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"SARS-CoV-2 airborne detection within different departments of a COVID-19 hospital building and evaluation of air cleaners in air viral load reduction"

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ABSTRACT

The pandemic of COVID-19 has brought in light the necessity for the development of novel detection methods for airborne transmitted pathogens, and the importance of effective clean air measures in hospital departments. In this study, airborne SARS-CoV-2 and particle matter (PM1, PM2.5) detection was performed in different areas of the COVID-19 building at the Ippokrateio University Hospital in Thessaloniki, Greece. More specifically, Sioutas cascade impactors were placed in the ICU (Intensive Care Unit) and HDU (High-Dependency Unit) on the first floor, and at the corridor and rooms at the COVID-19 clinic on the second floor. Furthermore, TECORA air pumps were placed at the building entrance to measure for PM1 and PM2.5. Afterwards, in a COVID room with confirmed air viral load an air cleaner was placed to examine the effect on viral load reduction. Results showed that no viral copies were detected in the air of ICU and HDU departments, in which negative pressure air filtration with HEPA filters is applied. On the contrary, viral load was effectively detected in rooms and corridors of the COVID floor and ranged from 25,9 to 1123,7 copies/m^3. PM1 filters showed 77.8 % viral positivity, and PM2.5 filters were 38.5 % virus positive. Moreover, air viral load in the COVID room with an air cleaner showed a reduction of up to 98.1 %. In conclusion, SARS-CoV-2 was effectively detected in the air of different areas in the COVID building after continuous sampling ranging between 24 h and 7 days, and it was shown how important and effective air cleaners are as first-line measures against pathogen airborne transmission in hospital environments.

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1. Introduction

The recent pandemic named COVID-19 caused has caused numerous of deaths worldwide and brought in top priority the necessity for the development of novel early warning and detection systems concerning airborne transmitted pathogens (Chang et al., 2023). The causative biological factor of the pandemic has been identified as a highly novel coronavirus named Severe Acute Respiratory Syndrome Virus, SARS-CoV-2 (Chen et al., 2020; Zhou P. et al., 2020). The capability of highly infectious SARS-CoV-2 variants to evade vaccine and antibody-mediated immune responses highlighted the great importance of risk assessment and management for preventing respiratory viruses and airborne pathogens transmission (Shrestha et al., 2022; Sun et al., 2022). In addition, the highy mutation rate of the virus increases the upraise of novel Variants of Concern (VoC) so the optimization of standard detection and viral clearance systems upgrades to a necessity (Banerjee et al., 2021; Sun et al., 2022).

Healthcare personnel in hospitals and other public health installations were the first line of defence at the pandemic compared to other public servers, and it is shown that except the protective measures, the type of ventilation with an air supply of more than 16 air changes per hour rate are highly significant in reducing viral infection (Ribaric et al., 2021). Furthermore, another factor that increases the necessity to develop air detection and viral clearance systems is the high rate of endonosocomial pathogen transmissions since it was shown that 11–15 % of SARS-CoV-2 infections of hospitalized COVID-19 patients were acquired in hospitals (Read et al., 2021; Rickman et al., 2021). Secondary attack rates reached 39 % when non–COVID-19 patients shared multi-bed patient rooms with asymtomatic COVID-19 patients dispite the minimal preventive measures such as closed curtains and 2 m bed distance (Karan et al., 2021). Previous studies have reported the importance of indoor air quality in hospital environments assessing air quality by targeting respiratory viruses such as influenza A/B and respiratory syncytial virus (RSV) on the one hand, and particle matter and volatile organic compounds on the other (Hanna et al., 2023; Riveron et al., 2023; Zhou B. et al., 2022). SARS-CoV-2 has been shown to be transmitted not only via respiratory droplets (1–1000 μm) but also via aerosols (>1 μm) through the air, where viral particles can remain viable for several hours and the survival duration is strain-related (Araf et al., 2022; Chatterjee et al., 2023).

Aerosol dispersal of viral particles significantly affects patient recovery as particles can be transmitted in over 1 m distance, whereas respiratory droplet transmission reaches shorter distances (<1 m) (Stern et al., 2021). In addition, viral particles can attach on particle matter and remain active for longer periods, and part of them can land in human mucosa cavities and establish infections (Ma et al., 2021). Studies have shown that particle matter (PM) > 10 μm can remain in the air for 5 min, and PM < 5 μm can travel away from its generation source and a proportion of them can reach human bronchi and alveoli via inhalation (Milton, 2020). Few studies have detected SARS-CoV-2 in hospital environment, but the sampling periods were restricted from minutes to few hours with non-stable air flow rates and did not compare the effect of an air cleaner at the same environment (Dubey et al., 2021; Jin et al., 2021; Groma et al., 2023; Konatzii et al., 2023; Setti et al., 2020; Liu et al., 2020; Pivato et al., 2021; Razzini et al., 2020; Stern et al., 2021).

Thus, ventilation conditions in indoor environments play an important role in virus mitigation and transmission control (Hanna et al., 2023). In hospital environments it is quite critical to eliminate viral airborne transmission to reduce patient mortality and days in the hospital (Feng et al., 2021; Ohbe et al., 2021). Previously, SARS-CoV-2 virus particles were detected in indoor hospital air in the ICU, and was shown that virus can be found in aerosol for days after patients are tested negative (Jin et al., 2021; Konatzii et al., 2023; Mehmood et al., 2020; Nor et al., 2021). Moreover, it was shown that new coronavirus strains are emerging continuously and in combination to other infectious pathogens have an enormous effect on the hygiene personnel, so it is of vital need to develop rapid tools to detect highly pathogenic airborne viruses in indoor air and effectively disinfect patient rooms and hospital areas (Chatterjee et al., 2023).

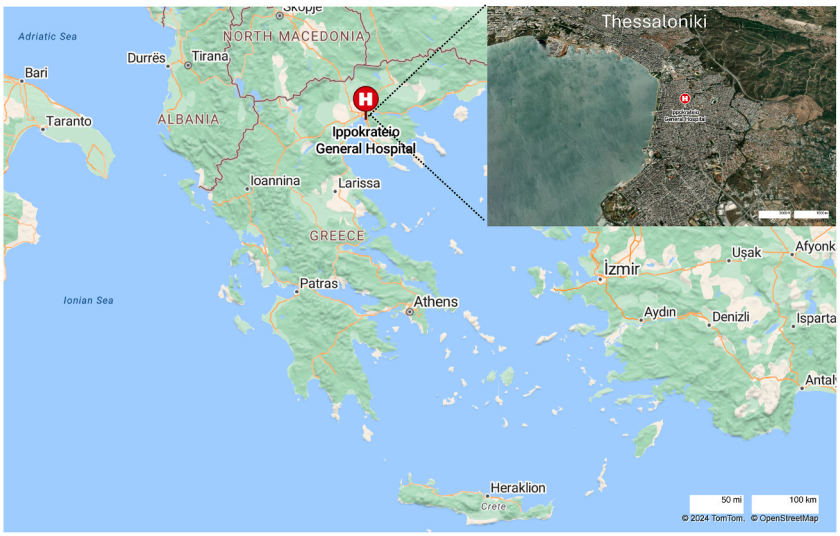

Fig. 1. Geographical location of Ippokrateio University Hospital in Thessaloniki, Greece.

In this work a systematic monitoring of airborne SARS-CoV-2 in typical areas within contaminant, emergency, and clean hospital zones was conducted, using an impactor and by developing a method to detect SARS-CoV-2 from environmental air filters. In addition, in order to develop a novel detection system including the airborne transmission risk assessment and management in designated COVID-19 hospitals and emerging pathogen units, the effect of air cleaners in the reduction of virus in the air was tested in areas confirmed positive for viral load.

The present study was performed from December 2021 to May 2022 during the outbreak of SARS-CoV-2 in Thessaloniki and the goals were: a) to detect viral load in indoor air in different areas and floors of a separate COVID building in a hospital located close to the city center of Thessaloniki, b) to evaluate the effect of an air-cleaner in the reduction of viral load in the presence of patients, and c) to examine the correlation between viral presence in the air and particle matter burden.

2. Material and methods

2.1. Experimental designation and bioaerosol collection

The study has been conducted at the Ippokrateio General University Hospital (40°36′45″N 22°57′42″E) (Fig. 1) in Thessaloniki, Greece. All COVID patients during the pandemic were treated at the Building C (Fig. 2).

For these experiments different samplers for bioaerosol and particulate matter collection were used, and all experiments were performed from December 2021 to May 2022. To obtain viral genomes and detect SARS-CoV-2 we used the Sioutas cascade impactor sampler (SKC, Inc., USA) attached to a respective sample pump (SKC, Inc., USA) with a fixed air filtration volume rate at 9 L per minute (L/min), which is able to separate and collect airborne particles in five ranges, Stage A: >2.5 μm, Stage B: 1.0–2.5 μm, Stage C: 0.5–1.0 μm, Stage D: 0.25–0.50 μm, and Stage E: <0.25 μm. Stages A to D use a 37-mm, 2.0-μm PTFE (Teflon) filter, and Stage E has an after-filter type 25-mm, 0.5-μm PTFE (Teflon) with laminated PTFE support. The impactor was working for 48 or 72 h continuously and in some cases till 7 days, and collected filters were placed in 15-ml conical tubes containing phosphate buffer saline (PBS, Thermo-Scientific, USA), and immediately stored at a −80°C freezer till further analysis. Filters were subjected to RNA extraction using an in-house Trizol-based method and the PureLink Viral RNA/DNA Mini kit (Thermo-Scientific, USA). RNA was eluted in 30 μl nuclease-free water and the concentration and integrity were evaluated using a NanoDrop Spectrophotometer (Thermo-Scientific, USA). Furthermore, to collect and measure PM2.5 and PM1 concentrations and to detect SARS-CoV-2 in the respective filters low air flow samplers (ENCO PM, TCR TECORA, Italy) were placed at the entrance of the COVID building (Fig. 2.). The used sampling heads meet the EN 14,907 standard and operated at a flow-rate of 38.3 L/min, with a collection time of 24 or 48 h. Samples were collected on PTFE (Teflon) membrane filters with a polymethylpentene (PMP) supporting ring (PALL Life Sciences, Ø 47 mm, pore size 2 μm, USA). PM mass concentrations were calculated by weighing the filters before and after sampling, and separate filters were collected for virus detection and subjected to the same RNA extraction method as the filters from Sioutas impactor. Despite the fact there was a five-month sampling period which ranged from December to May the mean ambient temperature at the Ippokrateio Hospital urban environment was 10.2 °C while average relative humidity was measured at 72,3 %.

Bioaerosol sampling was performed in two diferent hospital floors at the COVID building. The ICU and HDU units are located at the first floor, and the COVID clinic is located at the second floor, both of them renovated at 2020. The aforementioned units at the first

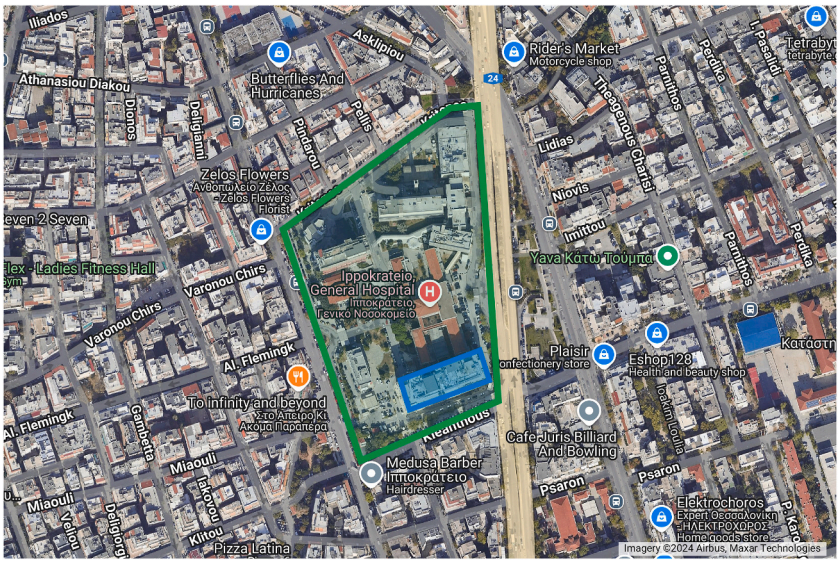

Fig. 2. Urban location of the Hospital within at the Thessaloniki city center. Green line shows the total area covered by the Hospital and blue line shows the COVID Building C where the sampling was performed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

floor were equiped with negative pressure room ventilation and HEPA filtration, and with isolation rooms before entering the main area, in which the personnel wears personal protective equipment (PPE) (Fig. 3). The sampling points are displayed in Fig. 3, including the personnel changing room and the drug preparation room. In addition, Sioutas impactor was placed at the center of ICU and HDU units at a height of 1.5 m, at a maximum distance of 2 m from lying patients. During the study, beds in both units were at least 80 % occupied by SARS-CoV-2 positive patients so the airborne virus transmission rate due to respiration was considered stable. Both units and all the floor area were cleaned three times per day at a steady hour schedule, and the personel was wearing continuously the appropriate PPE, meaning isolation gown, latex non-sterile gloves, N95 face mask, face shields, and head and boot covering. The ventilation rate in both ICU and HDU areas was 30 air changes per hour (ACH).

Furthermore, at the COVID clinic at the second floor, a Sioutas impactor was placed in four different sampling points including a convetional patient room (room number 204), the clinic corridor, the consumables storage room, and the personel restroom (Fig. 4). The patient room 204 has five beds in it, and all of them were full of patients (age $>$ 62 years old) during the sampling period. All patients when admitted to the hospital had a positive PCR SARS-CoV-2 test and were subjected to daily antigen SARS-CoV-2 rapid tests (Boson Biotech Ltd., China), and remained positive during the study period with typical COVID-19 symptoms. In addition, aerosol sampling was installed at an average distance of 1.5 m beside each patients' bed, at the height of the lying patients' heads, and to avoid the disturbing of the patients, sound filtration measures were taken so the average decibel measurement was stable at around 60 db. In room 204 (Fig. 4) sampling was performed in "open window" conditions, as this was the hospital regulation for the COVID clinic.

2.2. Virus detection and sequencing

To detect SARS-CoV-2 from environmental filter samples, the Genesig COVID-19 2G Real-Time PCR assay (Primerdesign, UK) was used, and the RT-qPCR was performed using an AriaMx Real-time qPCR instrument (Agilent, USA). The kit detects the S and ORF1ab genes of SARS-CoV-2 with a sensitivity of $<$0.4 copies/μl. During the RNA extraction process an internal standard control from the kit was used, and the positive control contained 1.25×10^5 SARS-CoV-2 gene synthetic DNA copies per μl. Positive control showed a Cq at 16.8 cycles for Cy5 channel (S gene) and at 16.83 cycles for FAM channel (ORF1ab gene). Due to the five-month sampling period we sought to sequence positive SARS-CoV-2 RNA samples to identify the dominant variant during experiments and whether there was a strain transition within sampling period.

Five positive samples from December to May were sequenced using previous methodology (Karthikeyan et al., 2022; Pechlivanis et al., 2022). Briefly, all samples were confirmed for SARS-CoV-2 positive RNA using the Vircell SARS-CoV-2 RealTime PCR Kit which targets the N and E viral genes and uses an amplification control which is associated to the sample extraction (human RNAse-P gene) to check the absence of carry-over of amplification inhibitors and the correct reverse transcription and amplification set-up. It is based on the reverse transcription (RT) and amplification of specific fragments of SARS-CoV-2 and SARS-related corona viruses by real-time PCR, targeting a specific fragment of the N gene for SARS-CoV-2 and a generic fragment of the E gene which is positive for SARS-CoV-2 and other SARS-related coronaviruses. The technique is divided into two main steps: RNA extraction and reverse transcription and amplification detection with specific oligo-pairs and probes. Coronavirus RNA is detected in FAM (N) and Cy5 (E) channels while the internal control is labelled with HEX/VIC (human P-RNAse). The QIAseq SARS-CoV-2 Primer Panel is a multiplexed PCR primer set for whole-genome amplification of SARS-CoV-2. Based on primer sequences, the QIAseq SARS-CoV-2 Primer Panel

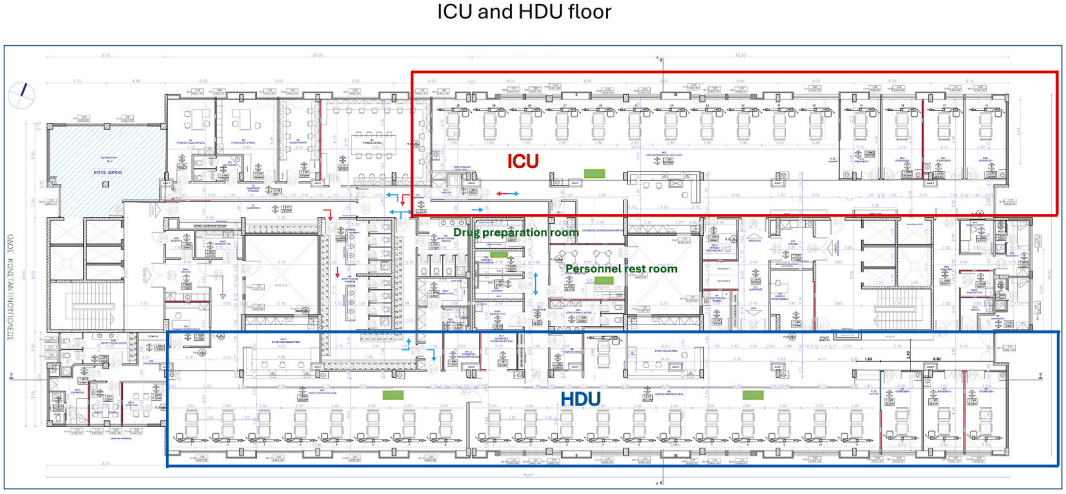

Fig. 3. First floor of the Building C (COVID Building) of the Ippokrateio University Hospital of Thessaloniki. Red rectangle shows the area of the Intensive Care Unit (ICU), and blue rectangle is diplayed by High-Dependency Unit (HDU). Green boxes shows the sampling points within the Units and the other rooms. During the study all beds were filled with patients. Blue and red arrows show positive or negative movement respectively from decontaminated to contaminated areas. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

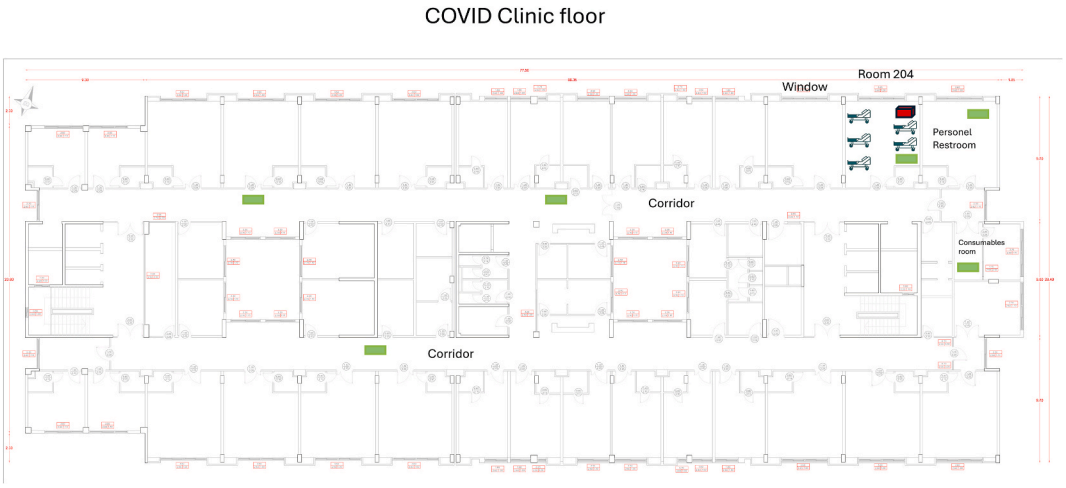

Fig. 4. COVID clinic patient floor (2nd) of the Building C (COVID Building) of the Ippokrateio University Hospital of Thessaloniki. Green boxes shows the sampling points within the different rooms of the clinic. The red box in the Room 204 shows the location of the air cleaner. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

amplifies 400 bp amplicons into two PCR pools that together cover the entire SARS-CoV-2 genome. Using the QIAseq FX DNA Library Kit, the amplicons from the QIAseq SARS-CoV-2 Primer Panel are brought within the length requirements to perform sequencing on Illumina ® instruments. Constructed libraries were analyzed in terms of quality on the Fragment Analyzer™ Automated 12 Capillary System using the dsDNA 915 Reagent Kit (Agilent Technologies, CA, USA), following the manufacturer's instructions, and in addition libraries were pooled and cleaned up using AMPure XP magnetic beads (Beckman Coulter, Brea, CA) with a volume ratio of 0.8x, following the manufacturer's instructions. Paired-end sequencing (2 × 150 cycles) was performed on a MiSeq system (Illumina, CA, USA).

Bioinformatic analysis pipeline included filtering corresponding reads to specific primers using Cutadapt, ensuring the removal of non-significant sequences. Afterwards, Trim Galore is employed to further refine the data, trimming low-quality bases and adapter sequences. The pre-processed reads are then aligned, using Burrows-Wheeler aligner (BWA-MEM). In addition, BAM file management is carried out with SAMtools, facilitating efficient manipulation and organization of alignment files.

Descriptive statistics are generated using SAMtools to gain insights into read mapping and coverage. Variant calling is performed utilizing Mutect2 to identify genomic variants accurately. Finally, custom R scripts are employed for visualization and reporting, allowing for comprehensive analysis and interpretation of the sequencing data.

2.3. Evaluation of air cleaners in hospital environment in air viral load reduction

To evaluate the effect of air cleaners on the reduction of air viral load, an air cleaner was introduced in a room with virus positive-patients at the COVID clinic, in which the detection of SARS-CoV-2 was confirmed prior to the introduction of the air cleaner. For this study the Airocide (APS GCS-25 model) air purifier (www.airocide.com) was used, which operates using photocatalytic oxidation technology developed by NASA in combination to ultraviolet irradiation (UV), and there is no HEPA filter in it. It is suitable for the removal of harmful pathogens and major allergens, such as dust from the atmosphere, and it fits for rooms up to 185 m^2. The model that was used uses a patented reaction chamber with hollow glass rods coated in TiO_2, and when ultraviolet (UV) light emitting radiation at 254 nm from lamps inside the chamber hits the TiO_2, it creates hydroxyl radicals and superoxide ions. These elements oxidize volatile organic compounds (VOCs) and kill airborne pathogens. According to manufacturer, the clean air delivery rate (CADR) is 34 m^3/h. In addition, the air cleaner is certified that emits no ozone or other harmful chemicals. The air purifier was introduced at the room 204 (Fig. 4) which has five beds for patients and the room measurements for length, width and ceiling height are 7.1, 3.6 and 3 m respectively. Thus, the air cleaner in room 204 shows a 0.44 air changes per hour rate (ACH). The evaluation of the air cleaner on the air viral load reduction has been performed by measuring the total amount of gene copies/m^3 of each sampling day with and without the use of the air cleaner, so the final reduction percentage is measured applying the following equation:

$$\frac{Final\ Value - Starting\ Value}{Starting\ Value}\ x\ 100$$

where, *Starting Value* = sum of S or ORF1ab gene copies from all impactor stages from each sampling day without the use of air cleaner, and *Final Value* = sum of S or ORF1ab gene copies from all impactor stages from each sampling day with the use of air cleaner.

2.4. Statistical analysis

Statistical analysis of the results was performed using GraphPad Prism (10.5) version. Results from different impactor size bins were compared by applying two-way ANOVA (analysis of variance), and the Tukey test was used to determine statistically significant differences between particulate concentrations ($p < 0.05$ was considered statistically significant). Quantitative data (C_t value) were normalized and converted to copies/m^3 based on the standard control concentrations. A $p < 0.05$ was considered statistically significant.

Table 1
Overview of air sampling and virus detection in the different departments and floors of the Ippokrateio Hospital COVID building. Sampling duration is presented in minutes and volume in air liters per minute (L/min). Real-time quantitative polymerase chain reaction (RT-qPCR) has targeted S and ORF1ab genes of SARS-CoV-2 and results are shown in copies/m^3. Gene copies are presented as the sum calculation from all five stage-bins from each sampling period.

Hospital Areas	Sampling start date	Volume (L/min)	Sampling duration (min - h)	Total Volume (L)	RT-PCR	S gene copies/m^3	ORF1ab copies/m^3
ICU (1st floor)							
	December 21, 2021	9	4310–72	38790	Negative	0	0
	December 24, 2021	9	2855–48	25695	Negative	0	0
	December 26, 2021	9	4290–72	38610	Negative	0	0
	December 29, 2021	9	7217–120	64953	Negative	0	0
	January 03, 2022	9	2875–48	25875	Negative	0	0
	January 05, 2022	9	2875–48	25875	Negative	0	0
	January 07, 2022	9	5715–96	51435	Negative	0	0
	January 11, 2022	9	1425–24	12825	Negative	0	0
HDU (1st floor)							
	January 14, 2022	9	4295–72	38655	Negative	0	0
	January 17, 2022	9	2895–48	26055	Negative	0	0
	January 19, 2022	9	2895–48	26055	Negative	0	0
	January 24, 2022	9	4125–69	37125	Negative	0	0
ICU Drug preparation room (1st floor)							
	March 18, 2022	9	4320–72	38880	Negative	0	0
	March 21, 2022	9	2880–48	25920	Negative	0	0
ICU Personel restroom (1st floor)							
	March 29, 2022	9	4050–68	36450	Positive	8,4	3,3
	April 01, 2022	9	7530–126	67770	Positive	5,1	4,6
	April 06, 2022	9	2715–45	24435	Negative	0	0
COVID Clinic Room (2nd floor)							
	January 27, 2022	9	1440–24	12960	Positive	679,2	586,4
	January 31, 2022	9	2865–48	25785	Positive	885,3	323,1
	February 02, 2022	9	3035–51	27315	Positive	1123,7	876,6
	February 04, 2022	9	4300–72	38700	Positive	964,4	514,7
COVID Clinic Corridor (2nd floor)							
	February 07, 2022	9	2855–48	25695	Positive	211,6	116,1
	February 09, 2022	9	2880–48	25920	Positive	308,3	157,2
	February 11, 2022	9	4335–72	39015	Positive	220,8	195,6
	February 14, 2022	9	2865–48	25785	Positive	403,7	226,8
	February 16, 2022	9	2825–48	25425	Positive	379,5	310
	February 18, 2022	9	4320–72	38880	Positive	481,8	277,3
COVID Clinic Personel Restroom (2nd floor)							
	February 21, 2022	9	10080–168	90720	Positive	156,1	68,5
	February 28, 2022	9	2880–48	25920	Positive	101,4	51,8
	March 02, 2022	9	2880–48	25920	Positive	92,7	25,9
COVID Clinic Consumables storage room (2nd floor)							
	March 04, 2022	9	5790–96	52110	Positive	133,4	118,2
	March 08, 2022	9	4350–72	39150	Positive	76,1	49,6
COVID Clinic Room 204 without Aircleaner (2nd floor)							
	May 01, 2022	9	2850–48	25650	Positive	550,5	420,1
	May 03, 2022	9	5680–96	51120	Positive	159,5	136,4
COVID Clinic Room 204 with Aircleaner (2nd floor)							
	May 06, 2022	9	5640–96	50760	Positive	3,4	5,3
	May 10, 2022	9	**Sampling duration (min - h)**	27000	Positive	10,4	5,2

3. Results

3.1. Viral airborne detection in different hospital areas

A total of 36 sampling days were recorded, where the first day was on the December 21, 2021 and the last one was on the May 10, 2022 (Table 1.). Each sampling day consists of five samples for each different stage of Sioutas impactor, so a total of 180 impactor air filters were analyzed, meaning RNA was extracted efficiently, and RT-qPCR was performed to detect SARS-CoV-2. The average Cq cycles for positive control gene copies were 16.86 for the S gene and 16.8 for the ORF1ab gene. In Table 1, all gene copies results are presented as the sum of gene copies from all the five impactor stage bins. The highest number of viral copies for S gene was shown at a Cq of 27.51, and for ORF1ab at a Cq of 28.63, whereas the lowest detection was shown at a Cq of 34.26 for the S gene and at a Cq of 34.15 for the ORF1ab gene. Further RT-qPCR results including relevant gel images, spectrograms, positive, negative and template controls can be found in supplementary files (SF_1). For ICU, ICU drug preparation room, and HDU, results showed that no SARS-CoV-2 genome was detected during the sampling period, which lasted from the 21st of December till the 24th of January, meaning that no viral copies were extracted in 70 air filters collected from the latter areas. The ICU personnel restroom was the only area on the first floor of the COVID building in which viral genome was detected after sampling for three days, and the total average as a sum calculation from all impactor stage bins was for the S gene 4,5 copies copies/m^3 and for the ORF1ab gene was 2,6 copies/m^3. Viral genome was detected in two out of three sampling days, with the third sampling day showing no viral genome (Table 1.).

In COVID clinic on the 2nd floor, all tested samples from the patient room 204, the personnel restroom, the consumables storage room, and the clinic corridor were positive for SARS-CoV-2 for both detected genes. Positive samples from each area result out of the sum of all size bins of Sioutas impactor from each sampling period and originate from 48- or 72-h measurements (except few measurements at 24- or 96-h as it is seen in Table 1. More specifically, for the S gene, copies ranged from 76,1 (consumables room) to 1123,7 (clinic room 204) copies/m^3, whereas for the ORF1ab gene, copies ranged from 25,9 (personnel restroom) to 876,6 copies/m^3 (clinic room 204) (Table 1). Concerning the different fractions, SARS-CoV-2 was detected in all different fractions and the highest viral loads were detected at stages A (>2.5 μm) and B (1–2.5 μm). At the room 204 and the COVID clinic corridor, viral genes were detected in all five stages of Sioutas impactor, whereas at the consumables and the personnel restroom areas there were sampling days that the lower stages D (0.25–0.5), and E (0-0.25) did not show viral gene copies. In almost all viral positive samples S gene copy numbers were higher than ORF1ab copies.

Furthermore, it was examined whether there was a specific size bin distribution of SARS-CoV-2 genome and thus, all results from positive samples from each different area of the COVID clinic at the second floor are shown in Fig. 5. Statistical analysis showed that while viral genome was detected in every area in almost all impactor stages, there were significant differences between areas. It is shown that viral copies were mainly concentrated at stages A (>2.5 μm), B (1–2.5 μm) and C (0.5–1 μm), and gene copy numbers were significantly different between room 204 and all the other areas of the COVID clinic including the corridor, the personnel restroom and the consumables room. For Stage A the mean copy number at room 204 was 246.4 S gene copies/m^3 compared to 61.2 and 65.7 S gene copies/m^3 for the clinic corridor and the consumables room respectively. Furthermore, at Stage B (1–2.5 μm) viral genome copies in the air of the corridor area were significantly different compared to the consumables room and the personnel restroom. There was no

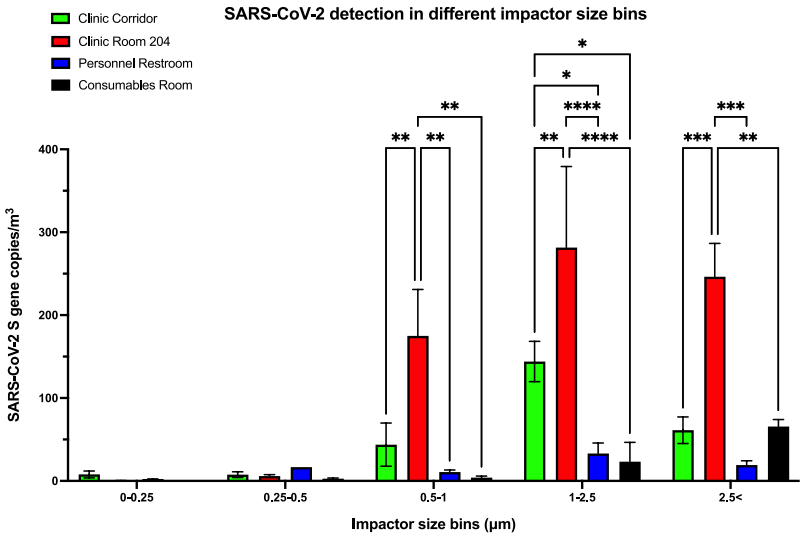

Fig. 5. Concentration of SARS-CoV-2 S gene (copies/m^3) in different size bins of Sioutas impactor. Different colors represent the different areas of the second floor of the COVID Building in Ippokrateio Hospital in Thessaloniki, Greece. The *x* axis represents the different size bins aerodynamic diameter in μm, and the *y* axis represents S gene copies per m^3. Stars denote p-value statistical differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

statistical difference in viral gene copies number between different areas in Stages D (0.25–0.5 μm) and E (0 - 0.25 μm).

Moreover, at room 204 the effect of air cleaners was evaluated by functioning continuously for 48 h in an "open windows" condition where windows were continuously open due to hospital regulations. In order to be in line with the epidemiology curve, the hospitalization numbers in Greece (Fig. 7A), and more specifically in the ICU of Ippokrateio Hospital (Fig. 7B), and since the last measurement of the air viral in room 204 was at the February 2, 2022, air viral load was measured again at the 1st and on the May 3, 2022 without the use of an air cleaner (Table 1.). Afterwards, two measurements with different sampling duration (96-h and 50-h) were performed at the 6th and the 10th of May with the use of an air cleaner. Results showed a reduction of up to 98,1 % for both S and ORF1ab genes in viral load compared to the sampling days where the air cleaner was not in function. More specifically, the average of the sum of gene copies from all impactor stages from both sampling days of S gene reduced from 355 to 6.9 copies/m^3 (98.06 %), and for ORF1ab gene reduced from 278.3 to 5.25 copies/m^3 (98.11 %).

3.2. SARS-CoV-2 sequencing

Sequencing results showed that there was no alteration at the dominant strain during our study period starting from December 2021 till May 2022. More specifically, confirmed SARS-CoV-2 samples were analyzed again for the N and the E gene using the Vircell-direct SARS-CoV-2 (SF_2). After showing positivity, libraries were constructed, and 2 out of the 5 positive samples produced high quality amplicons, passed the quality control and proceed to sequencing (SF_3). Bioinformatic analysis showed that all the different sub-lineages detected belong to the omicron strain lineage (SF_4, SF_5).

3.3. Particle matter measurements

To measure PM1 and PM2.5 at the COVID Building, two TECORA pumps were placed at the building entrance. In total, nine samples were collected for PM1, and thirteen samples were collected for PM2.5 respectively. Sampling period for PM ranged from the December 23, 2021 till the March 16, 2022. Results showed that for PM1 the average concentration was 21.02 ± 7.58 μg/m^3 and for PM2.5 was 22.61 ± 10.48 μg/m^3 respectively (Fig. 6). Concerning virus detection in PM filters, virus was detected in 7 out 9 samples in PM1 showing 77.8 % positivity and in PM2.5 filters only 5 out 13 samples were positive for SARS-CoV-2 showing 38.5 % positivity. Only in five sampling days PM1 and PM2.5 were collected the same day, and virus has been detected in all five days in PM1 filters and in two days in PM2.5 filters respectively.

4. Discussion

According to our knowledge, this is the first work to detect the virus in the air of a hospital environment using continuous 24-h or 48-h sampling periods that were extended to 7 days in a five-months range, and simultaneously to test the effectiveness of an air cleaner of TiO_2-UV light technology. In addition, this is the first attempt to detect SARS-CoV-2 in the air of a hospital environment in Greece. During the time period of the study (December 2021 – May 2022) the vaccination campaign in Greece has been completed as till the June 30, 2022 the 75,4 % (7.919.254 people) of the general population received one vaccine dose, 72,6 % received two vaccine doses and a 58,2 % received a booster third shot ("COVID-19 vaccination statistics"). The latter fact means that during our study the majority of patients were fully vaccinated against SARS-CoV-2 gamma strain with immunity being least effective against the dominant omicron strain, which showed a 3.2-fold higher transmission rate than delta strain (Araf et al., 2022).

One of the quality points of the current study is the sampling duration as it was set to continuous 24 or 48 h, whereas other studies varied from 15 min to 30 min, till 90 min to 4 h, and only few studies performed sampling using 12-h duration (Thuresson et al., 2024;

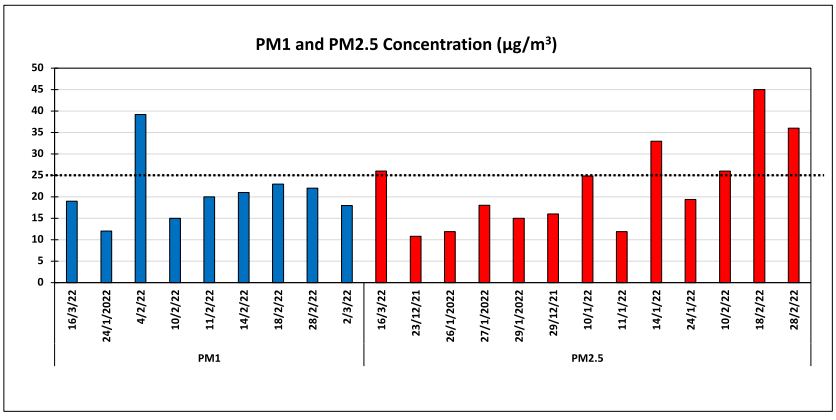

Fig. 6. PM1 and PM2.5 concentration in μg/m^3 in different samling days. TECORA pumps to measure PM were placed at the entrance of the COVID Building C in Ippokrateio Hospital in Thessaloniki, Greece. The dashed line shows the daily PM limits set by the Environmental Protection Agency (EPA).

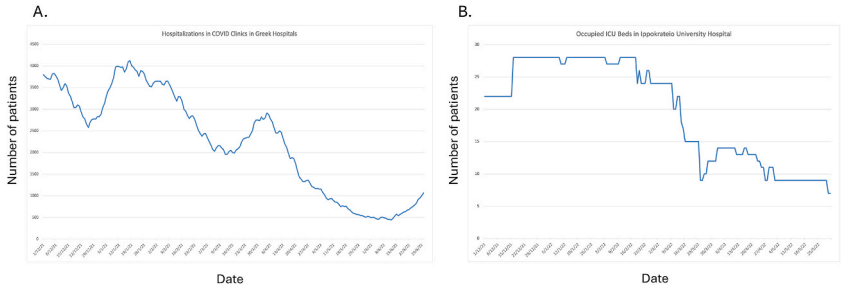

Fig. 7. Epidemiological curve of COVID hospitalizations during the study (December 01, 2021 - June 01, 2022. (A.) Number of hospitalizations in all Greek hospitals, and (B.) Number of occupied ICU beds in Ippokrateio University Hospital. All data originate from the Mandatory Notification System of the National Public Authority in Greece (www.eody.gov.gr).

Li et al., 2023; Shankar et al., 2022; Grimalt et al., 2022; Rufino de Sousa et al., 2022). Concerning constistency to other studies, our results can be compared to the latter shorter sampling periods and also to studies with similar sampling periods like Stern et al. who sampled five different spots for 48 h (Stern et al., 2021). The latter study used a micro-environmental cascade impactor fractionating particles in PM10, PM2.5 and ultrafine fraction, where viral RNA was detected in all fractions and the highest viral concetration detected was 51 viral copies/m^3 at the emergency unit. In our study the dispersion of SARS-CoV-2 concentration in the different hospital areas, showed that the highest concentration was detected in COVID clinic rooms displaying a high peak of 1123 copies/m^3, whereas at the corridor area showed 481 copies/m^3. The study of Grimalt using 4 h sampling periods showed 5 times higher concentrations in COVID rooms and at the corridor area reaching a maximum value of 5800 and 6200 copies/m^3. The same study showed a relevant high viral load in the ICU corridor reaching an average of 190 copies/m^3. Ribaric reviewed 51 studies and showed that the percentage of positivity in ICUs reached an average of 26,87 % but the average of viral copies reached only 1.58 copies/m^3 (Ribaric et al., 2021).

Moreover, our results showed that SARS-CoV-2 RNA most likely exists in particles ranging between 0.5 and 2.5 μm aerodynamic diameter, but also in ultrafine particles, which is consistent to another study where it was shown that viral RNA exists in 0.5–4 μm, and explains the long duration on viral positivity in air samples as smaller particles remain longer in the air (Groma et al., 2023). On the other hand, statistical analysis of the gene copies distribution in different size bins showed the significant difference of viral load in the air of a room with stable patients compared to a corridor area when people are not stable and contiously in motion. Despite the fact, that corridor air viral load was significanlty lower, in absolute numbers of genome copies virus was still present in high amounts. On the contrary, a recent study which concluded that the spread from patient rooms to the ward corridors is limited was conducted for a year (March 2020–May 2021) and on continuous 12-h sampling in corridors of two infectious disease SARS-CoV-2 wards. The results showed that only 2.6 % (20/784) of the collected samples were positive for viral genome, and despite copies were found in eight different fractions between 0.14 and 8.1 μm, no statistical difference between fractions was shown (Thuresson et al., 2024).

Due to “open windows” regulations at the COVID clinics of Greek Hospitals, it was not possible to detect the viral shedding reduction due to patients’ recovery, and furthermore during our study all the patients were daily confirmed to spread efficiently the virus. Nevertheless, another study conducted in two different house air-conditioned rooms with a single patient in each room, and used the same impactor we used, showed that at the first day of symptoms viral gene copies were detected in all size bins 10 to 100 times higher than in our study, and showed Cq values from 15.97 to 18.02 (Shankar et al., 2022). In addition, the highest amount was detected in Stage 4 (0.25–0.5 μm) but no significant differences were shown between fractions, and moreover sampling that was performed 4 days later showed no air virus detection in all stages, meaning that patients’ recovery should be taken into account when air viral distribution in different areas is assessed, as in our study gene copies were gathered mainly at impactor Stage A and the concentration was proportionally reduced in the corridor and other areas.

In this work, no virus has been detected in the ICU and the HDU rooms during the five-month sampling period, and it was shown that due to strict prevention measures in the ICU and the HDU and under negative pressure conditions viral load was not present in the air. Nevertheless, another work examing viral presence in the ICU found that SARS-CoV-2 was present in the air and it was shown that virus can be discharged in bioaerosol for days after patients are tested negative (Jin et al., 2021). The two units of Ippokrateio Hospital have been completely reconstructed and renovated in 2020, and negative pressure fans with HEPA filters were installed. Ribaric et al. reported that 53 % of the 51 studies showed the presence of SARS-CoV-2 in the ICU patient room indoor air but in the methodology they refer to different ventilation technologies and preventive measures (Ribaric et al., 2021). The fact that in our study both units were negative for viral presence is important as it was shown before that ICUs had significantly lower in-house mortality than HDUs among cardiac patients receiving non-invasive ventilation and intubation, and different structure between the two units has an effect on patient mortality (Wilcox et al. 2013; Ohbe et al., 2021).

In addition, viral sequencing showed that the dominant SARS-CoV-2 strain was of Omicron strain lineage, which is in line to another study on wastewaters, and shown that in Finland from November 2021 till February 2022 there was a transition from Delta to Omicron variant with a pick in viral load in December 2022 (Lipponen et al., 2024). In general, Omicron variant showed to have a 3.2–3.5-fold higher transmission rate compared to previous dominant strains, and a reduced effectiveness of vaccination in lowering risk of transmission, could be a likely contributor for the rapid propagation of Omicron (Allen et al., 2023). Thus, due to higher transmission rate of Omicron compared to other strains the virus in May remained in lower but significant levels in the air of room 204

which was full of patients and despite the fact that ICU admissions in the hospital were reduced compared to January to 8 from 27 and the hospitalizations in the country from more than 4000 to approximately 1000 (Fig. 7.).

Results from PM1 and PM2.5 analysis did not show any correlation to the virus positivity in the air. PM measurement stations have been placed at the COVID building entrance and at the two COVID floors, and for PM1 it was shown a 77.8 % of viral positivity, whereas for PM2.5 the positivity reached 38.5 %. The viral load of SARS-CoV-2 positive samples was irrelevant to PM burden as virus has been detected both in high and low particle matter concentrations. The latter results are in consistance to a study in Padua in Northern Italy where it was shown that the virus was not detected in 44 outdoor samples and virus monitoring on PM is not an early indicator of viral transmission (Pivato et al., 2021). Another study, showed that PM2.5 generated from human activities within healthcare conditions and in COVID patient wards can influence the indoor presence of SARS-CoV-2 but the degree of viral shedding depends on the number of symptomatic patients that are present in the room (Nor et al., 2021). In the latter study, the highest PM2.5 concentration reached 23.3 $\mu g/m^3$ and the mean concentration reached 17.5 $\mu g/m^3$, whereas in our study mean concentration reached 22.6 $\mu g/m^3$ and the highest concentration was 45 $\mu g/m^3$. Since, it is known from other studies that PM2.5 or PM10 suspended in the air can originate from the floor and different surfaces such as plastic and stainless steel, it is clearer now that particle matter can contribute to SARS-CoV-2 dispersal, but viral load is irrelevant to PM concentration (Bontempi E. 2020; Hagbom et al., 2015; Kujundzic et al., 2006). Another study, performed within a hospital environment in an HDU showed that viral RNA concentrations in the air are determined by indoor aerosol sources and do not appear to be dependent on total indoor PM concentrations (Groma et al., 2023). Thus, the main scientific question to be solved is whether viral RNA present in the air is infectious or originates from non-infectious virus particles, and additionally it should be examined which are the factors that contribute to the duration of infectious viral genetic material in the air. One study has retrieved viable virus at a range of 6–74 $TCID_{50}$ units/L from air samplers but further research is needed to clarify the duration and the parameters affecting the longevity of airborne viral particles (Lednicky et al., 2020).

It is already known that air cleaners can function in public areas such as schools, households, laboratories and industry buildings and they use several different physicochemical or biochemical technologies (Lu et al., 2022). In this work, the air cleaner that was used has a reaction chamber with a catalyst bed which contains titanium dioxide (TiO_2) and is surrounded by unique UV lamps. Thus, harmful particles are being destroyed by the catalyst excitation from the light coming from the lamps. The latter technology was shown before that effectively can remove xylene as an air pollutant but to our knowledge it is the first time that is tested against airborne viral pathogens (Rangkooy et al., 2020). Since the different clinical areas (ICU, HDU, surgery rooms) based on the microbial community level vary considerably, it is important to install the most efficient and less laborious equipment, meaning that air cleaners without the necessity of changing the HEPA filters every few months have an advantage compared to other technologies (Chen et al., 2023). The use of portable air purifiers (PACs) to reduse aerosols was studied before, and it was shown that can effectively reduce at least 46 % of aerosols compared to the non-usage of any mitigation measures for adverse environmental effects (Salmonsmith et al., 2023). Moreover, the effect of air cleaners in the reduction of clinical symptoms and the airborne viral load has been studied in Swiss schools where it was shown 77 % reduction in mean particle concentration, 93 % less coughing indicating fewer symptomatic students, and despite the fact virus was present in saliva samples (50/448), there was limited detection in bioaerosols samples (2/105) and in air cleaner HEPA filters (4/160) (Banholzer et al., 2024). The latter results can be correlated with the results from our study, since despite the fact that during sampling period all the five beds of the COVID room were occupied with positive patients, airborne viral detection was limited and significantly reduced to a maximum of 98.1 % when the air cleaner was operating, meaning that the air cleaner effect on the airborne viral load is irrelevant with the patient's viral burden and the release of live virus, and has a real-time effect on viral load.

Furthermore, another study has examined the combinatorial effect of two different air cleaning technologies which is a complex of the positive and negative oxygen ion purifier and the high-efficiency particulate air filter (HEPA), and after a 30-min purification period showed 98.44 %–100 % reduction in cigarette particle matter (PM), in staphylococcus albicans colony forming units (CFU), and inactivation of influenza virus under a simulated ward condition (Zhou et al., 2022). After the pandemic it become of great importance to use air cleaners that can effectively destroy airborne pathogens and intracellular components such as plasmids, DNA or RNA, and in parallel be of low energy cost and being able to function in 24-h periods in long-term duration without being toxic to humans. This work strongly enforces the usage of TiO_2 technology coupled to UV light and further research will shed light on the effectiveness against multiple pathogens that consist of the aerobiome either of human, animal or plant origin (Arjen de Groot et al., 2021; Robinson et al., 2020). The development of rapid detection and identification tools for air pathogens will help to develop early warning systems against pathogens and improve our understanding in microbial dispersal in the air.

As in all experimental protocols this study had some limitations. Due to hospital rules during COVID pandemic it was not possible to test the effect of the air cleaner in a "close window condition". Open windows in a patient room creates a continuous air-flow within the room and produce multiple air changes per hour. Nevertheless, it could be a hospital environment where window opening is not possible either to high urban pollution or due to building installations. In a patient room when windows are closed the external air flow is very limited and the air changes per hour depends on the ventilation. Subsequently the time the particles remain in the air is also limited meaning they drop faster at surfaces. Thus, in future experiments it should be examined whether the air cleaner can effectively clean air particles and viruses in a closed window condition, and determine which is the appropriate air changes per hour rate for a close window environment. In addition, it should be examined whether viral or other pathogen load is increased on room surfaces, on medical equipment or at the personnel clothing in parallel to air viral genome detection.

Another limitation is that extracted SARS-CoV-2 RNA was not treated in viral cultures to study the degree of virus viability and the rate of infectivity as it was suggested elsewhere where it was shown that all 6 virus positive out of 12 samples in room hospitals were negative after 4 passages in virus cultures (Ong et al., 2021).

In conclusion, in this study virus was effectively detected in the air of different departments of a COVID Building and viral load per

sampling day reached up to 1123 viral gene copies/m^3. Moreover, it was shown that negative pressure ventilation measures with HEPA filtration at the ICU and HDU areas can prevent SARS-CoV-2 airborne transmission as the virus could not be detected. Furthermore, it was demonstrated that air cleaners using TiO_2-UV light technology can reduce up to 98,1 % of viral load in the air of a COVID patient room with confirmed positive airborne viral RNA. In general, it was shown that in combination to behavioral compliance of strict safety health protocols, and the usage of appropriate air cleaners, airborne SARS-CoV-2 transmission can be significantly controlled, thus, reducing patient days in hospital and benefit public health care systems.

CRediT authorship contribution statement

Ilias S. Frydas: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marianthi Kermenidou:** Methodology, Data curation. **Maria Karypidou:** Methodology, Formal analysis. **Spyros Karakitsios:** Writing – review & editing. **Dimosthenis A. Sarigiannis:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaerosci.2025.106587.

Data availability

Data will be made available on request.

References

Allen, H., Tessier, E., Turner, C., Anderson, C., Blomquist, P., Simons, D., Løchen, A., Jarvis, C. I., Groves, N., Capelastegui, F., Flannagan, J., Zaidi, A., Chen, C., Rawlinson, C., Hughes, G. J., Chudasama, D., Nash, S., Thelwall, S., Lopez-Bernal, J., … Lamagni, T. (2023). Comparative transmission of SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants and the impact of vaccination: National cohort study, England. *Epidemiology and Infection, 151*, e58. https://doi.org/10.1017/S0950268823000420. PMID: 36938806; PMCID: PMC10125873.

Araf, Y., Akter, F., Tang, Y. D., Fatemi, R., Parvez, M. S. A., Zheng, C., & Hossain, M. G. (2022). Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. *Journal of Medical Virology, 94*(5), 1825–1832. https://doi.org/10.1002/jmv.27588. Epub 2022 Jan 23.

Arjen de Groot, G., Geisen, S., Jasper Wubs, E. R., Meulenbroek, L., Laros, I., Basten Snoek, L., Lammertsma, D. R., Hansen, L. H., & Slim, P. A. (2021). The aerobiome uncovered: Multi-marker metabarcoding reveals potential drivers of turn-over in the full microbial community in the air. *Environment International, 154*, Article 106551. https://doi.org/10.1016/j.envint.2021.106551. ISSN 0160-4120.

Banerjee, A., Mossman, K., & Grandvaux, N. (2021). Molecular determinants of SARS-CoV-2 variants. *Trends in Microbiology, 29*(10), 871–873. https://doi.org/10.1016/j.tim.2021.07.002. Epub 2021 Jul 27. PMID: 34373192; PMCID: PMC8313503.

Banholzer, N., Jent, P., Bittel, P., Zürcher, K., Furrer, L., Bertschinger, S., Weingartner, E., Ramette, A., Egger, M., Hascher, T., & Fenner, L. (2024). Air cleaners and respiratory infections in schools: A modeling study based on epidemiologic, environmental, and molecular data. *Open Forum Infectious Diseases, 11*(4), Article ofae169. https://doi.org/10.1093/ofid/ofae169. PMID: 38665173; PMCID: PMC11045022.

Bontempi, E. (2020). First data analysis about possible COVID-19 virus airborne diffusion due to air particulate matter (PM): The case of Lombardy (Italy). *Environmental Research, 186*, Article 109639. https://doi.org/10.1016/j.envres.2020.109639. ISSN 0013-9351.

Chang, Y., Wang, Y., Li, W., Wei, Z., Tang, S., & Chen, R. (2023). Mechanisms, techniques and devices of airborne virus detection: A review. *Int J Environ Res Public Health, 20*(8), 5471. https://doi.org/10.3390/ijerph20085471. PMID: 37107752; PMCID: PMC10138381.

Chatterjee, S., Bhattacharya, M., Nag, S., Dhama, K., & Chakraborty, C. (2023). A detailed overview of SARS-CoV-2 omicron: Its sub-variants, mutations and pathophysiology, clinical characteristics, immunological landscape, immune escape, and therapies. *Viruses, 15*(1), 167. https://doi.org/10.3390/v15010167. PMID: 36680207; PMCID: PMC9866114.

Chen, J., Ji, L., Xiong, G., & Ning, K. (2023). The distinct microbial community patterns and pathogen transmission routes in intensive care units. *Journal of Hazardous Materials, 441*, Article 129964. https://doi.org/10.1016/j.jhazmat.2022.129964. ISSN 0304-3894.

Chen, L., Liu, W., Zhang, Q., Xu, K., Ye, G., Wu, W., Sun, Z., Liu, F., Wu, K., Zhong, B., Mei, Y., Zhang, W., Chen, Y., Li, Y., Shi, M., Lan, K., & Liu, Y. (2020). RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. *Emerg Microbes Infect, 9*(1), 313–319. https://doi.org/10.1080/22221751.2020.1725399. PMID: 32020836; PMCID: PMC7033720.

Dubey, A., Kotnala, G., Mandal, T. K., Sonkar, S. C., Singh, V. K., Guru, S. A., Bansal, A., Irungbam, M., Husain, F., Goswami, B., Kotnala, R. K., Saxena, S., Sharma, S. K., Saxena, K. N., Sharma, C., Kumar, S., Aswal, D. K., Manchanda, V., & Koner, B. C. (2021). Evidence of the presence of SARS-CoV-2 virus in

atmospheric air and surfaces of a dedicated COVID hospital. *Journal of Medical Virology, 93*(9), 5339–5349. https://doi.org/10.1002/jmv.27029. Epub 2021 May 3. PMID: 33913527; PMCID: PMC8242543.

Feng, B., Xu, K., Gu, S., Zheng, S., Zou, Q., Xu, Y., Yu, L., Lou, F., Yu, F., Jin, T., Li, Y., Sheng, J., Yen, H.-L., Zhong, Z., Wei, J., & Chen, Y. (2021). Multi-route transmission potential of SARS-CoV-2 in healthcare facilities. *Journal of Hazardous Materials, 402*, Article 123771. https://doi.org/10.1016/j.jhazmat.2020.123771. ISSN 0304-3894.

Grimalt, O. J., Vílchez, H., Fraile-Ribot, P. A., Marco, E., Campins, A., Orfila, J., van Drooge, B. L., & Fanjul, F. (2022). Spread of SARS-CoV-2 in hospital areas. *Environmental Research, 204*(Part B), Article 112074. https://doi.org/10.1016/j.envres.2021.112074. ISSN 0013-9351.

Groma, V., Kugler, S., Farkas, Á., Furi, P., Madas, B., Nagy, A., … Osán, J. (2023). Size distribution and relationship of airborne SARS-CoV-2 RNA to indoor aerosol in hospital ward environments. *Scientific Reports, 13*, 3566. https://doi.org/10.1038/s41598-023-30702-z

Hagbom, M., Nordgren, J., Nybom, R., Hedlund, K. O., Wigzell, H., & Svensson, L. (2015). Ionizing air affects influenza virus infectivity and prevents airborne-transmission. *Scientific Reports, 5*, 11431. https://doi.org/10.1038/srep11431

Hanna, F., Alameddine, I., Zaraket, H., Alkalamouni, H., & El-Fadel, M. (2023). Airborne influenza virus shedding by patients in health care units: Removal mechanisms affecting virus transmission. *PLoS One, 18*(10), Article e0290124. https://doi.org/10.1371/journal.pone.0290124. PMID: 37878553; PMCID: PMC10599543.

Jin, T., Li, J., Yang, J., Li, J., Hong, F., Long, H., Deng, Q., Qin, Y., Jiang, J., Zhou, X., Song, Q., Pan, C., & Luo, P. (2021). SARS-CoV-2 presented in the air of an intensive care unit (ICU). *Sustainable Cities and Society, 65*, Article 102446. https://doi.org/10.1016/j.scs.2020.102446. ISSN 2210-6707.

Karan, A., Klompas, M., Tucker, R., Baker, M., Vaidya, V., & Rhee, C. (2021). The risk of SARS-CoV-2 transmission from patients with undiagnosed Covid-19 to roommates in a large academic medical center. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciab564. Published online.

Karthikeyan, S., Levy, J. I., De Hoff, P., et al. (2022). Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. *Nature, 609*, 101–108. https://doi.org/10.1038/s41586-022-05049-6

Konatzii, R., Schmidt-Ott, F., Palazis, L., Stagianos, P., Foka, M., Richter, J., Christodoulou, C., Sciare, J., & Pikridas, M. (2023). Exposure to airborne SARS-CoV-2 in four hospital wards and ICUs of Cyprus. A detailed study accounting for day-to-day operations and aerosol generating procedures. *Heliyon, 9*(3), Article e13669. https://doi.org/10.1016/j.heliyon.2023.e13669. ISSN 2405-8440.

Kujundzic, E., Matalkah, F., Howard, C. J., Hernandez, M., & Miller, S. L. (2006). UV air cleaners and upper-room air ultraviolet germicidal irradiation for controlling airborne bacteria and fungal spores. *J. Occup. Environ. Hygiene., 3*, 536–546.

Lednicky, J. A., Lauzardo, M., Hugh Fan, Z., Jutla, A., Tilly, Trevor B., Gangwar, Mayank, … Chang-Yu, Wu (2020). Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *International Journal of Infectious Diseases, 100*, 476–482.

Li, S., Guo, J., Gu, Y., Meng, Y., He, M., Yang, S., Ge, Z., Wang, G., Yang, Y., Jin, R., Lu, L., & Liu, P. (2023). Assessing airborne transmission risks in COVID-19 hospitals by systematically monitoring SARS-CoV-2 in the air. *Microbiology Spectrum, 11*(6), Article e0109923. https://doi.org/10.1128/spectrum.01099-23. Epub 2023 Nov 8. PMID: 37937995; PMCID: PMC10714815.

Lipponen, A., Kolehmainen, A., Oikarinen, S., Hokajärvi, Anna-Maria, Lehto, Kirsi-Maarit, Heikinheimo, Annamari, , … Pitkänen, Tarja, & WastPan Study Group. (2024). Detection of SARS-COV-2 variants and their proportions in wastewater samples using next-generation sequencing in Finland. *Scientific Reports, 14*, 7751. https://doi.org/10.1038/s41598-024-58113-8

Liu, Y., Ning, Z., Chen, Y., Guo, Ming, Liu, Yingle, Kumar Gali, Nirmal, … Lan, Ke (2020). Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature, 582*, 557–560. https://doi.org/10.1038/s41586-020-2271-3

Lu, S., Zhou, J., Wang, C., Meng, G., Li, Y., Jarin, M., Wu, Z., & Xie, X. (2022). Airborne pathogenic microorganisms and air cleaning technology development: A review. *Journal of Hazardous Materials, 424*(Part B), Article 127429. https://doi.org/10.1016/j.jhazmat.2021.127429. ISSN 0304-3894.

Ma, J., Qi, X., Chen, H., Li, X., Zhang, Z., Wang, H., Sun, L., Zhang, L., Guo, J., Morawska, L., Grinshpun, S. A., Biswas, P., Flagan, R. C., & Yao, M. (2021). Coronavirus disease 2019 patients in earlier stages exhaled millions of severe acute respiratory Syndrome coronavirus 2 per hour. *Clinical Infectious Diseases, 72*(10), e652–e654. https://doi.org/10.1093/cid/ciaa1283. PMID: 32857833; PMCID: PMC7499537.

Mehmood, K., Muhammad, S. I., & Abrar, M. M. (2020). Can exposure to PM2.5 particles increase the incidence of coronavirus disease 2019 (COVID-19). *Science of The Total Environment, 741*, Article 140441. https://doi.org/10.1016/j.scitotenv.2020.140441. ISSN 0048-9697.

Milton, D. K. (2020). A Rosetta stone for understanding infectious drops and aerosols. *J Pediatric Infect Dis Soc, 9*(4), 413–415. https://doi.org/10.1093/jpids/piaa079. PMID: 32706376; PMCID: PMC7495905.

Nor, N. S. M., Yip, C. W., Ibrahim, N., Mohd, Hasni Jaafar, Zetti, Zainol Rashid, Norlaila, M., … Nadzir, Mohd Shahrul Mohd (2021). Particulate matter ($PM_{2.5}$) as a potential SARS-CoV-2 carrier. *Scientific Reports, 11*, 2508. https://doi.org/10.1038/s41598-021-81935-9

Ohbe, H., Matsui, H., & Yasunaga, H. (2021). Intensive care unit versus high-dependency care unit for patients with acute heart failure: A nationwide propensity score-matched cohort study. *j intensive care, 9*, 78. https://doi.org/10.1186/s40560-021-00592-2

Ong, S. W. X., Tan, Y. K., Coleman, K. K., Tan, BH, Leo, YS, Wang, DL, … Marimuthu, K. (2021). Lack of viable severe acute respiratory coronavirus virus 2 (SARS-CoV-2) among PCR-positive air samples from hospital rooms and community isolation facilities. *Infection Control & Hospital Epidemiology, 42*(11), 1327–1332. https://doi.org/10.1017/ice.2021.8

Pechlivanis, N., Tsagiopoulou, M., Maniou, M. C., Anastasis, Togkousidis, Evangelia, Mouchtaropoulou, Taxiarchis, Chassalevris, & Anagnostis Argiriou & Fotis Psomopoulos. (2022). Detecting SARS-CoV-2 lineages and mutational load in municipal wastewater and a use-case in the metropolitan area of Thessaloniki, Greece. *Scientific Reports, 12*, 2659. https://doi.org/10.1038/s41598-022-06625-6

Pivato, A., Amoruso, I., Formenton, G., Di Maria, F., Bonato, T., Vanin, S., Marion, A., & Baldovin, T. (2021). Evaluating the presence of SARS-CoV-2 RNA in the particulate matters during the peak of COVID-19 in Padua, northern Italy. *Science of The Total Environment, 784*, Article 147129. ISSN 0048-9697.

Rangkooy, H. A., Jahani, F., & Siahi Ahangar, A. (2020). Photocatalytic removal of xylene as a pollutant in the air using ZnO-activated carbon, TiO2-activated carbon, and TiO2/ZnOactivated carbon nanocomposites. *Environ. Health Eng. Manag., 7*(1), 41–47. URL: http://ehemj.com/article-1-570-en.html.

Razzini, K., Castrica, M., Menchetti, L., Maggi, L., Negroni, L., Orfeo, N. V., Pizzoccheri, A., Stocco, M., Muttini, S., & Balzaretti, C. M. (2020). SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. *Sci Total Environ, 742*, Article 140540. https://doi.org/10.1016/j.scitotenv.2020.140540. Epub 2020 Jun 26. PMID: 32619843; PMCID: PMC7319646.

Read, J. M., Green, C. A., Harrison, E. M., Docherty, Annemarie B., Funk, Sebastian, Harrison, Janet, & and the ISARIC4C investigators. (2021). Hospital-acquired SARS-CoV-2 infection in the UK's first COVID-19 pandemic wave. *Lancet, 398*(10305), 1037–1038.

Ribaric, N. L., Vincent, C., Jonitz, G., Hellinger, A., & Ribaric, G. (2021). Hidden hazards of SARS-CoV-2 transmission in hospitals: A systematic review. *Indoor Air, 32*, Article e12968. https://doi.org/10.1111/ina.12968

Rickman, H. M., Rampling, T., Shaw, K., Martinez-Garcia, G, Hail, L, Coen, P, … Houlihan, CF. (2021). Nosocomial transmission of coronavirus disease 2019: A Retrospective study of 66 hospital-acquired cases in a London teaching hospital. *Clinical Infectious Diseases, 72*(4), 690–693.

Riveron, T. P., Wilde, M. J., Ibrahim, W., Carr, L., Monks, P. S., Greening, N. J., Gaillard, E. A., Brightling, C. E., Siddiqui, S., Hansell, A. L., & Cordell, R. L. (2023). Characterisation of volatile organic compounds in hospital indoor air and exposure health risk determination. *Building and Environment, 242*, Article 110513. https://doi.org/10.1016/j.buildenv.2023.110513. ISSN 0360-1323.

Robinson, J. M., Cando-Dumancela, C., Liddicoat, C., Weinstein, P., Cameron, R., & Breed, M. F. (2020). Vertical stratification in urban green space aerobiomes. *Environmental Health Perspectives, 128*(11), Article 117008. https://doi.org/10.1289/EHP7807. Epub 2020 Nov 25. PMID: 33236934; PMCID: PMC7687659.

Rufino de Sousa, N., Steponaviciute, L., Margerie, L., Nissen, K, Kjellin, M, Reinius, B, … Rothfuchs, AG (2022). Detection and isolation of airborne SARS-CoV-2 in a hospital setting. *Indoor Air, 32*, Article e13023. https://doi.org/10.1111/ina.13023

Salmonsmith, J., Ducci, A., Balachandran, R., Guo, L, Torii, R, Houlihan, C, … Lovat, LB (2023). Use of portable air purifiers to reduce aerosols in hospital settings and cut down the clinical backlog. *Epidemiology and Infection, 151*, e21. https://doi.org/10.1017/S0950268823000092

Setti, L., Passarini, F., De Gennaro, G., Barbieri, P., Perrone, M. G., Borelli, M., Palmisani, J., Di Gilio, A., Torboli, V., Fontana, F., Clemente, L., Pallavicini, A., Ruscio, M., Piscitelli, P., & Miani, A. (2020). SARS-Cov-2 RNA found on particulate matter of Bergamo in Northern Italy: First evidence. *Environmental Research, 188*, Article 109754. ISSN 0013-9351.

Shankar, S. N., Witanachchi, C. T., Morea, A. F., Lednicky, J. A., Loeb, J. C., Alam, M. M., Fan, Z. H., Eiguren-Fernandez, A., & Wu, C. Y. (2022). SARS-CoV-2 in residential rooms of two self-isolating persons with COVID-19. *Journal of Aerosol Science, 159*, Article 105870. https://doi.org/10.1016/j.jaerosci.2021.105870. ISSN 0021-8502.

Shrestha, L. B., Foster, C., Rawlinson, W., Tedla, N., & Bull, R. A. (2022). Evolution of the SARS-CoV-2 omicron variants BA.1 to BA.5: Implications for immune escape and transmission. *Reviews in Medical Virology, 32*(5), Article e2381. https://doi.org/10.1002/rmv.2381. Epub 2022 Jul 20. PMID: 35856385; PMCID: PMC9349777.

Stern, R. A., Koutrakis, P., Martins, M. A. G., Lemos, B., Dowd, S. E., Sunderland, E. M., & Garshick, E. (2021). Characterization of hospital airborne SARS-CoV-2. *Respiratory Research, 22*, 73. https://doi.org/10.1186/s12931-021-01637-8

Sun, C., Xie, C., Bu, G. L., Zhong, L. Y., & Zeng, M. S. (2022). Molecular characteristics, immune evasion, and impact of SARS-CoV-2 variants. *Signal Transduct Target Ther, 7*(1), 202. https://doi.org/10.1038/s41392-022-01039-2. PMID: 35764603; PMCID: PMC9240077.

Thuresson, S., Fraenkel, C.-J., Sasinovich, S., Medstrand, P., Alsved, M., & Löndahl, J. (2024). One year weekly size-resolved air sampling of SARS-CoV-2 in hospital corridors and relations to the indoor environment. *Indoor Air.* , Article 5578611. https://doi.org/10.1155/2024/5578611, 7 pages, 2024.

Wilcox, M. E., Chong, C. A. K. Y., Niven, D. J., Rubenfeld, G. D., Rowan, K., Wunsch, H., & Fan, E. (2013). Do intensivist staffing patterns influence hospital mortality following ICU admission? A systematic review and Meta-analyses*. *Critical Care Medicine, 41*(10), 2253–2274. https://doi.org/10.1097/CCM.0b013e318292313a

Zhou, B., Liu, T., Yi, S., Huang, Y., Guo, Y., Huang, S., Zhou, C., Zhou, R., & Cao, H. (2022). Reducing the effectiveness of ward particulate matter, bacteria and influenza virus by combining two complementary air purifiers. *Int J Environ Res Public Health, 19*(16), Article 10446. https://doi.org/10.3390/ijerph191610446

Zhou, P., Yang, X. L., Wang, X. G., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature, 579*, 270–273. https://doi.org/10.1038/s41586-020-2012-7