

1 **Title: Monitoring multi-pathogens and SARS-CoV-2 variants in aircraft and airport**
2 **wastewater**

3

4 Martin Tay¹, Benjamin Lee¹, Muhammad Hafiz Ismail¹, Jerald Yam¹, Dzulkhairul Maliki¹, Karina
5 Yew-Hoong Gin^{2,3,4}, Sae-Rom Chae^{5,6,7}, Zheng Jie Marc Ho⁵, Teoh Yee Leong^{5,6}, Lee Ching Ng^{1,8},
6 Judith Chui Ching Wong^{1‡}

7

8 ¹ Environmental Health Institute, National Environment Agency, Singapore

9 ²NUS Environmental Research Institute, National University of Singapore, Singapore

10 ³Energy and Environmental Sustainability for Megacities (E2S2) Phase II, Campus for Research
11 Excellence and Technological Enterprise (CREATE), Singapore

12 ⁴Department of Civil & Environmental Engineering, National University of Singapore, Singapore

13 ⁵Ministry of Health, Singapore

14 ⁶National Centre for Infectious Diseases, Singapore

15 ⁷ Saw Swee Hock School of Public Health, National University of Singapore, Singapore

16 ⁸School of Biological Sciences, Nanyang Technological University, Singapore

17

18 [‡] Corresponding author:

19 Judith Chui Ching Wong

20 Environmental Health Institute, National Environment Agency, Singapore

21 11 Helios Block #06-05/08, Singapore 138667

22 judith_wong@nea.gov.sg

23 **Abstract (276 Words)**

24 **Background**

25 As global travel resumed in COVID-19 endemicity, the potential of aircraft wastewater
26 monitoring to provide early warning of disease trends for SARS-CoV-2 variants and other
27 infectious diseases, particularly at international air travel hubs, was recognized. We therefore
28 assessed and compared the feasibility of testing wastewater from inbound aircraft and airport
29 terminals for 18 pathogens including SARS-CoV-2 in Singapore, a popular travel hub in Asia.

30 **Methods**

31 Wastewater samples collected from inbound medium- and long-haul flights and airport terminals
32 were tested for SARS-CoV-2. Next Generation Sequencing (NGS) was carried out on positive
33 samples to identify SARS-CoV-2 variants. Airport and aircraft samples were further tested for 17
34 other pathogens through quantitative reverse transcription polymerase chain reaction (RT-
35 qPCR).

36 **Results**

37 The proportion of SARS-CoV-2-positive samples and the average virus load was higher for
38 wastewater samples from aircraft as compared to airport terminals. Cross-correlation analyses
39 indicated that viral load trends from airport wastewater led local COVID-19 case trends by two
40 to five days. A total of ten variants (44 sub-lineages) were successfully identified from aircraft
41 wastewater and airport terminals, and four variants of interest (VOIs) and one variant under
42 monitoring (VUM) were detected in aircraft and airport wastewater 18-31 days prior to detection
43 in local clinical cases. The detection of five respiratory and four enteric viruses in aircraft

44 wastewater samples further underscores the potential to expand aircraft wastewater to monitoring
45 pathogens beyond SARS-CoV-2.

46 **Conclusion**

47 Our findings demonstrate the feasibility of aircraft wastewater testing for monitoring infectious
48 diseases threats, potentially detecting signals before clinical cases are reported. The triangulation
49 of similar datapoints from aircraft wastewater of international travel nodes could therefore serve
50 as a useful early warning system for global health threats.

51

52 **Introduction**

53 Globalisation, urbanisation and population mobility can accelerate the spread of infectious
54 diseases in a highly-connected world. This has been demonstrated in the COVID-19 pandemic
55 where air travel facilitated the rapid and extensive transmission of SARS-CoV-2 globally [1].
56 Although restrictions on international travel [2] and mandated clinical tests at airports for
57 incoming travelers [3] had some success in minimising new introductions of the virus [4-6] and
58 bought many countries or states time for implementation of vaccination, such controls had
59 adverse impacts on the economy of states and were thus not sustainable. Following the reopening
60 of borders, countries across the globe have experienced successive waves of COVID-19
61 transmission, largely driven by the continued emergence of new SARS-CoV-2 variants such as
62 Delta and Omicron BA.5, among others [7], which are more transmissible with high rates of
63 immune breakthrough. [8]. These waves of transmission continue to pose threats of increased
64 transmissibility, immune breakthrough [9] and varying disease severity [10, 11], bringing
65 uncertainty to society [12], economies [13], and health care systems [14]. Additionally,
66 respiratory viruses and infectious diseases like respiratory syncytial virus (RSV), tuberculosis
67 and influenza that have been previously contained due to early pandemic measures are now re-
68 emerging [15, 16]. This therefore underscores the need for comprehensive and timely public
69 health surveillance of circulating COVID-19 variants and other pathogens, to guide the
70 calibration of response measures and inform policy decisions [14].

71

72 Wastewater surveillance is an emerging tool for public health surveillance and has been used to
73 complement clinical surveillance in the monitoring of infectious diseases trends [17-19]. It has
74 been used to track the circulation of SARS-CoV-2, as well as other respiratory [20],

75 gastrointestinal [21], and other infectious diseases [22, 23]. It facilitates early case identification
76 [24], situational assessment [17], and monitoring of public health trends of various infectious
77 diseases in a population [25]. While wastewater monitoring has been largely focused on testing
78 samples collected from wastewater treatment plants and manholes to understand the prevalence
79 of infections in local communities, expanding the approach to aircraft wastewater holds
80 significant potential. The testing of flights from high-traffic destinations and strategic travel
81 nodes could provide an early indication of global transmission trends and serve as an early alert
82 system for emerging or re-emerging infections [1, 26-28].

83

84 Several countries including Australia [29], the United States (US) [30], the United Kingdom
85 [31], France, Denmark [32] and the United Arab Emirates [33] have conducted SARS-CoV-2
86 testing on aircraft wastewater. Among them, the US, France, and Denmark have further
87 conducted sequencing to identify variants from the aircrafts, serving as an indication of the
88 circulating variants originating from the inbound cities. Although the findings were focused on
89 SARS-CoV-2 monitoring, the studies collectively affirm the feasibility of the approach for
90 monitoring emerging infectious diseases threats, and experts advocate for the establishment of a
91 global wastewater surveillance consortium to promote alignment of methodologies and best
92 practices to facilitate monitoring and data sharing across regions [34].

93

94 In this study, we conducted aircraft and airport wastewater surveillance at Singapore Changi
95 airport which serves as a popular mall destination for locals [35] and a travel node in Asia [36].
96 We monitored 17 other pathogens beyond SARS-CoV-2 and its variants from January 2023 to

97 February 2023. In this pilot study, we detected nine other pathogens in aircraft wastewater
98 collected from medium- and long-haul flights, demonstrating the utility of aircraft wastewater
99 surveillance for monitoring respiratory and gastrointestinal diseases, in addition to the
100 monitoring of SARS-CoV-2 variants.

101

102 **Methods**

103 **Wastewater sampling from lavatory service truck**

104 Surveillance of inbound flights were conducted from January 2023 to February 2023, categorised
105 into 26 medium-haul flights (flight duration ≤ 8 h) sampled for the first month and 15 long-haul
106 flights (flight duration > 8 h) sampled for the second month (Table 1), originating from two
107 separate continents respectively. Categorisation of flight duration was guided by previous studies
108 [37, 38]. A lavatory service truck was designated for the collection of samples from aircraft.
109 From the lavatory service truck, samples were transferred into 250 mL screw-capped bottles
110 using a portable peristaltic pump (Masterflex, Germany) and transported to the laboratory for
111 testing. The collection tank of the lavatory service truck was disinfected and flushed between
112 collections. A total of 19 and 15 samples were collected from medium-haul and long-haul flights,
113 respectively. As only one lavatory service truck was deployed for this study, on occasions where
114 multiple flights were scheduled for collection on a single day, the samples from these flights
115 were pooled. Among wastewater samples collected from medium-haul flights, six samples
116 comprised pooled samples from two to three medium-haul flights each.

117

118

119 Wastewater sampling from manholes serving the airport terminal sites

120 Two autosamplers were deployed at two sites in Singapore Changi Airport to draw wastewater
121 samples from the sewage manholes covering the terminals which served both the long- and
122 medium-haul flights (Site 1: Terminals 1 and 2; Site 2: Terminal 3). Autosamplers were
123 programmed to collect four composite samples twice a week, with each composite sample
124 constituting sewage drawn from the sampling point every 15 min over a 6 h period. Samples
125 from each autosampler were transferred into 250 mL screw-capped bottles and transported to the
126 laboratory. A total of 136 (68 each) composite samples across two airport terminal sites were
127 collected.

128

129 Sample processing and nucleic acid extraction

130 Aircraft wastewater samples were centrifuged at 10,000 *g* for 20 min and the supernatant was
131 filtered through a 0.22 μm filter (Corning, Tewksbury, MA, USA). Wastewater samples
132 collected from the airport terminal sites were subjected to a single centrifugation step at 4,000 *g*
133 for 20 min. Virus concentration of the supernatant (~15 mL) was carried out using ultrafiltration
134 and RNA extraction as previously described [24, 39]. A duplex one-step quantitative reverse
135 transcription polymerase chain reaction (RT-qPCR), targeting both SARS-CoV-2 nucleocapsid
136 N1-gene and CPQ_056, was used to screen wastewater samples for the presence of SARS-CoV-
137 2 and rule out PCR inhibitors respectively. Primer and probe sequences were as previously
138 reported [40, 41]. PCR reactions were carried out in a final reaction volume of 20 μL containing
139 0.5 μM of N1 primers and 0.25 μM of N1 probe, 0.25 μM of CPQ_056 primers and 0.125 μM of

140 CPQ_056 probe, 1X Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabs,
141 Ipswich, MA, USA), 1X Luna® RT Enzyme Mix and 2.5 µL of template. Thermal cycling was
142 performed on the QuantStudio 5 machine (Applied Biosystems, USA) as described in the
143 Supplementary Material.

144

145 **Multiple-pathogen detection using panel assays and quantitative polymerase chain reaction**

146 Wastewater samples collected from 12 aircrafts, each originating from unique airports (medium-
147 haul, n=7; long-haul, n=5), between 8 January to 23 February 2023; and wastewater samples
148 collected weekly from the 2 airport terminal sites (n=12) within the same period, were tested
149 using the BioFire Respiratory Panel (RP2.1) assay as per manufacturer's instructions (BioFire
150 Diagnostics LLC, Saltlake, UT). Briefly, sample buffer was added to 300 µL of the ultrafiltration
151 retentate. The sample-buffer mixture was injected into a test pouch and loaded into the BioFire
152 Filmarray Torch Instrument (BioFire Diagnostics LLC, Saltlake, UT) for sample processing,
153 nucleic acid extraction, amplification, and molecular detection. RT-qPCR/qPCR analysis was
154 also performed on RNA extracts in accordance with methods described in Goh *et al.* for the
155 detection of Aichivirus, Adenovirus and Hepatitis A virus [42].

156

157 **Next Generation Sequencing and bioinformatic analyses of SARS-CoV-2 variants**

158 RNA extracts from wastewater samples with a minimum of 20 SARS-CoV-2 RNA copies/µL
159 were further subjected to Next Generation Sequencing (NGS). First strand cDNA synthesis was
160 performed followed by amplicon generation with the ARTIC v4.1 primers panel. The amplicon

161 generation performed is an adapted version of a previously published protocol [43], and the
162 adaptations are further described in the Supplementary Material.

163

164 The data was processed and mapped against the reference sequence (MN908947.3) using the
165 nfcore/viralrecon pipeline [44]. The aligned bam file was then parsed through Freyja [45] to
166 deconvolute the diversity of SARS-CoV-2 in each sample. Only samples that had a minimum of
167 80% genome coverage at a mapped read depth of 10X were used. SARS-CoV-2 variant
168 information of wastewater collected from water reclamation plants and clinical cases were
169 obtained from Singapore's National Environment Agency and Ministry of Health, respectively.

170

171 **Correlation of COVID-19 viral load in wastewater and clinical cases**

172 Correlation analysis of COVID-19 viral load in wastewater and clinical cases was carried out for
173 the period of August 2022 to October 2023. The number of COVID-19 clinical cases reported
174 globally was extracted from the complete Our World in Data COVID-19 dataset
175 (<https://ourworldindata.org/covid-cases>). The LOESS (locally estimated scatterplot smoothing)
176 method was applied to SARS-CoV-2 virus concentration detected in the community and airport
177 terminal sites to summarise the time series observations into a smoothed curve. The SARS-CoV-
178 2 virus concentration in community and airport terminal sites samples were obtained as
179 described in a previous study [17]. The association of viral load in wastewater with clinical cases
180 was assessed with the Pearson correlation coefficient between the respective wastewater datasets
181 and the global cases or local cases. To test the effectiveness of wastewater surveillance as a

182 leading indicator of resurgence of transmission, cross-correlation was performed between the
183 daily clinical cases in Singapore and the smoothed curves of the wastewater datasets.

184

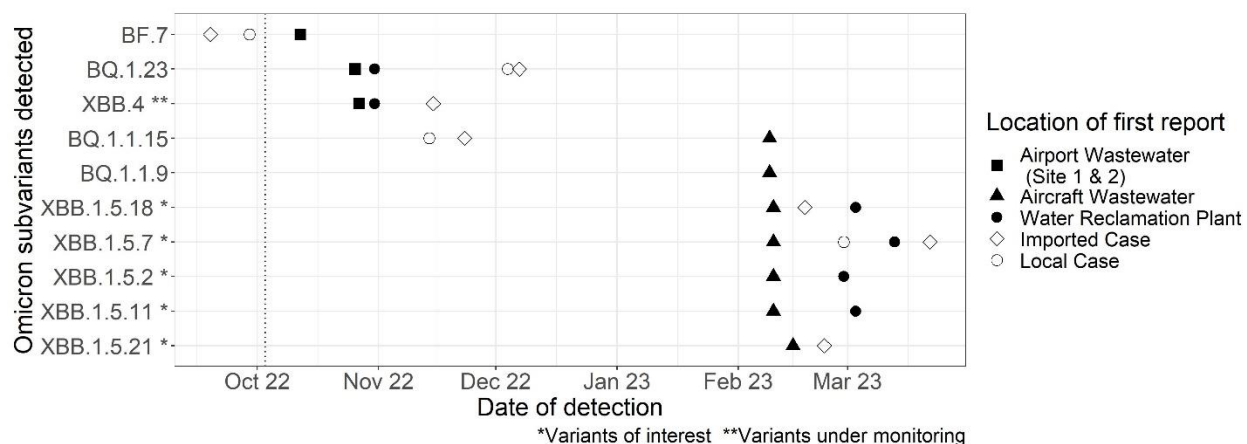
185 **Results**

186 **SARS-CoV-2 variants of concern**

187 SARS-CoV-2 RNA was detected in wastewater samples collected from both aircraft and airport
188 terminal sites. Five variants of interest (VOI) were first detected in aircraft wastewater collected
189 from long-haul flights (XBB.1.5.2, XBB.1.5.7, XBB.1.5.11, XBB.1.5.18, XBB.1.5.21), of which
190 four were eventually detected in the local water reclamation plants samples between 18 to 31
191 days later (XBB.1.5.2, XBB.1.5.7, XBB.1.5.11, XBB.1.5.18) (**Error! Reference source not**
192 **found.**). Among these VOIs, imported clinical cases for three sub-lineages (XBB.1.5.7,
193 XBB.1.5.18, XBB.5.21) were reported 8 to 40 days after it was detected in aircraft wastewater
194 (**Error! Reference source not found.**). Additionally, the XBB.4 variant under monitoring
195 (VUM) was first detected in wastewater collected from the airport terminal (Site 1) prior to
196 detection in water reclamation plants 4 days later, with the first imported clinical case reported
197 19 days later.

198

199



200

201 **Figure 1. Detection of Omicron subvariants in wastewater samples collected from aircraft,**
 202 **airport terminal sites, water reclamation plants, and in clinical samples of reported cases.**
 203 Detection of most SARS-CoV-2 variants in wastewater samples collected from aircraft (closed
 204 triangles) and airport terminal sites (closed squares), preceded the detection in wastewater
 205 samples collected from inland water reclamation plants (closed circles), and in clinical samples
 206 (imported cases: open diamond; local cases: open circles).
 207

208 A total of ten variants (44 sub-lineages) were successfully identified from NGS sequencing of
 209 wastewater samples collected from airport terminal sites (40 sub-lineages) and medium- and
 210 long-haul aircrafts (4 sub-lineages) (Table 1, Supplementary Material Table S1). All the variants
 211 identified were sub-lineages of the Omicron variant and several of these sub-lineages have been
 212 classified as VOIs and VUMs by the World Health Organization (WHO). The proportion of
 213 positive SARS-CoV-2 samples in aircraft wastewater was higher (60-68%) than in wastewater
 214 collected from airport terminal sites (13-41%) (Table 1).

215

216 **Table 1. Proportion of positive SARS-CoV-2 samples and variants identified in wastewater**
 217 **collected from aircrafts and airport terminal sites**

Collection Date (2023)	Site/ Flight	No. of Samples	SARS-CoV-2 Detected	Met Sequencing Analysis Requirements ¹	Variants Identified ²
January	Medium-Haul	19 ³	13/19 (68%)	1/19 (5.26%)	BA.5.2.48
	Airport (Site 1)	36	8/36 (22%)	0/36 (0%)	NA
	Airport (Site 2)	36	10/36 (27%)	1/36 (2.8%)	BA.2.3.20
February	Long-Haul	15	9/15 (60%)	3/15 (20%)	BQ.1.1 XBB.1.5
	Airport (Site 1)	32	4/32 (13%)	1/32 (3.13%)	XBB.2.4 XBB.1.5.X XBB.1.11.X
	Airport (Site 2)	32	13/32 (41%)	4/32 (12.5%)	XBB.1.5.X XBB.1.9.X XBB.1.11.X XBB.1.18.X XBB.1.22.X XBL

218

219 ¹ Only samples with $\geq 80\%$ genome coverage, 10 times read depth are analysed220 ²Detailed sub-lineages are provided in Supplementary Material Table S1221 ³ Six samples comprised pooled samples from two to three medium-haul flights

222

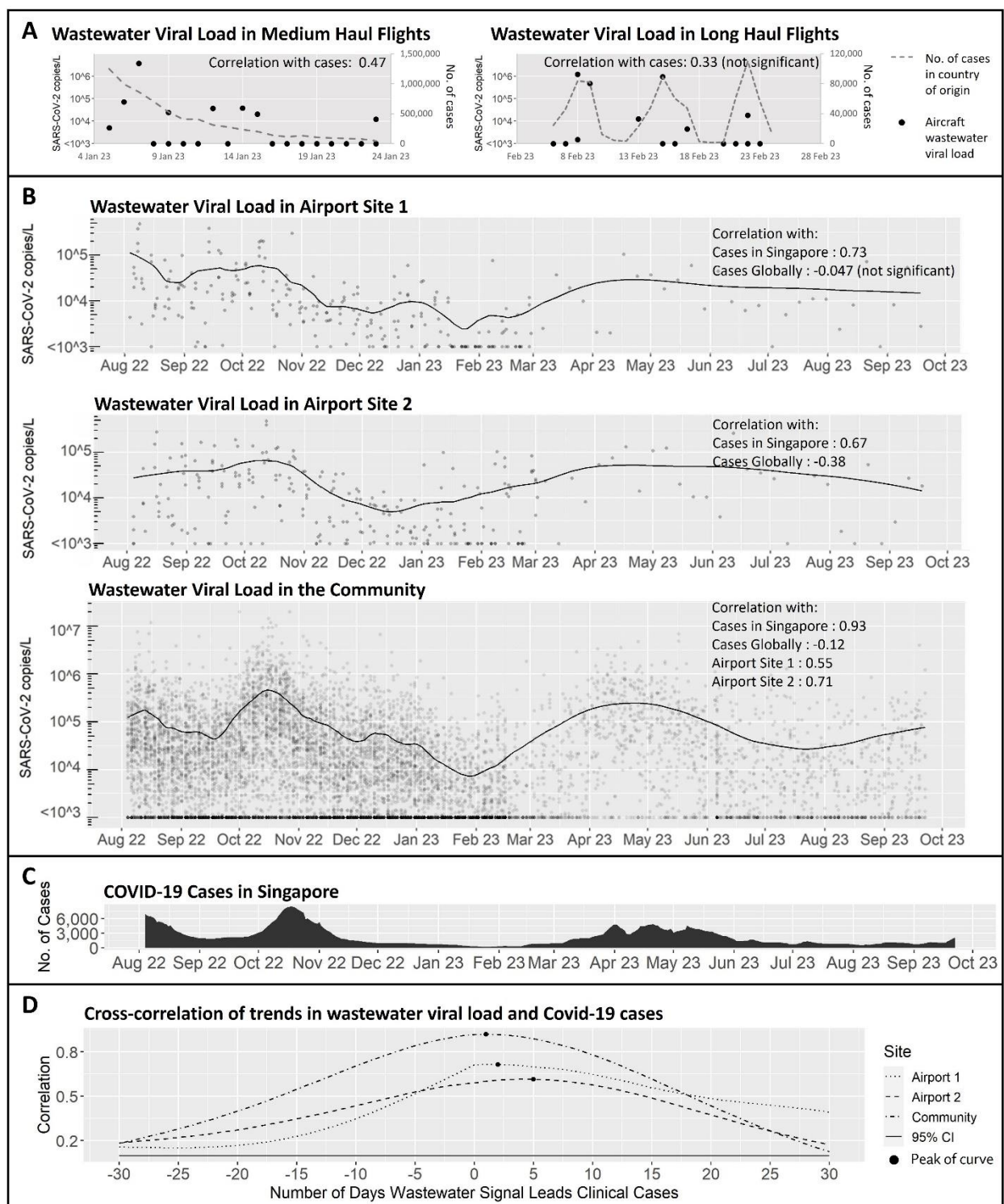
223 **Positive correlation of SARS-CoV-2 virus load from airport terminal sites with local**224 **COVID-19 cases**

225 Comparison of wastewater viral load trends at both the airport terminal sites revealed higher

226 positive correlation with local COVID-19 cases ($r = 0.73$ and 0.67) than those reported globally

227 (negative correlation, $r=-0.0047$, not significant, and -0.38) (Supplementary Material Table S2).
228 Positive correlation was also observed between the local wastewater viral load trend in the
229 community and that of both airport terminal sites ($r = 0.55$ and 0.71) (Supplementary Material
230 Table S3). Expectedly, the trend of local COVID-19 cases was more strongly associated with the
231 wastewater viral load trend from autosamplers deployed in the community compared to those
232 from airport terminal sites ($r = 0.93$ vs $r = 0.73$ and 0.67) (Supplementary Material Table S2)
233 [17]. Nevertheless, it was interesting to note that cross-correlation analyses suggested that the
234 wastewater viral load trends at the airport terminal sites led the trend in local COVID-19 cases
235 by two and five days, respectively, while the wastewater viral load trend in the community led by
236 a day (Figure C).

237



238

239 **Figure 2. SARS-CoV-2 virus load and COVID-19 cases.** Trend of SARS-CoV-2 viral load in
 240 (A) aircraft wastewater samples from medium- and long-haul flights, (B) Airport terminal sites
 241 and the community, and (C) COVID-19 cases reported in Singapore. (D) Cross-correlation of
 242 trends in wastewater viral load (airport terminal sites and community) and COVID-19 cases.

243

244 Detection of multiple pathogens in aircraft and airport wastewater samples

245 All aircraft wastewater samples screened for multiple pathogens yielded positive detections of
246 more than one pathogen. A total of five respiratory viruses (coronavirus NL63,
247 rhinovirus/enterovirus, influenza A virus, Parainfluenza type 3 virus, Respiratory Syncytial
248 Virus) and four enteric viruses (Norovirus, Aichivirus, Adenovirus, Hepatitis A) were detected,
249 with enteric viruses detected at higher proportions when compared with respiratory viruses (14-
250 86% vs 14-60%) (Figure 3).

251

252 Samples collected from the airport terminal sites had a higher proportion testing positive, and
253 yielded more pathogens (8-100%, n=14), when compared with aircraft wastewater samples (mid-
254 haul flights, 14-86%, n=8; long-haul flights, 20-60%, n=6). Both airport terminal sites and
255 aircraft wastewater samples had higher proportion of samples tested positive for enteric viruses
256 (14-100%) than for respiratory viruses (8-83%) (Figure 3).

		Mid-Haul Flights (n=7)	Long-Haul Flights (n=5)	Airport Terminal (n=12)
	Start Date:	8 Jan	6 Feb	9 Jan
	End Date:	23 Jan	23 Feb	23 Feb
Respiratory	Coronavirus 229E	0%	0%	42%
	Coronavirus HKU1	0%	0%	50%
	Coronavirus NL63	14%	0%	25%
	Coronavirus OC43	0%	0%	67%
	Human Metapneumovirus	0%	0%	0%
	Human Rhinovirus/Enterovirus	29%	60%	83%
	Influenza A	14%	0%	58%
	Influenza B	0%	0%	17%
	Parainfluenza 1	0%	0%	8%
	Parainfluenza 2	0%	0%	8%
	Parainfluenza 3	0%	20%	58%
	Parainfluenza 4	0%	0%	17%
	RSV	14%	20%	0%
Enteric	Norovirus (GII)	86%	40%	100%
	Aichivirus	29%	20%	92%
	Adenovirus	57%	40%	92%
	Hepatitis A Virus	14%	0%	0%

257

258 **Figure 3. Multi-pathogens detected in aircraft and airport terminal sites wastewater**
 259 **samples.** Samples collected from the airport terminal sites had a higher proportion testing
 260 positive and yielded more pathogens than aircraft wastewater samples. Both airport terminal sites
 261 and aircraft wastewater samples yielded higher proportions of samples testing positive for enteric
 262 viruses than for respiratory viruses.

263

264 Discussion

265 The detection of some SARS-CoV-2 variants in aircraft wastewater preceded the detection in
 266 wastewater samples collected from inland water reclamation plants and in clinical samples.
 267 Specifically, the detection of various VOIs (i.e. sub-lineages of XBB.1.5.X) in long-haul flights
 268 preceded the detections in inland wastewater and clinical samples by 31 days and 18 days,
 269 respectively. Furthermore, a VUM (XBB.4) was also detected in airport terminal sites'
 270 wastewater 4 and 19 days earlier than detections in inland wastewater and clinical samples,

271 respectively. Collectively, the findings suggest the usefulness of aircraft and airport wastewater
272 surveillance to potentially provide early signals of new virus introductions. This approach would
273 be particularly useful for monitoring more transmissible or deadly variants, especially if
274 surveillance was carried out at frequencies which could provide insights on likely introductions
275 to the country.

276

277 Sampling wastewater from airport terminals have been suggested as a proxy for monitoring
278 aircraft wastewater, particularly if there were capacity or resource constraints [46]. However, our
279 study revealed that the viral load trends from airport terminal sites' wastewater samples
280 correlated more strongly with the trends in community cases rather than global cases; potentially
281 providing an indication of the trend of COVID-19 infections in the community instead. Cross-
282 correlation analyses of viral load trends from airport terminal sites and community wastewater
283 samples showed that wastewater signals were observed to be lead indicators of local COVID-19
284 case trends, further highlighting the utility of wastewater surveillance as an important
285 supplemental to clinical surveillance systems. Notably, the detection of more pathogens in
286 wastewater from airport terminal sites compared to aircraft could possibly be due to the
287 combined wastewater coverage from travellers from multiple regions as well as the local
288 community. In our settings, we were limited by the availability of suitable sampling points in the
289 airport, and the sampling points could not segregate sewage lines serving the aircrafts from those
290 serving the airport. As the airport in Singapore comprises retail outlets that are popular with the
291 local community [35], and are staffed mostly by resident workers, these could partially explain
292 the corroboration of trends in wastewater viral load and community cases. While the testing of
293 our airport terminal sites' wastewater samples may provide limited insights on the global

294 situation, its utility could be increased at airports with segregated sampling points and with lower
295 traffic from the community.

296

297 The successful detection of respiratory and enteric viruses in aircraft wastewater samples
298 underscores the utility of aircraft wastewater monitoring for other infectious diseases beyond
299 SARS-CoV-2. The monitoring of medium- and long- haul flights also revealed unique trends of
300 the respective regions. For example, norovirus and adenovirus were found at a higher proportion
301 in medium-haul flights originating from the same continent, as compared to rhinoviruses/
302 enteroviruses which were detected at a higher proportion in long-haul flights. Overall, these
303 trends could reflect the epidemiology and circulating viruses of the originating regions. When
304 medium- and long-haul flights were further compared, we found that the proportion of positive
305 SARS-CoV-2 samples (44% vs 60%) and the sequencing rate (1% vs 8%) were lower for
306 medium-haul flights. One possible explanation could be the lower likelihood of toilet visits, and
307 in turn, virus shedding, for passengers onboard shorter flights [37].

308

309 This study has a few limitations. The scope of the study was a pilot study limited to surveillance
310 of 19 medium- and 15 long-haul flights from January to February 2023. The toilet use patterns of
311 the travelers were unknown and it was not possible to infer virus shedding rates in wastewater,
312 and in turn, disease incidence. The study was also limited by the availability of sampling trucks
313 and the design of the sewer network at the airport. On the latter point, it was thus not possible to
314 segregate wastewater collected from travelers from that of the local community.

315

316 Despite the potential of aircraft wastewater monitoring, our study has revealed challenges and
317 the need for more efficient sampling and testing solutions. Sampling has been largely carried out
318 via lavatory service trucks or grab sampling [31-33], with only a few companies offering devices
319 capable of sampling directly from the aircraft with minimal disruption to the short turnaround
320 time (90-120 min for large aircraft and 25-40 min for smaller aircraft) [29, 30, 47, 48]. Aircraft
321 wastewater is less diluted than regular sewage and presents challenges in sample preparation
322 [29]; while the wastewater sample may have a higher viral or bacterial load, the concentrated
323 wastewater may lead to clogged virus concentration filters and inhibition from disinfectants,
324 among others. Variant analyses via NGS also have limited sensitivity in detecting SARS-CoV-2
325 in samples with low viral loads, especially in complex matrices such as aircraft wastewater.
326 Mutations within viral genomes may also affect the binding efficiency of amplification primers,
327 potentially leading to low coverage rates across the genome. The accuracy of deconvolution
328 tools, like Freyja used in this study, is also dependent on existing lineage information obtained
329 by prior clinical sequencing data and published sequences [45]. Lastly, the complicated process
330 of NGS sample preparation prior to its subsequent analyses, and the turn-around-time required
331 for sequencing may be a bottleneck, delaying the availability of results and subsequent public
332 health responses.

333

334 While conducting wastewater testing at airport terminals and aircrafts may offer an early
335 warning of diseases for the local community, modelling analyses reveal that data from a network
336 of airports are needed to provide early warning and situational awareness of emerging outbreaks
337 [49]. It is therefore needful to encourage innovation in wastewater sampling and testing
338 technologies, and to work as a network in a global initiative. This collaboration would yield

339 synergistic effect, enhancing global surveillance and situational awareness of emerging
340 pathogens.

341

342 **Conflict of Interest**

343 The authors have no conflicts of interest to declare.

344

345 **Author Contribution**

346 MT, BL, MHI: data curation, formal analysis, investigation, methodology, writing – original
347 draft. JY, DM, KYHG: formal analysis, investigation. SRC, ZJMH, TYL: conceptualization.

348 NLC: Supervision, conceptualization, methodology. JWCC: Supervision, conceptualization,

349 methodology, formal analysis, writing – original draft. All authors provided critical review and

350 revision of the manuscript and were responsible for the decision to submit for publication.

351

352 **Funding**

353 This study was funded by Singapore’s Ministry of Finance and the National Environment
354 Agency.

355

356 **Data Availability**

357 The data underlying this article will be shared on request and may be subject to approval and/or a
358 data sharing contract.

359

360

361 Acknowledgements

362 The authors thank the Wastewater-Based Epidemiology Branch and Molecular Biotechnology
 363 Branch of the Microbiology and Molecular Epidemiology Division in the National Environment
 364 Agency for their support in wastewater sampling and testing of SARS-CoV-2 and other
 365 pathogens. The authors appreciate Mr. Alvin Xie Cheng Goh for his assistance with variant
 366 monitoring of clinical samples. The authors also thank the Changi Airport Group and SIA
 367 Engineering company for facilitating the collection of aircraft wastewater samples. The authors
 368 appreciate the Public Utilities Board, Singapore's Water Agency, for their provision of water
 369 reclamation plant samples and their support in access to the sewer network.

370

371 References

- 372 1. Sokadjo, Y.M. and M.N. Atchadé, *The influence of passenger air traffic on the spread of*
 373 *COVID-19 in the world*. Transportation Research Interdisciplinary Perspectives, 2020. **8**:
 374 p. 100213.
- 375 2. Bielecki, M., et al., *Air travel and COVID-19 prevention in the pandemic and peri-*
 376 *pandemic period: A narrative review*. Travel Medicine and Infectious Disease, 2021. **39**:
 377 p. 101915.
- 378 3. Yokota, I., P.Y. Shane, and T. Teshima, *Logistic advantage of two-step screening*
 379 *strategy for SARS-CoV-2 at airport quarantine*. Travel Medicine and Infectious Disease,
 380 2021. **43**: p. 102127.
- 381 4. Le, T.-M., et al., *Framework for assessing and easing global COVID-19 travel*
 382 *restrictions*. Scientific Reports, 2022. **12**(1): p. 6985.
- 383 5. Wilson, N., et al., *Estimating the impact of control measures to prevent outbreaks of*
 384 *COVID-19 associated with air travel into a COVID-19-free country*. Sci Rep, 2021.
 385 **11**(1): p. 10766.
- 386 6. Ge, Y., et al., *Impacts of worldwide individual non-pharmaceutical interventions on*
 387 *COVID-19 transmission across waves and space*. International Journal of Applied Earth
 388 Observation and Geoinformation, 2022. **106**: p. 102649.
- 389 7. Beesley, L.J., et al., *SARS-CoV-2 variant transition dynamics are associated with*
 390 *vaccination rates, number of co-circulating variants, and convalescent immunity*.
 391 eBioMedicine, 2023. **91**.

- 392 8. Samieefar, N., et al., *Delta Variant: The New Challenge of COVID-19 Pandemic, an*
 393 *Overview of Epidemiological, Clinical, and Immune Characteristics*. Acta Biomed, 2022.
 394 **93**(1): p. e2022179.
- 395 9. McLean, G., et al., *The Impact of Evolving SARS-CoV-2 Mutations and Variants on*
 396 *COVID-19 Vaccines*. mBio, 2022. **13**(2): p. e0297921.
- 397 10. Yang, B., et al., *Comparison of control and transmission of COVID-19 across epidemic*
 398 *waves in Hong Kong: an observational study*. Lancet Reg Health West Pac, 2024. **43**: p.
 399 100969.
- 400 11. Abdool Karim, S.S. and T. de Oliveira, *New SARS-CoV-2 Variants — Clinical, Public*
 401 *Health, and Vaccine Implications*. New England Journal of Medicine, 2021. **384**(19): p.
 402 1866-1868.
- 403 12. UnitedNations. *COVID-19 SOCIO-ECONOMIC IMPACT*. 2023 [cited 2023; Available
 404 from: <https://www.undp.org/coronavirus/socio-economic-impact-covid-19>.
- 405 13. Wei, X., L. Li, and F. Zhang, *The impact of the COVID-19 pandemic on socio-economic*
 406 *and sustainability*. Environ Sci Pollut Res Int, 2021. **28**(48): p. 68251-68260.
- 407 14. OECD, *Ready for the Next Crisis? Investing in Health System Resilience*. 2023.
- 408 15. The Lancet Respiratory, M., *Patterns of respiratory infections after COVID-19*. The
 409 Lancet Respiratory Medicine.
- 410 16. Marani, M., et al., *Intensity and frequency of extreme novel epidemics*. Proc Natl Acad
 411 Sci U S A, 2021. **118**(35).
- 412 17. Wong, Y.H.M., et al., *Positive association of SARS-CoV-2 RNA concentrations in*
 413 *wastewater and reported COVID-19 cases in Singapore – A study across three*
 414 *populations*. Science of The Total Environment, 2023. **902**: p. 166446.
- 415 18. Chua, F.J.D., et al., *Co-occurrence of BA.1 and BA.2 at the start of Singapore's Omicron*
 416 *wave revealed by Community and University Campus wastewater surveillance*. Sci Total
 417 Environ, 2023. **875**: p. 162611.
- 418 19. Mohapatra, S., et al., *Wastewater surveillance of SARS-CoV-2 and chemical markers in*
 419 *campus dormitories in an evolving COVID - 19 pandemic*. J Hazard Mater, 2023. **446**: p.
 420 130690.
- 421 20. Boehm, A.B., et al., *Wastewater concentrations of human influenza, metapneumovirus,*
 422 *parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-*
 423 *acids during the COVID-19 pandemic: a surveillance study*. The Lancet Microbe, 2023.
 424 **4**(5): p. e340-e348.
- 425 21. Chacón, L., et al., *Wastewater-Based Epidemiology of Enteric Viruses and Surveillance*
 426 *of Acute Gastrointestinal Illness Outbreaks in a Resource-Limited Region*. Am J Trop
 427 Med Hyg, 2021. **105**(4): p. 1004-1012.
- 428 22. Wolfe, M.K., et al., *Use of Wastewater for Mpox Outbreak Surveillance in California*. N
 429 Engl J Med, 2023. **388**(6): p. 570-572.
- 430 23. Wong, J.C.C., et al., *Zika Surveillance Complemented with Wastewater and Mosquito*
 431 *Testing*. Social Science Research Network [Preprint] Available at SSRN:
 432 <https://ssrn.com/abstract=4512915> or <http://dx.doi.org/10.2139/ssrn.4512915>, 2023.
- 433 24. Wong, J.C.C., et al., *Non-intrusive wastewater surveillance for monitoring of a*
 434 *residential building for COVID-19 cases*. The Science of the total environment, 2021.
 435 **786**: p. 147419-147419.
- 436 25. Diamond, M.B., et al., *Wastewater surveillance of pathogens can inform public health*
 437 *responses*. Nature Medicine, 2022. **28**(10): p. 1992-1995.

- 438 26. Bogoch, I.I., et al., *Anticipating the international spread of Zika virus from Brazil*. The
439 Lancet, 2016. **387**(10016): p. 335-336.
- 440 27. Hosseini, P., et al., *Predictive Power of Air Travel and Socio-Economic Data for Early*
441 *Pandemic Spread*. PLOS ONE, 2010. **5**(9): p. e12763.
- 442 28. Tian, H., et al., *Increasing airline travel may facilitate co-circulation of multiple dengue*
443 *virus serotypes in Asia*. PLOS Neglected Tropical Diseases, 2017. **11**(8): p. e0005694.
- 444 29. Ahmed, W., et al., *Wastewater surveillance demonstrates high predictive value for*
445 *COVID-19 infection on board repatriation flights to Australia*. Environ Int, 2022. **158**: p.
446 106938.
- 447 30. Morfino, R.C., et al., *Notes from the Field: Aircraft Wastewater Surveillance for Early*
448 *Detection of SARS-CoV-2 Variants - John F. Kennedy International Airport, New York*
449 *City, August-September 2022*. MMWR Morb Mortal Wkly Rep, 2023. **72**(8): p. 210-211.
- 450 31. Farkas, K., et al., *Wastewater-based monitoring of SARS-CoV-2 at UK airports and its*
451 *potential role in international public health surveillance*. PLOS Glob Public Health,
452 2023. **3**(1): p. e0001346.
- 453 32. Qvesel, A.G., et al., *SARS-CoV-2 Variants BQ.1 and XBB.1.5 in Wastewater of Aircraft*
454 *Flying from China to Denmark, 2023*. Emerg Infect Dis, 2023. **29**(12): p. 2559-2561.
- 455 33. Albastaki, A., et al., *First confirmed detection of SARS-COV-2 in untreated municipal*
456 *and aircraft wastewater in Dubai, UAE: The use of wastewater based epidemiology as an*
457 *early warning tool to monitor the prevalence of COVID-19*. Sci Total Environ, 2021.
458 **760**: p. 143350.
- 459 34. Murakami, M., et al., *The growing need to establish a global wastewater surveillance*
460 *consortium for future pandemic preparedness*. J Travel Med, 2023. **30**(7).
- 461 35. Hamdi, R. *Singapore's Changi Is Changing the Idea of What an Airport Can Be*. 2019
462 [cited 2024; Available from: [https://skift.com/2019/03/11/singapores-changi-is-changing-](https://skift.com/2019/03/11/singapores-changi-is-changing-the-idea-of-what-an-airport-can-be/)
463 [the-idea-of-what-an-airport-can-be/](https://skift.com/2019/03/11/singapores-changi-is-changing-the-idea-of-what-an-airport-can-be/)].
- 464 36. Cripps, K. *Singapore's Changi Airport fully reopens Terminal 2 following dramatic*
465 *makeover*. 2023 [cited 2024; Available from: [https://edition.cnn.com/travel/singapore-](https://edition.cnn.com/travel/singapore-changi-airport-terminal-2-expansion/index.html)
466 [changi-airport-terminal-2-expansion/index.html](https://edition.cnn.com/travel/singapore-changi-airport-terminal-2-expansion/index.html)].
- 467 37. Jones, D.L., et al., *Suitability of aircraft wastewater for pathogen detection and public*
468 *health surveillance*. Science of The Total Environment, 2023. **856**: p. 159162.
- 469 38. Wilkerson, J.T., et al., *Analysis of emission data from global commercial aviation: 2004*
470 *and 2006*. Atmos. Chem. Phys., 2010. **10**(13): p. 6391-6408.
- 471 39. Mailepessov, D., et al., *Development of an efficient wastewater testing protocol for high-*
472 *throughput country-wide SARS-CoV-2 monitoring*. Sci Total Environ, 2022. **826**: p.
473 154024.
- 474 40. Niu, C., et al., *Interlaboratory assessment of quantification of SARS-CoV-2 RNA by*
475 *reverse transcription digital PCR*. Analytical and Bioanalytical Chemistry, 2021. **413**: p.
476 7195-7204.
- 477 41. Stachler, E., et al., *Quantitative CrAssphage PCR assays for human fecal pollution*
478 *measurement*. Environmental science & technology, 2017. **51**(16): p. 9146-9154.
- 479 42. Goh, S.G., et al., *Occurrence of microbial indicators, pathogenic bacteria and viruses in*
480 *tropical surface waters subject to contrasting land use*. Water Res, 2019. **150**: p. 200-
481 215.
- 482 43. D, D.N.A., et al., *COVID-19 ARTIC v3 Illumina library construction and sequencing*
483 *protocol v5*. 2020.

- 484 44. Patel, H., et al., *nf-core/viralrecon: nf-core/viralrecon v2.6.0 - Rhodium Raccoon*. 2023,
485 Zenodo.
- 486 45. Karthikeyan, S., et al., *Wastewater sequencing reveals early cryptic SARS-CoV-2 variant*
487 *transmission*. *Nature*, 2022. **609**(7925): p. 101-108.
- 488 46. Deere, D., et al., *Ad-hoc guidance: Wastewater sampling of aircrafts for SARS-CoV-2*
489 *surveillance: A guidance document for Member States*. 2023.
- 490 47. Wegrzyn, R.D., et al., *Early Detection of Severe Acute Respiratory Syndrome*
491 *Coronavirus 2 Variants Using Traveler-based Genomic Surveillance at 4 US Airports,*
492 *September 2021-January 2022*. *Clin Infect Dis*, 2023. **76**(3): p. e540-e543.
- 493 48. Wignall, A. *How it works: the aircraft turnaround*. 2022 [cited 2024; Available from:
494 <https://www.aerotime.aero/articles/32767-how-it-works-the-aircraft-turnaround>.
- 495 49. Jin, S., et al., *Feasibility of wastewater-based detection of emergent pandemics through a*
496 *global network of airports*. *PLOS Global Public Health*, 2024. **4**(3): p. e0003010.
- 497