Particulate matter ($PM_{2.5}$) as a potential SARS-CoV-2 carrier

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Abstract

The rapid spread of the SARS-CoV-2 in the COVID-19 pandemic had raised questions on the route of transmission of this disease. Initial understanding was that transmission originated from respiratory droplet from an infected host to a susceptible host. However, indirect contact transmission of viable virus by fomites and through aerosols has also been suggested. Herein, we report the involvement of fine indoor air particulate with the diameter of ≤ 2.5 μm ($PM_{2.5}$) as the transport agent of the virus. $PM_{2.5}$ was collected over four weeks during a 48 hours measurement intervals in four separate wards containing different infected clusters in a teaching hospital in Kuala Lumpur, Malaysia. Our results indicated highest SARS-CoV-2 RNA on $PM_{2.5}$ in the ward associated with a lavatory. We suggest a link between the virus-laden $PM_{2.5}$ and the ward's design. Patients' symptoms and numbers that govern the magnitude of viral shedding may also influence the number of airborne SARS-CoV-2 RNA on $PM_{2.5}$ in an enclosed environment.

Main Text

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)is primarily transmitted via respiratory droplets of varioussizes[1-3]. Large respiratory droplets (> 5 μm)transmission occur when a person is in close contact with someone(WHO 2014)[4] who has respiratory symptoms such ascoughing or sneezing[5], whereas finer virus-ladenrespiratory droplets and particulate matters (≤5 μm) can remain inthe air for an extended period and be carried over greaterdistances[6] > 6 m (such as the outbreak oftuberculosis, measles and chickenpox)[7]. Despite numerousstudies that have demonstrated the transmission route of SARS-CoV-2via respiratory droplets, evidence on aerosols-borne transmissionremains limited[1,8,9]. Recently, the transmission ofvirus via solid aerosols has been reported in several hospitals inWuhan. The study indicates positive detection of SARS-CoV-2 in arange of particulate matter (PM) from submicrometer and/orsupermicrometer [1,10] , suggesting that SARS-CoV-2 can betransported via solid aerosols. No correlation was found betweenvirus concentration and particulate matter as a function ofparticles diameter. Nevertheless, positive correlations between$PM_{2.5}$ and other respiratory viruses such as theinfluenza virus have been reported[11], emphasizing thepossibility of particulate matter as a transport carrier forSARS-CoV-2.

$PM_{2.5}$ is fine solid aerosols with a particle diameterof ≤ 2.5 μm that is suspended in ambient air. $PM_{2.5}$ inindoor environments is largely derived from common outdoor sourcessuch as motor-vehicles, biomass burning, and industrialemissions[12,13,14]. Prolonged exposure to$PM_{2.5}$ is particularly detrimental to human health asthis fine particulate matter can be easily inhaled and penetratedeep into the lungs[15,16]. In the air, $PM_{2.5}$is known to have a significantly longer lifetime where it can besuspended at an extended period compared to respiratory liquiddroplets. This longer lifetime of particles may pose a significantviral exposure to healthcare personnel, especially in indoorenvironments. $PM_{2.5}$ can also be deposited in

indoorenvironments such as hospitals' flooring[17,18] and anysurface materials[19,20]. This fine particulate matter isreadily propagated by tiny turbulent eddies in the air that arisefrom physical activities such as human movements andwalking[21,,22]. Considering the fact that the viabilityof SARS-CoV-2 on many types of surfaces have been reported(*e.g.*, on metals for 48 hours, plastic for 72 hours,cardboard for 24 hours, and copper for 4 hours)[23,24], itis likely that the virus on the surface can be potentially lodgedon the $PM_{2.5}$ and redistributed/transported back into theair.

Recent findings based on measurements of air particles havesuggested that SARS-CoV-2 could be re-suspended in the air whenhealthcare workers remove their personal protective equipment(PPE)[2,5]. Furthermore, it is also suggested thatsuspended tiny dust in the air could couple with microorganisms ofdiameter < 5 μm during aerosolization[7]. Since thediameter of the SARS-CoV-2 is two orders of magnitude smaller -approximately 70–90 nm[25], the mechanism/mode of theairborne transport is still unclear and, therefore, worthexploring. In this study, we hypothesize the possible role of$PM_{2.5}$ as a carrier (or transport agent) for SARS-CoV-2.In this study, we aim to investigate the possible link between$PM_{2.5}$ and SARS-CoV-2 from several wards in ahospital.

Indoor $PM_{2.5}$: We measured$PM_{2.5}$ using an in-house air qualitysensor[12,26], *AiRBOXSense* (see SI forspecifications). This was in tandem with sampling of$PM_{2.5}$ on filter papers using a Low Volume Sampler (LVS)(see SI for details). Air samplings were done for 48 hours in thisstudy to maximise the potential capture of virus loading on$PM_{2.5}$. Typically, $PM_{2.5}$ measurements aretaken in lesser intervals (*e.g.*, 8 and 24hours)[23]. We also used quartz microfiber filters (poresize of 0.6-0.8 um) in this study instead of nanofiber filters(commonly used in virus filtration) to enable us to link SARS-CoV-2transmission via $PM_{2.5}$ (see SI for details).

All $PM_{2.5}$ measurements and samplings were taken inCOVID-19 wards. General details of patients and how they weresegregated in wards are described in Table 1 and SI. The highestconcentration of indoor $PM_{2.5}$ was measured in generalward B (Table 1) (13.27 μgm^{-3}), while the lowestconcentration was measured in general ward D (2.63μgm^{-3}). General ward B was occupied by a cluster ofpatients from the same institution and was observed to have themost activity among the patients. Higher $PM_{2.5}$concentrations can be contributed by physical activities such asmovements of health workers and patients[21,28,29]. The$PM_{2.5}$ concentrations measured in this study are slightlylower than reported in a European urban hospital[30].

Virus RNA analysis: Filter membranes collectedfrom the LVS from different wards were used in the detection ofSARS-CoV-2 RNA. The results from the Reverse TranscriptionQuantitative Real Time Polymerase Chain Reaction (RT-qPCR) showedpositive detection of the SARS-CoV-2 nucleocapsid (N) gene. Onlythe N1 nucleocapsid gene was successfully detected. According tothe Emergency Use Authorization (EUA)[31], detection ofeither the N1 or N2 gene is considered positive for the presence ofSARS-CoV-2 (Orig3n, Inc. 2020). We detected positive results forSARS-COV-2 genes in the single room

Ward A (74 ± 117.1 copiesuL^{-1}) and General Ward B (10 ± 7.44 copiesuL^{-1}). The viral genomes extracted consist of the totalviral genome from the filter paper. Therefore, the high standarddeviation reported may be a result of the heterogenous mixture ofgenomic RNA in the RT-qPCR template and the presence of therelatively low SARS-CoV-2 genome. Nonetheless, the cycle threshold(CT) value was <40 (Orig3n Inc. 2020), confirming the positivedetection of SARS-CoV-2 in our samples (Table 1). Due tooperational restriction imposed by the hospital, the sample sizewas limited and a very low viral RNA yield was recovered. Theuniqueness in the result is that viral RNA was still able to bedetected in the single occupancy ward (Ward A). Ward A is a smallenclosed room (22 m^2) with a lavatory attached. Thefrequent use of the lavatory resulted in the increase ofviral shedding activity. We suspect that virus-ladenPM$_{2.5}$ generated from the shedding activity circulatedwithin the enclosed room despite low PM$_{2.5}$ concentration(11.25 µg m^{-3}), thus explaining the spike in the data.The degree of viral shedding (from the patients) due to symptomssuch as coughing, sneezing, diarrhoea, etc. has been reported toinfluence the number of virus particles in theenvironment[1,5]. It is suggested that the increased virusparticles (due to shedding) in a poorly ventilated environmentmight increase the virus-PM$_{2.5}$assemblage[9,19,34]. A study done by [5]reported that they were not able to detect SARS-CoV-2 in all oftheir tested air samples. However, they highlighted that theirshort sampling time of 15 min - 4 h might not represent total airvolume in the ward and the presence of SARS-CoV-2 might havepossibly been diluted during air exchanges in the ward. Thislimitation was modified in our study by extending the air samplingduration.

SARS-CoV-2 RNA was also detected in General Ward B. General WardB is a larger room (100 m^2) consisting of 18 occupiedbeds with two air purifying units installed at a distance of 8 maway from the LVS. The amount of SARS-CoV-2 collected in theparticulate matter is significantly lower than from Ward A despitethe higher number of patients and concentration of PM$_{2.5}$(17.58 µgm^{-3}). Such a low concentration of viral load inthe aerosol could be attributed to the fact that all the leading tominimal virus shedding; although higher particulate matter readingin relation to the occupants' activities that promotes there-suspension of PM$_{2.5}$ from the floor andsurfaces[35,36].

Virus-laden PM$_{2.5}$ was not detected in Wards C and Ddespite having similar ward size. The number of patients in Ward Cis similar to Ward B, whereas the number of patients in Ward D ishalf of that of Wards C. The patients in Ward C and Ward D werealso diagnosed with mild symptoms. The non-detection of the virusin these wards may be due to very low virus shedding from thepatients. Another possible factor to explain the absence ofSARS-CoV-2 RNA in PM$_{2.5}$ is that the LVS in Ward C (andalso Ward D) was positioned adjacent to an air purifier. Althoughthe effectiveness of air-purifier in removing PM$_{2.5}$remains unclear, air-filtration has been reported to reduce viralloading in air[35,36].

Our results clearly indicated that SARS-CoV-2 RNA is presentwithin PM$_{2.5}$ particles. Hence, it is crucial todetermine whether these RNAs came from intact virus particles orare merely RNA from non-infectious virus particles. The detectionof SARS-CoV-2 viral RNA on surfaces was previously reported on acruise ship, the Diamond Prince, even after 17 days after theevacuation of passengers[37]. In addition, the Centres

forDisease Control and Prevention (CDC) pointed out that theinfectivity of the detected particles was still uncertain. A studycarried out in a CDC facility showed that SARS-CoV-2 was able toremain infectious up to 72 hours on some surfaces[24].Thus, it is suggested that infectious virus be determined byculturing of virus residing on the $PM_{2.5}$ ontoappropriate cell culture. Although our study could not show adirect link between the concentration of $PM_{2.5}$ andSARS-CoV-2. We did find that $PM_{2.5}$ generated fromhuman activities in healthcare facilities can influence thepresence of SARS-CoV-2 RNA in indoor environments. Furthermore, thedegree of viral shedding from symptomatic patients may alsoinfluence the presence of SARS-CoV-2 RNA on $PM_{2.5}$.Therefore, we recommend that all possible precautions againstairborne transmission in indoor environments should be takenseriously.

Declarations

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Author contributions M.S.M.N conceptualized theidea. M.S.M.N, N.S.M.N, N.I and Z.Z.R aided in the study design.M.S.M.S performed the indoor air sampling procedures. N.S.M.N Y.C.Wand NI did the qRT-PCR and rRT-PCR analyses. M.S.M.N, N.S.M.N,Y.C.W and L.C.Y wrote the early versions of the manuscript. Allauthors contributed equally to the data analysis andinterpretations. All authors co-wrote the final version of thepaper.

Competing interests The authors declare nocompeting interests.

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Table

Table 1|Summary of the data collected atHCTM.

Ward[a]	$PM_{2.5}$ (µgm-3[3])[b]	SARS-CoV-2 RNA[c]	No. of occupiedbeds	Area[d]	Remarks[e]
Singleroom Ward A	11.25 ±2.05	Detected	1	~ 22m^2	Withoutair purifier
GeneralWard B	17.58 ±4.27	Detected	18	~ 100m^2	Two airpurifiers (LVS sampler located far from the airpurifier)
GeneralWard C	14.66 ±5.59	Notdetected	17	~ 100m^2	One airpurifier
GeneralWard D	7.57 ±1.37	Notdetected	8	~ 100m^2	One airpurifier

[a] Selected wards thatwere sampled consisting of different patient clusters: Single roomward A, an executive ward that hosts only one COVID-19 patient;General ward B was occupied an institutional cluster; General WardC was occupied by patients arriving from overseas; and General WardD was occupied by migrant workers (see SI fordetails).
[b]Average 48 hourlyconcentrations (with standard deviation) of $PM_{2.5}$measured in different wards at HCTM.
[c] Detection of SARS-CoV-2RNA on captured $PM_{2.5}$ at differentwards.
[d] Ward area is anapproximate measurement and not the officialmeasurement.
[e] SeeSI for details on air purifiers.

Figures

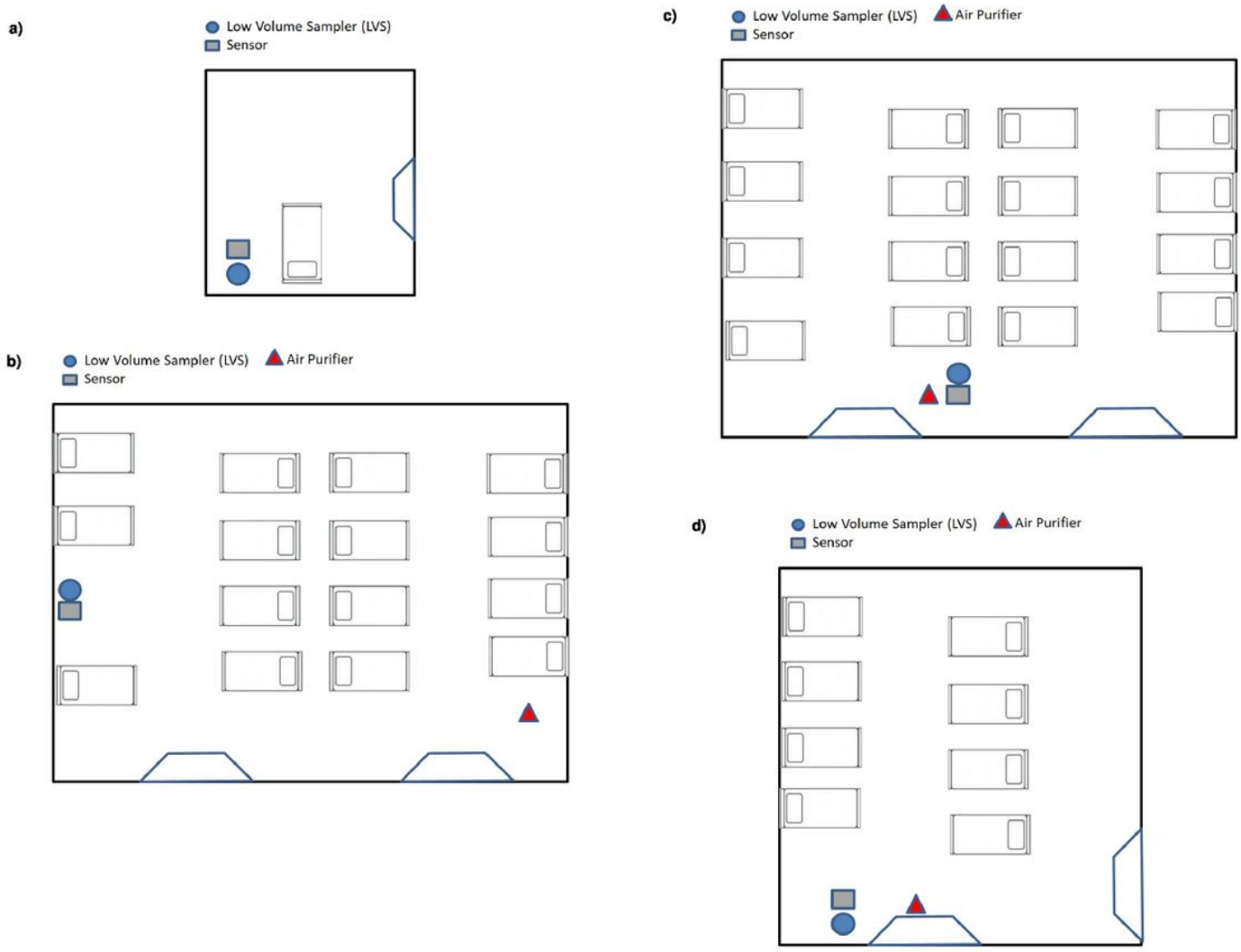

Figure 1

Characteristics of the wards and instrumentation deployment in this study a) Single room A and b) General ward B c) General ward C d) General ward D.

Supplementary Files

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