-qPCR was used to detect SARS-CoV-2 RNA and a fecal indicator virus. Aircraft wastewater samples had a positivity rate of 93% for SARS-CoV-2 RNA. More recently, the CDC contracted with a biotech company to investigate the feasibility of detecting SARS-CoV-2 variants through wastewater surveillance of international long-haul flights arriving at JFK International Airport in New York City.[66] Wastewater samples were collected from 88 international flights arriving from the United Kingdom, Netherlands, and France from August 1 to September 9, 2022. Samples were screened using RT-PCR with low-Cq value samples subjected to amplicon-based whole-genome sequencing. Among these 88 samples, 81% were positive for SARS-CoV-2 RNA by RT-PCR with 40% of the RT-PCR positive samples yielding genomes that were all identified as Omicron sub-lineages. The program demonstrated aircraft wastewater surveillance could provide a resource-efficient approach to monitor SARS-CoV-2 variants without direct traveler involvement or disruption of routine airport operations.

These ten studies consistently demonstrate the suitability of aircraft wastewater testing for public health surveillance of a diverse array of pathogens using both PCR and sequencing techniques. Given the proof of concept established by the existing body of studies, the next concern is the scale that would be required to achieve a reasonably representative sample of pathogens circulating via intercontinental travelers.

Estimated Scale of an Aircraft Wastewater Surveillance System

As previously mentioned, other modeling efforts have attempted to estimate the likelihood of detecting a single infected passenger entering a country by using wastewater surveillance of aircraft.[50] Here we use a simple Monte Carlo simulation to estimate the number of long-haul flights that must be sampled to achieve 10% coverage of long-haul passengers, which we assume to be reasonably representative of intercontinental air travel. Based on the seat configurations of typical long-haul aircraft and intentional sampling from larger ones, we modeled the number of seats on a long-haul flight as between 350 to 410 (uniform distribution). Again, assuming full flights could be selected for sampling, we modeled the occupancy rate of those seats to range between 85 to 100% (uniform). Finally, the probability of any passenger "participating" in the wastewater sample by defecating in the onboard toilet is estimated at 25 to 36% based on a previous survey of long-haul flight passengers in the UK.[50] With each of these inputs modeled as a uniform distribution (all values in the range are equally likely), a Monte Carlo simulation with 10,000 draws indicates that the range (10th to 90th percentile) of passengers included in a lavatory wastewater sample from a long-haul flight is 87 to 130 with a mean value of 107. If the pathogen of interest were shed in urine and the participation rate thereby increased to 75 to 100% (presumably more passengers urinate during flights), then the mean number of passengers included in the wastewater sample would increase to 308 per flight. A sensitivity analysis of the simulation indicates that 74% of the variation in the number of passengers surveyed per long-haul flight is attributable to the likelihood of participation (i.e., defecation or urination during flight), followed by the flight occupancy (14%), and the number of seats (13%).

The number of long-haul flights to be sampled can be estimated by dividing the target proportion of long-haul flight passengers, we assume 10%, by the estimated number of passengers contributing to each aircraft wastewater sample. In 2019 there were 1.93 billion international air travelers worldwide[67]; however, the majority of these travelers are not on long-haul flights (i.e., transcontinental travel). Using a long-haul flight proportion of 4%[68] and assuming the average long-haul flight has twice as many passengers as other flights yields an estimated 196 million long-haul flight passengers in 2019 (roughly 10% of total international travelers). Assuming a fecal-shed pathogen, surveying 10% of the global annual long-haul flight passengers via aircraft wastewater would require sampling roughly 2,900 to 4,400 flights per week (Table 1). If the pathogen were shed in urine, and the participation rate (urination into the onboard toilet) were 75 to 100%, then the number of flights sampled to account for 10% of long-haul travelers would range from 1,000 to 1,500 flights per week.

The number of flights to be sample can also be considered on a country-by-country basis. Given its geographical location, a majority of the international travelers arriving in the US are likely to be long-haul flight passengers. In 2021 there were 50,852,096 international passenger arrivals to the US.[69] As shown in Table 1, surveying 10% of these passengers for a fecal-shed pathogen via aircraft wastewater would require sampling an average of 925 flights per week. In the case of a urine-shed pathogen, covering 10% of international arrivals would require collecting wastewater samples from 269 to 381 flights per week. Of the international arrivals to the US in 2021, 68% arrived via 10 airports. In principle, these airports could be prioritized as aircraft wastewater surveillance hubs. Assuming the sampling density at each airport is proportional to the international passenger arrival volume, wastewater sampling operations at these airports would require sampling between 170 (JFK) to 43 (IAD) flights per week in the case of a fecal-shed pathogen or between 59 to 16 for a urine-shed pathogen (Table 2).

Table 1. The estimated number of long-haul flights to be sampled per week to achieve a 10% coverage of all long-haul flight international passengers based on Monte Carlo simulation of a fecal-shed pathogen and a urine-shed pathogen**.

	Fecal-shed Pathogen **# flights sampled/week** **10% LH passenger survey rate** Mean 10th - 90th Percentile	**Urine-shed Pathogen** **# flights sampled/week** **10% LH passenger survey rate** Mean 10th - 90th Percentile
2019 Global Long-haul Passengers (196 million total)	3,570 (95% CI: 3,540 - 3,600) 2,910 - 4,350	1,240 (95%CI: 1,230 - 1,250) 1,040 - 1,470
2021 US International	925 (95% CI: 918 - 931)	322 (95% CI: 319 - 324)

Passengers (50.9 million total)	756 – 1,030	269 - 381

**Means and 95% confidence intervals are from bootstrapping a Monte Carlo simulation with 200 simulations of 1,000 draws each. Credible intervals from the 10th to 90th percentile are from a single simulation of 10,000 draws.

Table 2. The estimated number of long-haul flights to be sampled per week at each of the top ten US airports for international arrivals to achieve a 10% survey rate of total international passengers based on Monte Carlo simulation of fecal-shed and urine-shed pathogens in aircraft wastewater.

U.S. Airport (annual international passenger volume, 2021)[70]	**Fecal-shed Pathogen** **# flights sampled/week** **10% LH passenger survey rate**	**Urine-shed Pathogen** **# flights sampled/week** **10% LH passenger survey rate**
JFK (6.36 million)	170	59
MIA (5.93 million)	159	55
LAX (3.87 million)	104	36
EWR (3.23 million)	87	30
IAH (3.21 million)	86	30
DFW (2.91 million)	78	27
ATL (2.78 million)	74	26
ORD (2.64 million)	71	25
FLL (1.96 million)	53	18
IAD (1.63 million)	43	16

The modeling results reported here are crude estimates aimed to determine the scale required to achieve a 10% long-haul passenger sampling rate and are agnostic to important infectious disease characteristics such as prevalence, reproduction rate, incubation period, shedding period, etc. Given the estimated scale required for a general-purpose aircraft wastewater surveillance system, it may be desirable to tailor aircraft wastewater surveillance to specific infectious disease emergence or re-emergence scenarios in real time. Various models utilizing flight network data in combination with infectious disease characteristics have described the propagation of transmission chains along international flight paths, such as early projections for the spread of COVID-19.[71] The Global Epidemic and Mobility computational model (GLEaM), has been used to predict the spread of epidemic diseases and has been used to consider the spread of pandemic diseases such as H1N1.[72,73] GLEaM is publicly available via the GLEaMviz Simulator (http://www.gleamviz.org/) and could be used to design and deploy adaptive aircraft wastewater surveillance programs in response to real-time disease emergence.[74] An adaptive framework such as this has been previously proposed for vector-borne disease surveillance using the Vector-Borne Disease Airline Importation Risk tool.[75]

Following the identification of an emerging epidemic of concern, modeling results could be integrated with rapid response capabilities to establish aircraft wastewater testing at strategic nodes based on the relevant infectious agent characteristics and epidemic location. It should be noted that in this application scenario, the rapid startup and commissioning of aircraft wastewater sample collection and testing at new locations will not be trivial, given the sample collection logistics. It may be advantageous to adopt a posture of readiness with ongoing routine aircraft wastewater surveillance at key nodes in the international air travel network to maintain the system in a ready state.

Aircraft Wastewater Sampling Logistics

Depending on the desired passenger participation rate, the use of aircraft wastewater to survey pathogens among international travelers could require the sampling of hundreds to thousands of flights each week. An efficient and well-organized sample collection program is critical to success. There are important challenges associated with sample collection and transport from the strictly controlled airport space to the centralized laboratory for testing. Access to the ramp where aircraft are serviced is restricted to airport operators. Anyone entering the ramp must undergo a security assessment and badging process or be escorted by authorized personnel. Accessing these spaces depends on the ongoing collaboration of several entities, including airlines, airport authorities, terminal operators, and ground servicing companies, which in turn depends on the support of the appropriate authorities. Thus, it is critical that any sustainable aircraft wastewater surveillance program be led by government public health agencies with support from private sector and research partners.

Once access to the aircraft and secured airport space is established, sample collection is complicated by the aircraft ground servicing operations and the limited time available to perform them. When the aircraft arrives at the ramp, there are several service activities that must be performed in tandem within specified time frames, including passenger disembarking, baggage handling, aircraft cleaning, refueling, and any required maintenance. Ideally, the wastewater sample collection should take place as a component of the routine lavatory servicing. To accomplish this in their program, Qantas[76] designed and manufactured a sample collection device that connect to the outlet on the aircraft lavatory service panel and then connects with the standard lavatory waste dump hose. Sample collection protocols to perform end-to-end operations in a safe and efficient manner that is integrated into routine aircraft servicing must also be carefully considered. During the CDC program, the sample collection added three minutes, on average, to the aircraft ground handling times with zero spills.[66] More recently the European Union (EU) Health Security Committee and Integrated Political Crisis Response has published ad hoc guidance to detail considerations such as coordinating with partners, sampling locations, and sample collection methods for aircraft wastewater sampling for SARS-CoV-2.[77]

Aircraft Wastewater Analytical Approaches

The form of an aircraft wastewater surveillance system for public health surveillance (e.g., number of airports, number of flights to be sampled, required analytical endpoint, time to results, etc.) will ultimately be determined by its intended purpose, which could evolve over time and space. The scoping model developed in a previous section indicates a throughput of up to hundreds of weekly flights per airport for routine surveillance of international travelers. Accommodating this volume would most likely require automation and a high -throughput analytical workflow, e.g. using robotic liquid handlers and high-affinity capture technologies that couple seamlessly to detection/quantification technologies. A variety of concentration methods have been used for SARS-CoV-2 in aircraft wastewater, including electronegative membrane filtration, centrifugal ultrafiltration, Concentrating Pipette, syringe filter, PEG precipitation, and in one case no concentration (i.e., direct extraction). However, the throughput required for scaling must remain a key consideration.[58] Affinity capture magnetic nanoparticles have proven effective for high-throughput SARS-CoV-2 capture from municipal wastewater and for aircraft wastewater analysis.[66,78]

If aircraft wastewater sampling results will be used to inform follow-up diagnostic testing of individual passengers or quarantine, the time to results for the selected workflow must be carefully considered.[60] To circumvent some of the logistical difficulties associated with moving samples in and out of secure areas and shorten the time to results, it may be possible to set up small scale laboratories at key airports within the air travel network. For rapid response capabilities, it may be feasible to devise a mobile microbiology laboratory within a unit load device container that can be conveniently transported by wide body aircraft and readily deployed for aircraft wastewater surveillance at airports worldwide. Even inflight sample sequencing has been suggested based on the near real-time analysis in response to Ebola virus.[60,79] No matter what workflow (pre-treatment, concentration, extraction) is used, the end point analytical method must achieve the appropriate sensitivity and specificity for the public health decisions or interventions to be made. Thus, any aircraft wastewater surveillance system must be carefully designed with the desired actionable decision in mind. In general, there are two categories of analysis available – target specific methods and target agnostic methods.

Target specific methods include techniques typically used for diagnostic assays including PCR-based techniques (qPCR, RT-qPCR, dPCR, etc.). These techniques have been widely used for wastewater surveillance. While they can be very sensitive and specific, they require the design of reagents specific to the target of interest *a priori*, which precludes their usefulness for measuring unknown targets such as an entirely novel pathogen. Additionally, many qPCR platforms only allow testing for up to six targets simultaneously in a single experimental run. Nonetheless, PCR-based techniques could be very useful for sensitive screening of aircraft wastewater for known pathogens, especially in highly parallel and multi-target formats such as TaqMan array cards (TAC), fluidigm BioMark HD real-time PCR or microarrays.[80,81] Other analytical techniques that warrant further investigation include loop-mediated isothermal amplification (LAMP) and helicase-dependent amplification (HDA) which can return results in shorter time frames and have been implemented on lateral flow test strips in clinical and environmental settings.[82–84] More novel techniques that could become relevant include mass spectrometry for the detection of proteins relevant to specific pathogens[85], enzyme-linked immunosorbent assays (ELISA) to detect specific antibodies[86], or clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic assays.[87,88]

In contrast to targeted diagnostic techniques (e.g., PCR, LAMP, CRISPR), next generation sequencing (NGS) can offer semi-targeted or even untargeted analytical capabilities.[89] As has been previously suggested, such capabilities could be very desirable for broad genomic surveillance of aircraft wastewater.[90] However, the strengths and limitations of these techniques must be carefully considered in light of the intended use. Compared to PCR techniques, sequencing techniques are often less sensitive, require more time to produce results, and are more costly.[91] Sequencing approaches are diverse, but for wastewater surveillance they are typically either untargeted (shotgun metagenomic or metatranscriptomic), targeted (tiled amplicon-based approach to selectively amplify and sequence the genome of a pathogen of interest) or semi targeted (hybridized probe-capture methods).[89] Although proper implementation requires considerable expertise and care, the targeted method generates deep sequencing or amplicon-based (pseudo-targeted via amplification) depending on the intended analyte.[89] Previous studies of aircraft wastewater have used shotgun sequencing techniques to characterize ARGs among bacteria.[58,59] Since bacteria are abundant in wastewater, shotgun sequencing is a reasonable approach for target agnostic characterization. In the case of viruses, which are much less abundant in wastewater, metagenomic or metatranscriptomic characterization often requires the use of enrichment probes as has been done for both municipal and aircraft wastewater.[60,92] These probes are often designed using a large database of genomic sequences to achieve inclusion of the broadest range of viruses possible.[60] Sequences of newly emerging viruses may not be present in such databases and could be inadvertently excluded via enrichment probe techniques. Shotgun techniques have been applied to analyze the wastewater virome, but the resulting metagenomes are likely a conservative estimate of virus occurrence and diversity and identifying entirely novel genomes is less likely.[93,94] Many studies reporting the early detection of novel SARS-CoV-2 variants via municipal wastewater used enrichment probes or tiled amplicon-based sequencing (i.e. semi-targeted).[95–97] One study noted that without the use of a semi-targeted viral enrichment panel they only detected 40 SARS-CoV-2 read pairs despite starting with an average of approximately 4,400 GC.[97] Just like PCR-based diagnostic assays, amplicon-based approaches require *a priori* knowledge of the intended target genome. Therefore, semi-targeted amplicon-based techniques must be carefully considered for the detection of novel pathogenic viruses via wastewater, since the performance would depend on how much the novel genomic sequence diverges from the wild type sequences used to design the tiled primer sets. Every published NGS study of SARS-CoV-2 in aircraft wastewater to date has used tiled amplicon sequencing and alignment with existing databases to characterize the lineages present in the sample.[63,64,66] While untargeted shotgun sequencing may yield detections for more abundant pathogens in aircraft wastewater, the detection of more rare pathogens may depend on the efficiency of viral probe capture panels for genomes that deviate from wild types.[89] For this reason, the most reliable form of aircraft wastewater surveillance in the near term is most likely to be informed of emerging pathogens by clinical reporting with genomic characterization via clinical samples followed by rapid implementation of well-integrated PCR-based and targeted or semi-targeted NGS techniques.

Challenges, Limitations, and Future Research Opportunities

Aircraft wastewater surveillance presents a unique set of challenges and limitations, both technical and procedural, that should be acknowledged and, in some cases, further investigated.

Among the primary challenges is that not all passengers on-board the aircraft will use the lavatory toilet. The likelihood of each passenger contributing a biological sample to the aircraft waste tanks depends entirely on the shedding pathway relevant to the pathogen. For fecal shedding, as is hypothesized for SARS-CoV-2, the likelihood of passengers defecating in the onboard toilets during flight is a critical constraint. Self-report survey data indicate for short-haul flights this likelihood is low, while for long-haul flights it increases, but is still well below 50%. However, in one study flights with just a single COVID-19 case onboard still demonstrated a 75% positivity rate via wastewater, which draws scrutiny to the fecal-centric shedding or to the likelihood of defecating among COVID-19 cases.[55] For pathogens likely to be shed in urine, the probability of an individual passenger contributing a sample on a long-haul flight may reach close to 100%. It is clear there is still great uncertainty around this fundamental element of aircraft wastewater surveillance and further investigation is warranted.

Another critical, yet uncertain, technical challenge is the possibility of residual contamination in the aircraft waste tanks from one flight leg to another. Such cross-contamination could yield false positives regarding the presence of infections among the passengers onboard. Ground crews rinse and prime the waste tanks with disinfectant blue juice between flights, but the efficacy of this procedure in removing residuals, especially given the sensitivity of PCR techniques, is uncertain. In the only study to investigate residual cross contamination, disinfectant rinsate samples (n = 28) collected following routine waste tank servicing demonstrated a 7% positivity rate by RT-qPCR, although the concentrations of SARS-CoV-2 RNA were much lower (frequently below the limit of quantification) than the actual wastewater samples.[55] Recently, aircraft wastewater has demonstrated high positivity rates (>80%) for SARS-CoV-2 RNA in both the United States and United Kingdom (Farkas 2023; Morfino 2023).[65,66] Better characterizing the likelihood of residual cross-contamination through larger studies would decrease the uncertainty surrounding these observations and greatly increase confidence in the application of aircraft wastewater surveillance for additional pathogens in the future.

Aircraft wastewater also faces additional challenges that are universal to wastewater surveillance. Further research is warranted to determine optimal and standardized protocols for sample collection and analysis.[98] The time required to obtain results and sample analysis costs are also important considerations that must be addressed to make such programs feasible and cost-effective, especially if follow-up testing of individual passengers is intended. The stability of relevant analytes, such as DNA and RNA, should also be investigated to ensure the signal is persistent in wastewater during the flight in the presence of disinfectants, low temperatures, and mixing induced by aircraft movement. While much of the published aircraft wastewater work to date has focused on SARS-CoV-2, it is important to expand and validate the approach to include other pathogens, such as enteric and respiratory pathogens and potentially even arboviruses and hemorrhagic fevers (Ahmed et al. 2023 under review).

If aircraft wastewater surveillance can produce reliable data for human pathogens, the next obstacle is the scale required to achieve a useful sample of international travelers. An endeavor of this magnitude would require substantial international collaboration and support as already noted in a recent review.[90] To decrease the analytical throughput, it may be possible to composite wastewater samples collected from arriving aircraft based on geographically related points of origin. Alternatively, it may be possible to collect the wastewater samples from the triturator as it is discharged from the lavatory service truck into the municipal collection system. This would require the development of an inventory system to track which flights have been collected into each service truck and which service trucks have been sampled upon discharge to the triturator.

Wastewater collected from airport terminals has also been used for early detection of SARS-CoV-2[99] and its variants[100] and, more recently, for mpox.[101] For both airport and aircraft wastewater, there are important epidemiological considerations. For wastewater from airports, it can be difficult, if not impossible, to know whether the signal has originated from a passenger arriving on a flight or a local worker. In the case of newly emerging or novel pathogens, this distinction could be less relevant as the infectious agent may not be expected among the local population. A key advantage of aircraft versus airport wastewater is, in the absence of cross-contamination, the data from aircraft wastewater can be definitively linked to a person that was onboard the flight, for which there are manifests. Follow-up surveillance actions could be implemented based on contact tracing.[51] It should be noted, however, that the origin and destination of the flight may not match the origin and destination of the traveler as they could be in transit between locations.[60]

Beyond these technical challenges and limitations, there is also the critical issue of securing permission to collect aircraft wastewater samples and the willingness of ground handlers to perform the collection as part of the routine servicing. As currently formatted, collecting wastewater samples from individual aircraft would require the ongoing permission and cooperation of the relevant commercial airline. As has been noted elsewhere, IATA has expressed support for "a global approach to managing infectious diseases".[90,102] Rebuilding and maintaining customer confidence has been a critical element for the recovery of the airline industry following COVID-19, and customers expect airlines to continue to take actions to mitigate the risks of infectious disease associated with air travel.[103,104] Participation in aircraft wastewater surveillance programs could be a compelling and less intrusive opportunity for commercial airlines to engage with risk mitigation for infectious diseases. However, dependence on the goodwill of commercial entities may not be sufficient to secure the future of a global aircraft wastewater public health surveillance system. In this case, international governments should consider taking action to incentivize public health surveillance opportunities as a global public good.[105] The potential high value of such a system for informing other forms of public health surveillance in a multi-layered approach warrants further investigation and development of a global scale aircraft wastewater surveillance program as has been previously suggested and elaborated herein.[90]

Conclusions

Aircraft wastewater offers a strategic opportunity for efficient and non-disruptive public health surveillance of globally circulating pathogens. While such an approach was proposed as early as 2015, published reports of aircraft wastewater surveillance for COVID-19 have made a compelling demonstration of such a system. However, the viability of such system depends largely on its intended use. Previous analyses of aircraft wastewater as a way to identify infected individuals and prevent disease importation indicate the efficiency for this purpose is limited.[50,51] Two alternatives may offer more potential. First, in the case of known epidemics with pandemic potential, aircraft wastewater surveillance could be targeted toward important nodes via integrated network and disease modeling. Second, as a more general case, aircraft wastewater surveillance is an opportunity to collect a representative sample of pathogens circulating via international travelers. Importantly, the general use case would allow the system to remain in a ready state for rapid response to localized epidemics and could provide useful intelligence to other traveler disease programs.

The scope of a global aircraft wastewater program for routine public health surveillance demands international collaboration, knowledge sharing, and investment. Such investments are trivial compared with the costs of epidemics ($60 billion USD/year to the global economy) and the COVID-19 pandemic, which cost an estimated $11 trillion USD to global governments in 2021 alone.[106] Losses were particularly hard felt for the travel industry with an estimated $168 billion USD loss for commercial airlines in 2020 and an estimated $935 billion USD loss for the tourism industry over the first 10 months of 2020.[107,108] The potential of aircraft wastewater surveillance as an new layer of public health response justifies the cooperation of commercial airlines and the investment of international governments to deliver public good.

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