

Rapid Environmental Monitoring, Capture, and Destruction Activities of SARS-CoV-2 and Bacterial Pathogens During the COVID-19 Health Emergency

Review began 10/02/2023
Review ended 10/06/2023
Published 10/11/2023

© Copyright 2023
Talukdar et al. This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 4.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Debjyoti Talukdar¹, Roberto Marchetti², Rosaria E. Pileci³

1. Medical Research, Mkhitar Gosh Armenian-Russian International University, Yerevan, ARM 2. Internal Medicine, Laboratori Clodia Diagnostics & Services, Bolzano, ITA 3. Research and Development, U-Earth Biotech Ltd, Milan, ITA

Corresponding author: Debjyoti Talukdar, drtalukdard@aol.com

Abstract

Background: The SARS-CoV-2 pandemic is a health emergency for occupational healthcare workers at COVID-19 hospital wards in Italy. The objective of the study was to investigate the bioreactor's effectivity in monitoring and improving air quality via detection, capture, and destruction of the SARS-CoV-2 virus and bacterial pathogens, reducing the risk of transmission among healthcare workers.

Methods: Bioreactors are a demonstrated effective biomonitoring system. We implemented a methodological approach wherein they were placed at various hospitals treating COVID-19 patients in Italy. The detection of the SARS-CoV-2 virus was achieved through rapid biomonitoring testing of the solutes from the AIRcel bioreactors via SARS-CoV-2 rapid test antigen and consecutive reverse transcription-polymerase chain reaction (RT-PCR) analysis with the multiplex platform (XABT) and the real-time PCR rotor-gene.

Results: The marked presence of the SARS-CoV-2 virus was found in multiple water samples via the detection of ORF1ab + N and/or E gene involved in gene expression and cellular signaling of the SARS-CoV-2 virus. The AIRcel bioreactors were able to neutralize the virus and bacterial pathogens effectively as traces of the viruses and bacteria were no longer found in multiple solute samples after an overnight period.

Conclusions: Transmission of COVID-19 via bioaerosols, transmitted by infected patients, remains a viable threat for health workers. AIRcel bioreactors allow for rapid biomonitoring testing for early virus detection within the environment, reducing the risk of exponential contagion exposure and maintaining good air quality without endangering health workers. This same protocol can also be extended to public spaces as a bio-monitoring hotspot tool for early detection.

Categories: Infectious Disease

Keywords: bacterial coinfection in covid-19, public health and safety, rt-pcr, air transmission, inhalation exposure, infectious diseases, bioaerosol, sars cov-2, biomonitoring, bioreactor

Introduction

Coronaviruses are part of a large family of viruses that infect humans and animals and are classified into four genera (α , β , γ , and δ). Human coronaviruses belong to α and β genera [1]. The current outbreak of β -coronavirus disease, emerged in Wuhan in December 2019 (COVID-19) and was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a pandemic (June 28, 2020 WHO report), which, at the time of paper submission, is affecting more than 55.6 million people, with more than 1.3 million deaths in 231 countries [2-4]. This pandemic outbreak, with regard to the ease and astonishing speed with which the virus spreads, has highlighted the necessity to implement preventive surveillance systems for viruses that are proactive and allow rapid isolation actions.

The significance of SARS-CoV-2 viral transmission via aerosols (airborne transmission) has been intensely discussed and it is now accepted to be among the pathways of viral transmission, together via larger respiratory droplets, and direct contact with contaminated surfaces [5,6]. Previously, other coronaviruses, (e.g., SARS and MERS) have been shown to disperse via aerosols and have been determined to cause nosocomial infections as well as extensive hospital outbreaks [7-9]. Even if the relative contributions of the different transmission modes remain unclear, the current evidence is sufficiently clear to justify engineering controls targeting airborne transmission as part of an overall strategy to minimize infection risk indoors. Doubtless, this need is particularly important in hospitals and other healthcare facilities managing COVID-19 patients.

In the first part of this article, some unpublished results from a sampling campaign at Saronno Hospital in

How to cite this article

Talukdar D, Marchetti R, Pileci R E (October 11, 2023) Rapid Environmental Monitoring, Capture, and Destruction Activities of SARS-CoV-2 and Bacterial Pathogens During the COVID-19 Health Emergency. Cureus 15(10): e46851. DOI 10.7759/cureus.46851

2011 are presented. These results show the AIRcel's efficiency at capturing and destroying particulate matter and airborne pathogenic bacteria. In the second part of the article, we tested the AIRcel bioreactor efficiency in detecting, capturing, and destructing the SARS-CoV-2 virus. The AIRcel units were placed in three different hospitals in Milan, Italy during the COVID-19 health emergency. Some water samples from the AIRcels were collected and sent to the laboratory to be tested for SARS-CoV-2 through real-time RT-PCR. Finally, we developed U-Alert, a protocol to achieve early detection of SARS-CoV-2 virus within the environment via rapid biomonitoring testing through rapid antigen test on the AIRcel water. Given the very promising results, we recommend health organizations and local governments install such protective measures to contain the spread of the virus in the interest of the people.

This article was previously posted to the medRxiv preprint server on November 27, 2020.

Materials And Methods

AIRcel bioreactor technology

'AIRcel' is a biological air treatment device manufactured by a team of researchers at U-Earth Biotech Ltd, a biotech company located in the UK and Italy, specializing in biological air purification in work environments. AIRcel is a bioreactor with a smart operating system that captures macro and micro molecules by ionic differentiation and, with the help of the active bio-oxidation process inside, recirculates 'purified' air outside the reactor. The unit is replenished weekly with water (which condenses inside the unit) and with a monthly dose of U-Ox additive (non-pathogenic, non-GMO), which is a consortium of microorganisms that digest air contaminants. These bioreactors, in correlation with a biofilter technology, consist of various phases in close contact such as a solid, liquid, and gas phase. The bioreactor itself is considered the solid phase, the liquid phase consists of water, and treated air is considered the gas phase. The usual biofilter design consists of physical support for biomass, whereas the patented plastic bioreactor consists of an optimized configuration in order to enhance the biomass-degrading activity, 'digesting' captured contaminants by bio-oxidation. Pollutants are attracted and captured via liquid-gas mixing through the reservoir tank which contains electrically grounded water that cleans and purifies the air. In these bioreactors, charged particles attract the contaminants and any odors generated, and they are removed through electrical grounding wherein organic compounds are oxidized [10]. Generally, pollutants move from high concentration to low concentration enabling the process to repeat itself as a sustainable technology wherein air quality is maintained. Unlike other air treatment systems, it does not require pressure membrane filters or high temperatures to operate. By attracting airborne pathogens carried by aerosols in rooms, which ordinarily deposit on surfaces, it helps maintain hygienic ventilation.

Instrumentation during the Saronno Hospital campaign in 2011

The campaign at Saronno Hospital in 2011 included the installation of one AIRcel 600 and four AIRcel 85 systems (two models of air purifiers that only differ in dimensions and capacities) into a highly frequented hospital area of 1,000 sqm with a footfall of around 1,300 people per day. The units were placed in visiting rooms, the central booking office, and a waiting area for 100 people. To verify the effectiveness of the system, monitoring activity on indoor air quality was performed for a one-month duration (from August 2, 2011 to September 5, 2011). The monitoring activity was also performed on processed water quality to evaluate the fate of contaminants captured. The trial was carried out during normal operating hours, with no sealing of the indoor environment (door and windows open or depending only on staff/patients' needs).

For particle sampling, the AEROTRAK™ Portable Airborne Particle Counter (ISO 21501-4:2007) was used for cleanroom particles classification following ISO 14644-1:1999 (0.3; 0.5; 1; 2; 3; 5 μm). For microbiological air sampling, the SAS Super IAQ Surface Air System (model 90593), which conveys a known volume of air during a fixed period on Petri Plates filled with Standard Plate Count Agar (PCA) was used. Samples were then cultivated and the colonies formed were counted after characteristic intervals. Microbiological analysis and standard chemical analysis (including Fluorides, Chlorides, Ca, Mg, K, Cu, Fe, Mn, As, total organic carbon, and conductivity analysis) were performed on water samples collected from the AIRcels water.

Instrumentation during the SARS-CoV-2 pandemic 2020

Ten AIRcel units per hospital were placed in three different hospitals in Milan, Italy during the COVID-19 health emergencies. At Sesto San Giovanni (Multimedica) Hospital (Figure 1a), the AIRcels were placed in the COVID-19 dialysis and visiting rooms. At San Raffaele Hospital (Figure 1b - note: the air extraction was present on the wall behind every bed), the units were placed in the emergency room (ER) and the COVID Intensive Care dedicated ward, used for the most serious cases, equipped with all the proper ventilation requirements (contamination most likely occurred during the application of ventilators on intubated patients). AIRcel bioreactors were also placed in other COVID-19 treatment areas including San Giuseppe Hospital (Figure 1c) in the obstetrician, ER, and canteen areas.



FIGURE 1: AIRcels setting at San Raffaele Hospital (a), Sesto San Giovanni (Multimedica) Hospital (b), and San Giuseppe Hospital (c) in Milan during the COVID-19 health emergency.

AIRcel - biological air treatment device

Multiple real-time PCR kit

Periodically, some water samples were extracted from the AIRcels and sent to our laboratory for SARS-CoV-2 detection based on conventional RT-PCR analysis. RT-PCR analysis involves radioactive isotope markers to detect genetic materials of the SARS-CoV-2 pathogen. The analysis is based upon biomolecular assay and is sensitive for mRNA detection. The target sequences have been identified and made public since December 2019; therefore, the manufacturers of the detection kits were able to market products useful for identifying the target sequence responsible for the pandemic in the early months of 2020.

For the real-time PCR, we used a detection system called (XABT) Multiple Real-Time PCR kit for the detection of 2019-nCov adapted by our research laboratory in Bolzano by Beijing Applied Biological Technologies Co. Ltd. The kit is based on a multiplex platform capable of simultaneously detecting an extended group of β -coronaviruses. This commercial kit consists of two distinct master mixes identified by tube A and tube B. The first is specific to the target SARS-CoV-2 gene ORF1ab + N, while the second contains the generic β -coronavirus E gene. We tested the kit with extremely diversified types of samples: in addition to the classic buccal, oropharyngeal samples, and fecal samples, solute samples were extracted from AIRcel bioreactors. To test the solutes (water + U-Ox additive) from the AIRcels, the QIAGEN's Pathogen extraction kit supplied by QIAGEN, (Hilden Germany) was used.

SARS-CoV-2 antigen biological sampling tests

The AIRcel water samples were also tested with the rapid antigen tests. The SARS-CoV-2 rapid test cassette

antigen (nasopharyngeal swab) is a rapid qualitative membrane-based immunoassay for the detection of SARS-CoV-2 antigens typically in human nasopharyngeal swab samples. In this study, we showed a variant of this test used on environmental samples on a water basis. The SARS-CoV-2 antibody-coated region of the test line, when in contact with the sample, reacts with the test SARS-CoV-2 antibody-coated particles. The mixture then migrates upward on the membrane by capillary action and reacts with the SARS-CoV-2 antibody in the test line region. If the sample contains SARS-CoV-2 antigens, a colored line will appear in the test line region as a result of the reaction. Positive results indicate the presence of viral antigens, but verification with a molecular method in the laboratory with PCR protocol is necessary for definitive confirmation. From the tests carried out on solute samples (water + U-Ox additive) taken from the AIRcels it is possible to verify in only 15 minutes whether the device has captured and incorporated suspended airborne particle “droplets” contaminated by SARS-CoV-2, circulating within the range of action of the device itself.

Results

During the COVID-19 crisis: technology testing for SARS-CoV-2 at Multimedica, San Raffaele, and Sacco Hospitals

In total 68 samples were processed in three distinct test sessions between April and June 2020, using the QIAGEN Rotor-Gene thermal cycler. The result of the RT-PCR showed a marked presence of the target β -coronavirus E gene for 19 of the 68 samples, while the target ORF1ab + N was detected in seven samples. In particular, at Sacco Hospital, the test results show the detection of ORF1ab + N and/or E genes in 15 samples out of 40.

Further experimental activity and related tests were conducted on the acquired samples. Twelve water samples of 100 ml each were selected and collected from hospital units that tested positive for both viral targets, and they were treated with 1 mL of U- Ox microbial additives on an equal volume of sample to test the short-term degradation capacity in vitro. In five out of 12 samples, traces of the viruses were no longer found after the overnight period. These results indicate the system's ability to capture virus droplets and destroy them inside the reactor. Based on these results we interpreted the results shown in Table 1 in the same way: in many samples, the SARS-CoV-2 virus was first captured and then digested by the AIRcel.

(a)	Multimedica Hospital			
Sample 1	30/04/2020	29/05/2020	04/06/2020	Notes
SARS-CoV-2	Detected	Digested	Not Detected	The water was not changed b/w the 2 testing rounds
β -coronavirus E gene	Detected	Digested	Detected	The water was not changed b/w the 2 testing rounds
Sample 2				
SARS CoV-2	Not Detected	Not Detected	Not Detected	-
β -coronavirus E gene	Detected	Digested	Not Detected	The water was not changed b/w the 2 testing rounds
(b)	San Raffaele Hospital			
Sample 1	30/04/2020	29/05/2020	-	Notes
SARS CoV-2	Not Detected	Detected	-	-
β -coronavirus E gene	Not Detected	Detected	-	-
Sample 2				
SARS CoV-2	Not Detected	Detected	-	-
β -coronavirus E gene	Detected	Digested	-	The water was not changed b/w the 2 testing rounds
Sample 3				
SARS CoV-2	Not Detected	Not Detected	-	-
β -coronavirus E gene	Detected	Detected	-	-
Sample 4				
SARS CoV-2	Not Detected	Not Detected	-	-
β -coronavirus E gene	Not Detected	Not Detected	-	-
(c)	Sacco Hospital			

Sample 1	04/06/2020	17/08/2020	-	Notes
SARS CoV-2	Detected	Not Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 2				
SARS CoV-2	Not Detected	Not Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 3				
SARS CoV-2	Not Detected	Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 4				
SARS CoV-2	Detected	Digested	-	The water was not changed b/w the 2 testing rounds
β-coronavirus E gene	Detected	Digested	-	The water was not changed b/w the 2 testing rounds
Sample 5				
SARS CoV-2	Not Detected	Detected	-	-
β-coronavirus E	Not Detected	Not Detected	-	-
Sample 6				
SARS CoV-2	Not Detected	Not Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 7				
SARS CoV-2	Not Detected	Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 8				
SARS CoV-2	Not Detected	Not Detected	-	-
β-coronavirus E gene	Detected	Detected	-	-
Sample 9				
SARS CoV-2	Not Detected	Not Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-
Sample 10				
SARS CoV-2	Not Detected	Not Detected	-	-
β-coronavirus E gene	Not Detected	Detected	-	-

TABLE 1: Test results show in Multimedica Hospital the detection of ORF1ab + N and/or E gene in four samples (a); test results show in San Raffaele Hospital the detection of ORF1ab + N and/or of ORF1ab + N and/or E gene in all six samples (b); test results show in Sacco E gene in 15 samples out of 40 (c).

The number of samples gathered from three different hospitals namely Multimedica Hospital, San Raffaele Hospital, and Sacco Hospital were different as they involved the detection of SARS-CoV-2 (ORF1ab, E & N genes) based on the number of samples extracted from the AIRcel bioreactors units placed in those hospitals in Milan, Italy, during COVID-19 health emergencies.

The AIRcel bioreactors underwent major improvements to better address the capture/decomposition process since 2011 as per the Italian Health and Environment Authority guidelines and validation. This study also

involved inputs from Azienda Sanitaria Locale (Local Health Authority, Milan, Italy), and ARPA Lombardia (Environment Authority). As demonstrated in Figures 2a-2d, 3, and Table 2, these bioreactors demonstrate that during monitoring activities in a highly frequented hospital environment, they captured bacterial pathogens in varying sizes ranging from 0.3 to 5 μ m and reduced the airborne load of these pathogens as signified as illustrated as reduction in colony forming units (CFU).

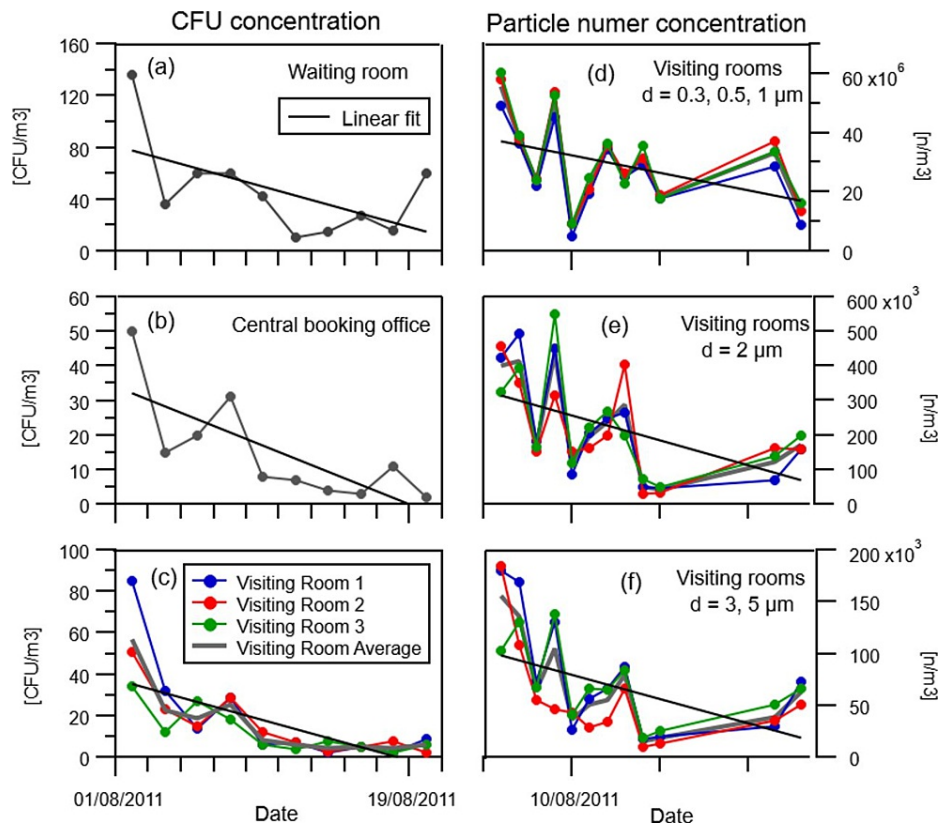
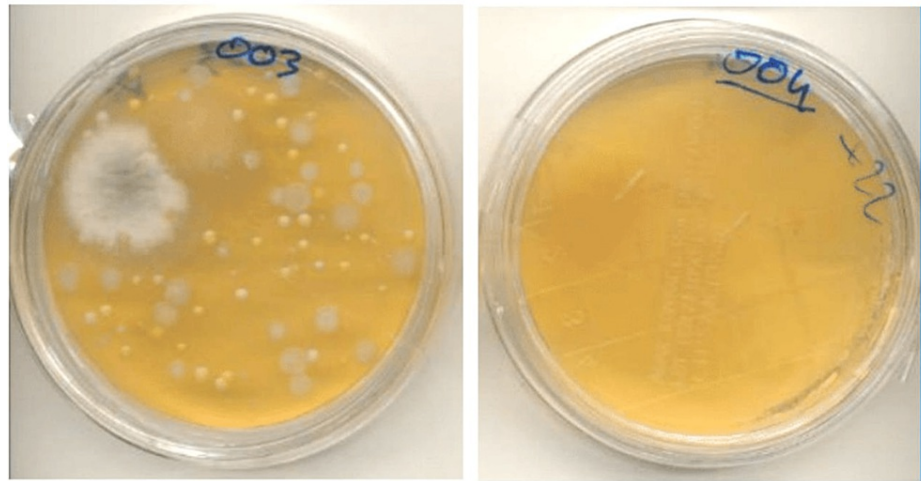


FIGURE 2: The left side of the figure shows the abatement in CFU concentration in the waiting room (a), central booking office (b) and visiting rooms (c). The right side of the graph illustrates the abatement in particle number concentration in the visiting rooms per different particle size ranges: 0.3, 0.5 and 1 μ m (d); 2 μ m (e) and 3, 5 μ m (f).

CFU - Colony forming units



BEFORE

AFTER

FIGURE 3: An example of some Petri Plates filled with Standard Plate Count Agar before and after the AIRcel placement.

Standard Plate Count Agar (PCA)

Bacteria counts concentration	Monitoring area	Average reduction	Max reduction
	Visiting rooms	87%	95%
	Central booking office	87%	98%
	Waiting room	57%	87%
Particles number concentration	Monitoring area	Average reduction	Max reduction
0.3; 0.5; 1.0 μm	Visiting rooms	51%	86%
	Central booking office	37%	83%
	Waiting room	35%	85%
2 μm	Visiting rooms	49%	89%
	Central booking office	33%	74%
	Waiting room	40%	80%
3; 5 μm	Visiting rooms	60%	90%
	Central booking office	47%	70%
	Waiting room	43%	63%

TABLE 2: Average and maximum (max) reduction per cubic meter per bacteria counts and particle number per different size ranges.

Figure 3 demonstrates bacterial content in Petri plates filled with Standard Plate Count Agar before placement of AIRcel bioreactors and after placement of these bioreactors in various hospital premises in Milan, Italy. It shows the significant reduction of bacterial pathogens from the surrounding environment. Lastly, Table 2 demonstrates the reduction of bacterial pathogens per cubic meter. It also shows a reduction in bacterial count concentration in various monitoring areas in three hospital premises namely Multimedita Hospital, San Raffaele Hospital, and Sacco Hospital, Milan, Italy, in terms of average reduction, and maximum reduction.

The main results of the campaign are shown in Figure 2 and Table 2. On the left side of the figure, the abatement of bacteria counts (CFU) concentration over the month of the campaign monitoring is shown

(also shown in Figure 3). Overall, in the waiting room, there is an average reduction of 57% in the CFU concentration with a maximum reduction of 87% (Figure 2a, Table 2); an average reduction of 87%, and a maximum reduction of 98% in the central booking office (Figure 2b, Table 2), and an average reduction of 87% and a maximum reduction of 95% in the visiting rooms (Figure 2c, Table 2). On the right side of the figure, the abatement in the particle number concentration in the visiting rooms per different particle ranges is shown.

As shown in Table 2, remarkable reductions in the number concentration of particles, from an average value of 37% to 69% were obtained per all the size classes. It is important to point out that this abatement trend was obtained in normal operating hospital activities, while usually the air purifier tests are run in a laboratory that does not fully represent real case scenarios.

Pathogenic bacteria such as Legionella, Escherichia coli, Enterobacter species, Enterococcus, Salmonella, and single-stranded RNA virus species like Enterovirus counts were checked in the processed water of the AIRcel after two and three months of system activity (October 5, 2011 and November 8, 2011), and the results were compared with tap water supplied in the hospital. The aim was to verify possible changes in the qualitative characteristics of the AIRcel water and if potential changes could be related to the activity of AIRcel in capturing the aero-dispersed pollutants. The findings showed Staphylococcus and Pseudomonas presence (in lower concentrations than the maximum values referred to drinkable water) in the AIRcel water sample and not in the tap water of the hospital. No bacteria presence was found in the second water sample on November 8, 2011. The results were interpreted by all the institutions involved, as a capture of the aero disperse Staphylococcus and Pseudomonas bacteria by the AIRcel and their successive digestion.

Discussion

Many recent literature studies reported the detection of SARS-CoV-2 in hospital air samples [11-14]. Among these works, a study performed air sampling in the general hospital ward and detected SARS-CoV-2 PCR-positive particles of sizes >4 µm and 1-4 µm in some rooms, despite these rooms having 12 air changes per hour [13]. Scientists have demonstrated that speaking and coughing produce a mixture of both droplets and aerosols in a range of sizes, that these secretions can travel together for up to 4-8 meters, that it is feasible for SARS-CoV-2 to remain suspended in the air and viable for hours, that SARS-CoV-2 RNA can be recovered from air samples in hospitals, and that poor ventilation prolongs the amount of time that aerosols remain airborne [11,15].

For this reason, in order to improve indoor air quality and reduce the risk of transmission, the WHO recommended a minimum ventilation rate of 288 m³ per person [16]. However, even where the ventilation systems run at optimal rate there are still frequent cases of airborne bacteria and viruses causing hospital infections. To contain the spread of the virus and reduce transmission and infection rates, air purifiers can act as a supplementary measure [17]. Studies show that air purifiers in dental clinics have reduced the transmission of airborne pathogens and exposure to health workers via droplets and aerosols [18]. Unfortunately, most air purifiers that capture air pollutants by using ventilation, seem not to be completely effective. In a 2003 work, five experiments were conducted to assess how aerosolized bacteria and spores respond like particulate contaminants to the primary electrical forces in a room [19]. Most are too small to respond to gravity and ventilation, thus remaining suspended in the air causing potential contagion. This concept can help to better understand viruses' possible spreading dynamics.

In this work, the AIRcels, air purifiers manufactured by a team of researchers at U-Earth Biotech Ltd, were used. The AIRcel attracts the pollutants through a concentration gradient and not through ventilation, then captures them into a water tank where charged particles are removed by electrical grounding and the organic compounds are oxidized. This technology has undergone intensive field testing in hospitals (surgery rooms, ERs, and labs), waste treatment plants [20], and heavy-duty manufacturing environments [21,22] since 2011, proving itself excellent at capturing and destroying a very broad range of contaminants.

Currently, the reference method for the detection of SARS-CoV-2 is the reverse transcription polymerase chain reaction (RT-PCR). Although RT-PCR has high sensitivity and specificity, it requires skilled personnel and a long period of data processing and analysis [23]. Several types of SARS-CoV-2 real-time RT-PCR kits have been developed and approved [24,25]. In this work, the multiplex platform (XABT) Multiple Real-Time PCR Rotor-Gene was used. For qualitative immediate detection of SARS-CoV-2, rapid antigen tests are also available [26]. The RT-PCR test is conducted using different types of specimens, including sputum, nasopharyngeal swabs, pharyngeal swabs, and saliva. Both RT-PCR and rapid antigen tests are principally performed on symptomatic patients and on those who were in close contact with positive patients. However, many virus carriers are asymptomatic and without the ability to screen them quickly and effectively, they can have the potential to increase the risk of disease transmission if no early effective measures are implemented [27,28]. Therefore, there is an urgent need for non-invasive systems that can be used to localize the source of the infection and act as an early warning.

In this regard, a research article illustrates the wastewater-based epidemiology (WBE) analysis, as an effective approach to predict the potential spread of the infection by testing for infectious agents in

wastewater, since feces and urine from disease carriers in the community contain many biomarkers [29]. Wastewater collected in epidemic circulation areas like Milan and Rome were found to be positive for the SARS-CoV-2 virus, according to clinical data, RNA was detected with six samples out of 12 testing positive [30].

This grants AIRcels full-fledged inclusion as a valid support for biomonitoring of the areas where they are installed. AIRcels were found to be effective in hospital wards where COVID-19-positive patients or suspected asymptomatic patients were kept for observation. The air purifiers reduced the viral load by capturing and destroying the SARS-CoV-2 virus in their vicinity and therefore reduced exposure to the virus.

Another session of experiments was conducted to test a new rapid diagnostic kit for the detection of SARS-CoV-2 antigen, on samples that already tested positive for the specific target when processed with the real-time PCR-RT. The purpose of the experiment was to correlate the same methods used to detect the SARS-CoV-2 target for human use to a new environmental level, in order to have a useful detection system for the AIRcel users to run on their own and get results in less than 15 minutes. Both positive and negative samples were then compared to verify the sensitivity of the rapid kit and its use since the CE IVD validation required its use for diagnostic purposes only. The same quantity as foreseen by the protocol, validated with a nasopharyngeal swab, was tested by directly entering the solute extracted from the AIRcel water tank into the test panel cassette of the chromatographic support used to perform the test. All the samples gave the same results as the RT-PCR. These very promising results allowed us to develop a protocol we called U-Alert for continuous environmental biomonitoring and quick virus detection of SARS-CoV-2 RNA through analysis of the recirculating water of the AIRcel, first with the rapid antigen diagnostic kit, then by confirmation of the results via RT-PCR. In this way, the complete virus detection protocol provides early warning that quickly confirms (within 24 hours) the presence of the viral targets being detected.

Limitations

The study was limited to data in terms of 10 AIRcel bioreactor units placed in three different hospitals in Milan, Italy, during COVID-19 health emergencies. Therefore, the study is limited to projecting the efficacy of AIRcel bioreactor combating COVID-19 based on the data gathered from Milan, Italy.

Conclusions

This study has demonstrated the capability of AIRcel bioreactors to capture and digest viruses such as SARS-CoV-2 and β - coronaviruses. It is also important to note that it is possible to carry out this checking activity periodically, on a scheduled basis, for the placed AIRcels. The continuous monitoring activities can provide useful data on the biological activity of bioreactors over time, to evaluate how the microbial community present in the devices interacts with the virus. In this study, AIRcel bioreactors have demonstrated an analytical approach to maintain air quality and quantify the presence of viral targets through efficient biomonitoring leading to the capture and destruction of SARS-CoV-2 and β -coronaviruses. The use of AIRcels as an environmental monitoring tool, especially if combined with the rapid detection system of the SARS-CoV-2 antigen supplied with the air purifier, provides valid support to the user, by giving the possibility of carrying out a preventive diagnosis not only on air quality but also of the actual presence of the coronavirus (with U-Alert protocol), which will then be eventually confirmed with the RT-PCR technique. Detecting the presence of inactivated SARS-CoV-2 in the AIRcels' water tanks, for example in a school, while lowering the viral charge suspended in the air, could also effectively inform on the possible presence of positive individuals in selected classrooms that require further testing. Accurate detection is crucial for prevention.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Debjyoti Talukdar, Roberto Marchetti, Rosaria E. Pileci

Acquisition, analysis, or interpretation of data: Debjyoti Talukdar, Roberto Marchetti, Rosaria E. Pileci

Drafting of the manuscript: Debjyoti Talukdar, Roberto Marchetti, Rosaria E. Pileci

Critical review of the manuscript for important intellectual content: Debjyoti Talukdar, Roberto Marchetti, Rosaria E. Pileci

Supervision: Debjyoti Talukdar, Roberto Marchetti, Rosaria E. Pileci

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

The authors wish to thank specific individuals who assisted in this study along with hosting the AIRcel units in COVID-affected areas, providing samples and assistance crucial for the study: Ospedale San Raffaele team, and specifically Dr. Elena Bottinelli and Dr. Matteo Moro, Ospedale Multimedica: Dr. Matilde Ardizzi, Eng. Varisco Dr. Andrea Vergani and Dr. Davide Pasetti. Ospedale Sacco: Dr. Matteo Letzger, Dr. Pietro Olivieri, and Dr. Gabriele Luongo. Special thanks to Betta Maggio and the UEarth Team for providing background information on the technology, AIRcel equipment, and inspiration for this study.

References

- Cui J, Li F, Shi ZL: Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol.* 2019, 17:181-92. [10.1038/s41579-018-0118-9](https://doi.org/10.1038/s41579-018-0118-9)
- Chan JF, Yuan S, Kok KH, et al.: A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet.* 2020, 395:514-23. [10.1016/S0140-6736\(20\)30154-9](https://doi.org/10.1016/S0140-6736(20)30154-9)
- Huang C, Wang Y, Li X, et al.: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020, 395:497-506. [10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)
- Weekly epidemiological update and weekly operational update. (2020). Accessed: February 1, 2023: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>.
- Morawska L, Cao J: Airborne transmission of SARS-CoV-2: the world should face the reality. *Environ Int.* 2020, 139:105730. [10.1016/j.envint.2020.105730](https://doi.org/10.1016/j.envint.2020.105730)
- van Doremalen N, Bushmaker T, Morris DH, et al.: Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med.* 2020, 382:1564-7. [10.1056/NEJMc2004973](https://doi.org/10.1056/NEJMc2004973)
- Booth TF, Kournikakis B, Bastien N, et al.: Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. *J Infect Dis.* 2005, 191:1472-7. [10.1086/429634](https://doi.org/10.1086/429634)
- Cho SY, Kang JM, Ha YE, et al.: MERS-CoV outbreak following a single patient exposure in an emergency room in South Korea: an epidemiological outbreak study. *Lancet.* 2016, 388:994-1001. [10.1016/S0140-6736\(16\)30623-7](https://doi.org/10.1016/S0140-6736(16)30623-7)
- Kim SH, Chang SY, Sung M, et al.: Extensive viable Middle East Respiratory Syndrome (MERS) coronavirus contamination in air and surrounding environment in MERS isolation wards. *Clin Infect Dis.* 2016, 63:363-9. [10.1093/cid/ciw239](https://doi.org/10.1093/cid/ciw239)
- La Rosa G, Mancini P, Bonanno Ferraro G, et al.: SARS-CoV-2 has been circulating in northern Italy since December 2019: evidence from environmental monitoring. *Sci Total Environ.* 2021, 750:141711. [10.1016/j.scitotenv.2020.141711](https://doi.org/10.1016/j.scitotenv.2020.141711)
- Guo ZD, Wang ZY, Zhang SF, et al.: Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. *Emerg Infect Dis.* 2020, 26:1583-91. [10.3201/eid2607.200885](https://doi.org/10.3201/eid2607.200885)
- Lednický JA, Lauzard M, Fan ZH, et al.: Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int J Infect Dis.* 2020, 100:476-82. [10.1016/j.ijid.2020.09.025](https://doi.org/10.1016/j.ijid.2020.09.025)
- Chia PY, Coleman KK, Tan YK, et al.: Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nat Commun.* 2020, 11:2800. [10.1038/s41467-020-16670-2](https://doi.org/10.1038/s41467-020-16670-2)
- Santarpia JL, Rivera DN, Herrera VL, et al.: Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci Rep.* 2020, 10:12732. [10.1038/s41598-020-69286-3](https://doi.org/10.1038/s41598-020-69286-3)
- Bourouiba L: Turbulent gas clouds and respiratory pathogen emissions: potential implications for reducing transmission of COVID-19. *JAMA.* 2020, 323:1837-8. [10.1001/jama.2020.4756](https://doi.org/10.1001/jama.2020.4756)
- World Health Organization: Natural ventilation for infection control in health-care settings. World Health Organization, Geneva; 2009.
- Zhao B, Liu Y, Chen C: Air purifiers: a supplementary measure to remove airborne SARS-CoV-2. *Build Environ.* 2020, 177:106918. [10.1016/j.buildenv.2020.106918](https://doi.org/10.1016/j.buildenv.2020.106918)
- Chen C, Zhao B, Cui W, Dong L, An N, Ouyang X: The effectiveness of an air cleaner in controlling droplet/aerosol particle dispersion emitted from a patient's mouth in the indoor environment of dental clinics. *J R Soc Interface.* 2010, 7:1105-18. [10.1098/rsif.2009.0516](https://doi.org/10.1098/rsif.2009.0516)
- Utrup LJ, Werner K, Frey AH: Minimizing pathogenic bacteria, including spores, in indoor air. *J Environ Health.* 2003, 66:19-26, 29.
- Shim JS, Jung JT, Sofer S, Lakhwala F: Oxidation of ethanol vapors in a spiral bioreactor. *J Chem Technol Biotechnol.* 1995, 64:49-54. [10.1002/jctb.280640109](https://doi.org/10.1002/jctb.280640109)
- Zanni S, Bonoli A: Indoor air quality in waste treatment: environmental issue and biotechnology application for air. *WSEAS Trans Environment Development.* 2014,
- Zanni S, Bonoli A: Indoor air quality in waste treatment: environmental issue and biotechnology application for air pollution containment, a case study. *WSEAS Trans Environment Development.* 2014, 10:529-41.
- Zanni S, Bonoli A, Mancini M: Abatement and bio-digestion of airborne contamination in precision

- mechanics: the case study of Beretta firearms. IWWATV. 2015,
24. Shen M, Zhou Y, Ye J, Abdullah Al-Maskri AA, Kang Y, Zeng S, Cai S: Recent advances and perspectives of nucleic acid detection for coronavirus. *J Pharm Anal.* 2020, 10:97-101. [10.1016/j.jpha.2020.02.010](https://doi.org/10.1016/j.jpha.2020.02.010)
 25. Iglói Z, Leven M, Abdel-Karem Abou-Nouar Z, et al.: Comparison of commercial realtime reverse transcription PCR assays for the detection of SARS-CoV-2. *J Clin Virol.* 2020, 129:104510. [10.1016/j.jcv.2020.104510](https://doi.org/10.1016/j.jcv.2020.104510)
 26. van Kasteren PB, van der Veer B, van den Brink S, et al.: Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. *J Clin Virol.* 2020, 128:104412. [10.1016/j.jcv.2020.104412](https://doi.org/10.1016/j.jcv.2020.104412)
 27. Hirotsu Y, Maejima M, Shibusawa M, et al.: Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs, including from seven serially followed patients. *Int J Infect Dis.* 2020, 99:397-402. [10.1016/j.ijid.2020.08.029](https://doi.org/10.1016/j.ijid.2020.08.029)
 28. Long QX, Tang XJ, Shi QL, et al.: Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med.* 2020, 26:1200-4. [10.1038/s41591-020-0965-6](https://doi.org/10.1038/s41591-020-0965-6)
 29. Gao M, Yang L, Chen X, et al.: A study on infectivity of asymptomatic SARS-CoV-2 carriers. *Respir Med.* 2020, 169:106026. [10.1016/j.rmed.2020.106026](https://doi.org/10.1016/j.rmed.2020.106026)
 30. Mao K, Zhang H, Yang Z: Can a paper-based device trace COVID-19 sources with wastewater-based epidemiology?. *Environ Sci Technol.* 2020, 54:3733-5. [10.1021/acs.est.0c01174](https://doi.org/10.1021/acs.est.0c01174)