Pathogen Epidemics Investigation through Aircraft Wastewater Surveillance

MGI's ATOPlex Technology and DNBSEQ-G400 Sequencing Platform Decipher the Genome of the Omicron Variant

CSIRO Land and Water of Australia, in collaboration with MGI and other institutes successfully detected and traced the SARS-CoV-2 variant Omicron in aircraft wastewater based on the MGI ATOPlex multiplex PCR technology and DNBSEQ sequencing platform for the first time.

This work was published in the journal *Science of the Total Environment* in 2022, under the title of "Detection of the Omicron (B.1.1.529) variant of SARS-CoV-2 in aircraft wastewater"[1].

Recommended applications: Pathogenic microorganisms -SARS-CoV-2

Recommended model: DNBSEQ-G400RS

- ### SARS-CoV-2 targeted sequencing specialist

  MGI's SARS-CoV-2 amplicon-based library preparation technology, DNBSEQ sequencing platform and the supporting analysis software facilitate the identification, genotyping and tracing of COVID-19.

- ### Efficient and high-quality sequencing data output

  DNBSEQ sequencing technology exhibits many excellent features such as high accuracy, low repeat rate and low index hopping rate.

- ### Compatible with automated sample preparation system

  MGI's SARS-CoV-2 targeted sequencing solution is compatible with MGI's self-developed automated equipment, thus endowing efficient sample extraction and library preparation.

Background

On November 25, 2021, the National Institute for Communicable Diseases (NCID) of South African detected B.1.1.52, a new SARS-CoV-2 subtype, based on the genome sequencing results of 22 COVID-19 patients in Gauteng province[2]. The new mutation in this subtype leads to greater infectivity, virulence and immune escape ability in the human body, and reduces the effectiveness of previous defenses against COVID-19. Thus, the Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE), World Health Organization (WHO) classified the subtype as a variant of concern (VOC) and named it Omicron the next day. There are more than 50 mutation sites in the genome of the Omicron, of which more than 30 mutation sites are located on spike proteins that bind to host cell receptors[3]. More seriously, preliminary analysis of SARS-CoV-2 repeated infection in South Africa suggested that Omicron had a higher probability of repeated infection than other VOC variants.

On the day when the subtype was named Omicron, travel-related cases of Omicron were found in such regions as Belgium, Hong Kong and Israel. On November 27, 115 confirmed cases were reported worldwide[4]. In order to avoid virus transmission, more than 50 countries including Australia took such measures as travel restrictions and strengthening border management. However, on November 29, 2021, the first positive case of Omicron found on a repatriation flight from South Africa, was reported in Australia.

Monitoring airliner wastewater, especially when travel became the transmission path of sudden VOCs, may be an effective means to monitor VOCs. In the early days of the COVID-19 outbreak, cases of SARS-CoV-2 virus were successfully detected in the aircraft wastewater of international flights[5]. Recent scientific studies indicated that the monitoring of airline wastewater had an accuracy of up to 83.7% for the monitoring of negative nucleic acid of COVID-19 in international flights[6].

Research description

In this study, the Omicron variant was successfully detected in the aircraft wastewater of an international flight arriving in Australia based on MGI's ATOPlex platform and DNBSEQ sequencing technology for the first time. This finding provides further evidence of aircraft wastewater as an infectious vector, which also confirms the important role of aircraft wastewater as an independent and non-invasive monitoring site for coronavirus.

Materials and Methods

Sample collection and RT-qPCR analysis

In this experiment, a total of 12 aircraft wastewater samples (A1-A12) were collected from international flights arriving in Australia. Subsequently, 50 ml of each sample was centrifuged, the supernatant was concentrated, washed and underwent RNA extraction. Finally, the extracted RNA was washed with buffer.

Previous studies showed that the RT-qPCR detection of N genes in SARS-CoV-2 could be used for the detection and quantification of RNA in SARS-CoV-27. The detection of the target S gene del (69-70) mutation could initially determine the presence of the Omicron variant. Thus, the presence of the Omicron subtype in wastewater samples should be verified first.

Library preparation and sequencing

In this study, the sequencing library was constructed using MGI's ATOPlex technology and brand A's ARTIC V3 technology. For the former, the ATOPlex SARS-CoV-2 full-length genome panel was used to construct the short amplicon (240-333 bp) library. During library construction, the input RNA was reversely transcripted into cDNA; then the Lambda bacteriophage DNA (200 GC) was added as a control into each sample to ensure that each sample could produce enough amplification products for sequencing; DNA/cDNA samples were purified and quantified after two rounds of PCR amplification (concentration ≥ 4 ng/μL).

The above libraries were equimolarly mixed and underwent single-stranded DNA circularization to form ssCirDNA using the MGIEasy Dual Barcode Circularization Module. Afterwards, the DNA nanoball (DNB) library was generated by rolling circle amplification and underwent paired-end 100 sequencing (PE100) on the MGI DNBSEQ-G400 genetic sequencer.

Bioinformatics analysis

After sequencing, the ATOPlex based sequencing data were processed according to the SARS-CoV-2_MultiPCR_v1.0 work flow (https://github.com/MGItech-bioinformatics/SARS-CoV-2_Multi-PCR_v1.0). The data were processed as follows: The primer sequences were filtered and the rest data was aligned to the SARS-CoV-2 genome, Lambda bacteriophage DNA or GAPDH. The data size of SARS-CoV-2 in each sample was normalized with the data size of the bacteriophage. The data size of the variant was calculated by the Freebayes platform based on the comparative result of BWA mem version 0.7.13-r1126. In addition, the consistent sequence of the genome of each sample was generated according to the variation informa-

tion and the genome of the SARS-CoV-2 variant

Finally, the virus genome sequences detected in aircraft wastewater were compared with the latest SARS-CoV-2 genomes for clade distribution, mutation identification, genome quality inspection, construction of phylogenetic tree and visual comparison. The overall process of this study is shown in Fig. 1.

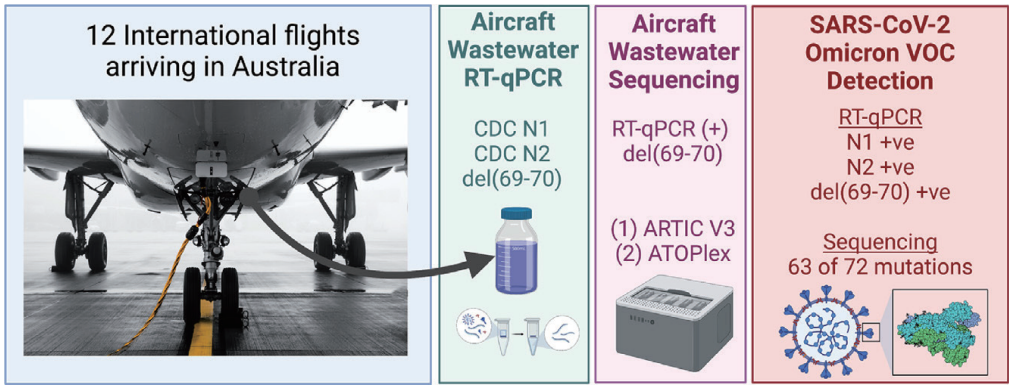

Fig. 1 The overall process of this study.

Sample collection	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
A total of 12 aircraft wastewater samples were collected from international flights arriving in Australia (A1-A12).	 ATOPlex RNA Multiplex PCR-based Library Preparation Set  DNBSEQ-G400 genetic sequencer	SARS-CoV-2_ MultiPCR_v1.0 Freebayes platform BWA	Clade distribution, Mutation identification, Genome quality inspection, Construction of phylogenetic tree.

Results

RT-qPCR was used to detect whether the samples contained SARS-CoV-2

RT-qPCR analysis results suggested that for the SARS-CoV-2 US CDC N1 and N2 tests, SARS-CoV-2 virus was present in the A3 and A12 samples. Except for the A12 sample, no other aircraft wastewater samples were amplified according to del (69-70) analysis. For the A12 sample, the mean Cq value of the del (69-70) test was 35.5±0.17, suggesting that the Omicron variant BA.1 (the Omicron virus is classified as BA.1 and BA.2, of which BA.1 can be confirmed by SGTF) was detected in this sample (Table 1).

Flight No.	Departure port– sampling port	Flight duration	Sampling date	Number of passengers	Mean ± SD log_{10} GC/50 mL of wastewater		Mean Cq ± SD
					US CDC N1	US CDC N2	del(69–70)
A1	JNB-DRW	~ 14 h	11/04/2021	193	<ALOD	<ALOD	<ALOD
A2	JNB-DRW	~ 14 h	19/08/2021	166	<ALOD	<ALOD	<ALOD
A3	IST-DRW	~ 14 h 25 min	2/10/2021	142	2.98 ± 0.13	3.01 ± 0.27	<ALOD
A4	LAX-SYD	~ 15 h	03/11/2021	108	<ALOD	<ALOD	<ALOD
A5	LAX-SYD	~ 15 h	04/11/2021	Freighter service (no passengers)	<ALOD	<ALOD	<ALOD
A6	DEL-DRW	~ 11 h 40 min	07/11/2021	37	<ALOD	<ALOD	<ALOD
A7	LAX-SYD	~ 15 h	08/11/2021	109	<ALOD	<ALOD	<ALOD
A8	LAX-SYD	~ 15 h	09/11/2021	147	<ALOD	<ALOD	<ALOD
A9	DEL-DRW	~ 11 h 40 min	10/11/2021	77	<ALOD	<ALOD	<ALOD
A10	LAX-SYD	~ 15 h	14/11/2021	Freighter service (no passengers)	<ALOD	<ALOD	<ALOD
A11	LAX-SYD	~ 15 h	15/11/2021	191	<ALOD	<ALOD	<ALOD
A12	JNB-DRW	~ 14 h	25/11/2021	20	4.30 ± 0.02	4.19 ± 0.03	35.5 ± 0.17

JNB: O.R. Tambo International airport.
DRW: Darwin International Airport.
LAX: Los Angeles International Airport.
SYD: Sydney International Airport.
DEL: Indira Gandhi International Airport.
IST: Istanbul Airport.
ALOD: Assay limit of detection.

Table 1 The collected wastewater samples were analyzed by RT-qPCR for the presence of SARS-CoV-2 or Omicron subtype RNA.

ATOPlex-based sequencing had wider coverage and sensitivity

High-throughput sequencing was performed on the A12 sample using the ATOPlex and ATRIC V3 technology. The comparative results of the two different detecting approaches showed that ATOPlex had higher genome coverage than ATRIC V3: the coverage of ATRIC V3 was only 61%, while that of ATOPlex was up to 99%, and all Omicron mutations were detected by ATOPlex (20 mutations detected by ARTIC V3) (Fig. 2). The main reason for such a huge difference is that 259 pairs of primers were added in the ATOPlex workflow, and the multiplex primer method would not be interfered by Omicron mutations and could avoid the impact of primer loss. This also suggested that ATOPlex had higher sensitivity and accuracy to low-depth mutations (Fig.2).

The final results showed that due to the low genome coverage and fragmented sequencing of ARTIC V3, further confirmation of the virus strains was limited to some extent. The phylogenetic tree generated by sequencing based on ATOPlex is very close to the reference comparison, and this also proves that ATOPlex can recover as much genome information of the virus as possible, so as to accurately and rapidly trace virus variants.

The ATOPlex based sequencing results showed better performance

In addition, the reads of the ATOPlex platform is better than those of ATRIC V3; a total of 4.8 Gb sequencing data and 24M PE100 sequencing reads were obtained from A12 wastewater samples from ATOPlex panel, with Q30 > 88.3%. Reads were filtered according to the reads quality, adapter and unknown base (N) rate, 96.1% of the reads were used for down-stream analysis, suggesting the high quality of the data.

Cluster analysis tracing

Phylogenetic tree suggested that compared with wild-type strains, the A12 wastewater sample was clustered well with the clinical isolates of Australian Omicron. The clustering results showed that the virus genome in the A12 aircraft wastewater sample was the most closely related to the Omicron variant (21K) (Fig. 3).

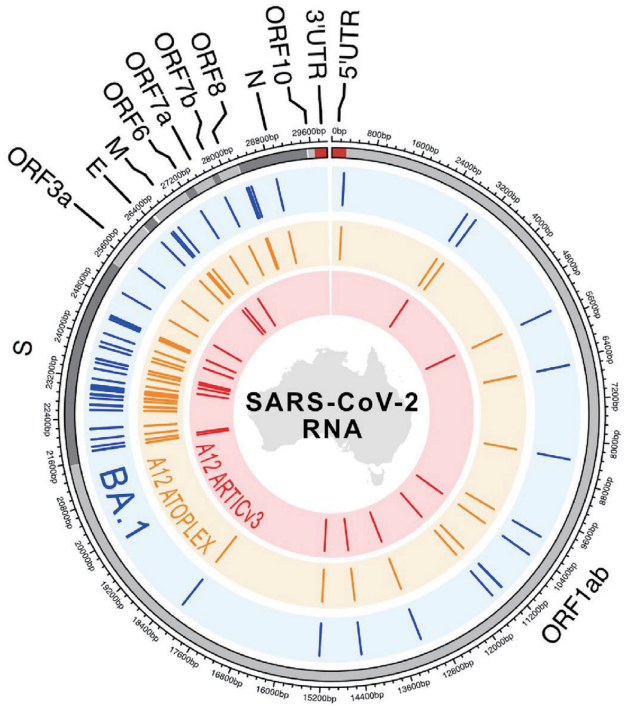

Fig. 2 Reference genome of Omicron BA.1 and the results of genome mutations of the same sample A12 detected by ATOPlex and ARTIC V3. The blue circle indicates the position of the mutation on the Omicron BA.1 genome, the yellow circle indicates the position of the mutation detected by ATOPlex platform, and the red circle indicates the mutation results detected by ARTIC V3.

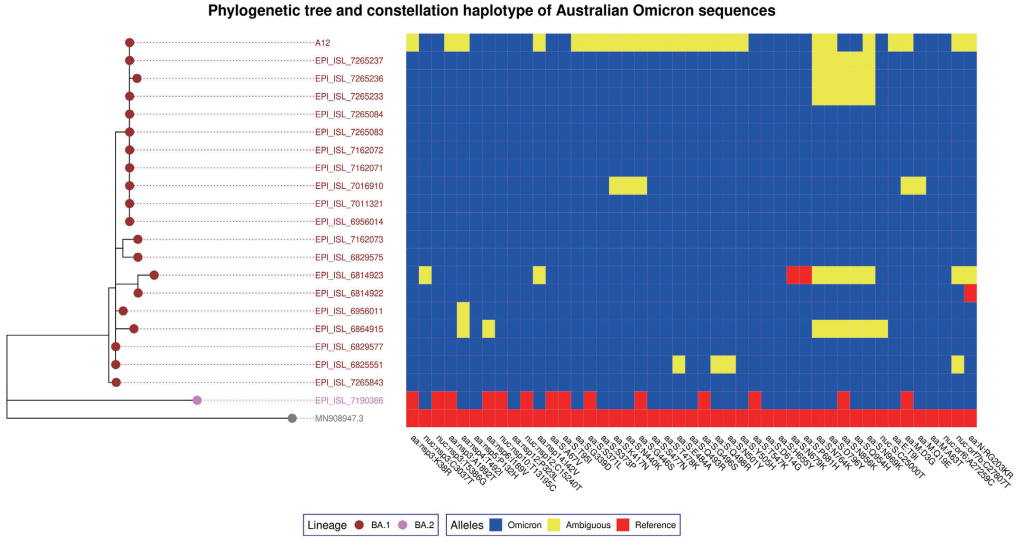

Fig. 3 Phylogenetic tree and constellation haplotype of the cluster of Australian Omicron sequences and aircraft wastewater Omicron sequences (A12). The blue indicates Omicron mutations, the yellow indicates ambiguous mutations, and the red indicates reference strains.

Conclusion

This study proves that aircraft wastewater is one of the important ways to monitor the global transmission of Omicron. In this study, the Omicron variant was successfully detected and traced in aircraft wastewater using the MGI's ATOPlex platform and DNBSEQ-G400 genetic sequencer.

In this study, targeted library construction was performed for the whole genome of the virus based on ATOPlex technology followed by pair-ended 100 (PE100) sequencing on DNBSEQ-G400. Compared with ARTIC V3, the ATOPlex platform shows higher sensitivity, coverage and accuracy, and thus is more suitable for the detection and tracing of SARS-CoV-2 in wastewater or similar environments.

Genetic Sequencer DNBSEQ-G400

References

1. Ahmed, W. et al. Detection of the Omicron (B.1.1.529) variant of SARS-CoV-2 in aircraft wastewater. *Sci Total Environ* 820, 153171, doi:10.1016/j.scitotenv.2022.153171 (2022).
2. National Institute for Communicable Diseases (NICD), 2021. New COVID-19 Variant Detected in South Africa. 25 November 2021. Accessed on 5 December 2021.
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5. Ahmed, W. et al. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers. *J Travel Med* 27, doi:10.1093/jtm/taaa116 (2020).
6. Ahmed, W. et al. Wastewater surveillance demonstrates high predictive value for COVID-19 infection on board repatriation flights to Australia. *Environ Int* 158, 106938, doi:10.1016/j.envint.2021.106938 (2022).
7. CDC, 2019. 2019-Novel Coronavirus (2019-nCoV) Real-Time rRT-PCR Panel Primers and Probes.

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G400RS	900-000170-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
	metargetCOVID	970-000228-00
Library Prep	ATOPlex RNA Multiplex PCR-based Library Preparation Set V3.1 (16 RXN)	940-000132-00
	MGIEasy Dual Barcode Circularization Kit (16 RXN)	1000020570
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)	1000016950
	CPAS Barcode Primer 3 Reagent Kit V2.0	1000011532

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