**The size distribution of SARS-CoV-2 genetic material in**

**airborne particles sampled in hospital and home care environments occupied by COVID-19 positive subjects**

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Abstract

Characterizing the size distribution of airborne particles carrying SARS-CoV-2 virus is essential for understanding and predicting airborne transmission and spreading of COVID-19 disease in hospitals as well as public and home indoor settings. Nonetheless, few data are currently available on virus-laden particle size distribution. Thus, the aim of this study is reporting the total concentrations and size distributions of SARS-CoV-2- genetic material in airborne particles sampled in hospital and home environments. A nanoMOUDI R122 cascade impactor (TSI, USA) was used to collect size-segregated aerosol down to the sub-micron range in home and in three different hospital environments in presence of infected patients in order to provide the concentration of airborne SARS-CoV-2 genetic material for each particle size range at different sampling locations. Providing one of the largest datasets of detailed size-fractionated airborne SARS-CoV-2 RNA to date, we found that 45.2% of the total sub- and super-micrometric fractions were positive for SARS-CoV-2 with its genetic material being present in 17.7% of sub-micrometric (0.18–1 μm) and 81.9% of super-micrometric (>1 μm) fractions. The highest concentration of SARS-CoV-2 genetic material in total suspended particles (5.6 ± 3.4 RNA copies m-3) was detected in the room occupied with patients with more severe COVID-19 symptoms collected during the patients’ high flow nasal oxygen therapy. The highest concentration at certain particle size fraction strongly depends on the sampling environment. However, the contribution of SARS-CoV-2 genetic material was in favour of super-micrometric compared to sub-micrometric particle size range. The evaluation of the individual risk of infection was carried out on the basis of the obtained data considering a hypothetical exposure scenario. The obtained results indicate the necessity of the protective masks in presence of infected subjects, especially while staying for longer period of time in the hospital environments.

Keywords: airborne virus transmission; indoor air; particle size-distribution; risk assessment; submicrometric particles

1. Introduction

The dynamic of the COVID-19 pandemic is complex in terms of transmission pathways, infection risks and treatment approach. Despite of all efforts made by the medical experts and scientific community, many challenges remain unsolved in order to better understand epidemiology, pathology and transmission of COVID-19 (Dinoi et al., 2021, Domingo et al, 2020, Nazaroff, 2022, Tellier, 2022).

Expiratory human activities such as coughing, sneezing, and speaking generate a large size spectrum of respiratory particles which size can range from the sub-micrometric range to several hundreds of µm (Morawska et al., 2009, Nazaroff, 2022, Seminara et al., 2020, Shen et al., 2022, Stadnytskyi et al., 2021). The aerodynamic diameter is an important parameter for airborne particles since it can serve as a vector of pathogens which could be carried away by air currents or air jets well beyond the separation distance recommended during the current pandemic (2 m) (Bourouiba, 2020, Milton, 2020). The lifetime of particles in the air is heavily dependent on the size: fine (< 2.5 μm) and especially sub-micrometric particles remain airborne for longer periods of time and may travel further than 2 m (Bahl et al., 2020).

 A large body of evidence was reported on detection and different aspects of SARS-CoV-2 airborne transmission. First, SARS-CoV- 2 was reported to remain viable in the air for up to 3 h, with a half-life of 1.1 h, in laboratory experiments (van Doremalen et al., 2020). In other field studies, viable SARS-CoV-2 was detected in air samples collected in hospital wards and rooms occupied by COVID-19 patients (Lednicky et al., 2020; Santarpia et al., 2022). Numerous studies have detected SARS-CoV-2 RNA in airborne samples of total suspended particles (TSP) without size fractionation (Binder et al., 2020; Ding et al., 2020; Kenarkoohi et al., 2020; Lednicky et al., 2020; Razzini et al., 2020; Santarpia et al., 2020); whereas other studies collecting TSP did not detect the presence of any forms of SARS-CoV-2 RNA in samples (Cheng et al., 2020; Faridi et al., 2020; Liu et al., 2020; Masoumbeigi et al., 2020; Ong et al., 2020). Even though the evidence suggests that smaller respiratory particles, may be a vector for COVID-19 (Morawska and Cao, 2020; Morawska and Milton, 2020; Noorimotlagh et al., 2020; Stadnytskyi et al., 2020) and that the size of the particles containing pathogens impacts the likelihood of infection (Thomas et al, 2013), little is known about the size distribution of SARS-CoV-2 genetic material and its airborne transmission through different particle size fractions in indoor and outdoor air. Few studies consider size distribution in hospital environment: Liu et al., (2020) found SARS-CoV-2 in two size ranges (between 0.25 and 1.0 μm and > 2.5 μm); del Real et al., (2022) in one size range (<2.5 μm); Santarpia et al. (2022) in three size ranges ( < 1 μm, 1–4 μm, and > 4.1 μm); Chia et al. (2020) in two size ranges (1–4 μm and > 4 μm); Stern et al. (2021b, 2022) in three size ranges (< 2.5 μm, 2.5–10 μm, and >10 μm) and Oksanen et al., (2022) in six size ranges (0.65–4.7 μm, 7.0–12.0 μm, >10 μm, and <100 μm). All of these studies found SARS-CoV-2 RNA positive samples in multiple size fractions, however, they all report a rough size distribution (up to 6 size ranges), without detailed information on virus distribution in sub-micrometric particles.

 Here we show for the first time the fine resolution of the size distribution of SARS-CoV-2 genetic material (originated from respiratory particles of COVID-19 positive patients) in airborne particles (down to the sub-micrometric size range) collected in different hospital and home care environments. Since there are still unanswered questions about the severity of risk of submicron particle-associated COVID-19 transmission and potential further infection, the evaluation of individual infection risk on the basis of hypothetical exposure scenario was also performed.

2. Materials and methods

2.1. Study design, setting and participants

This study is an experimental investigation on the concentration and aerodynamic characteristics of size-segregated airborneparticles containing SARS-CoV-2 genetic material in home care and different areas of hospital environment occupied with COVID-19 patients. Details about the patients in different environments studied can be found in SI Table 1. In order to collect proper amount of particles also in the sub-micrometric range, then allowing a proper RNA detection within, we decided adopting longer sampling times in different environments (2-7 days). Indeed, small particles present a negligible volume/mass, thus longer sampling periods are needed. We are aware that the sampled genetic material can be originated from both fresh respiratory particles and aged airborne particles (previously emitted by the infected subjects but then experiencing dimensional transformations, e.g. due to the evaporation phenomena). We also point out that the different samplings performed can present different temporal proximities with respect the viral shedding peak of the infected subject. Nonetheless, these methodological choices did not represent a limitation of the study as the main goal of the paper is showing the presence of SARS-CoV-2 genetic material in ambient particles as a function of their size.

Home care environment

Investigation in the home care environment was conducted in an apartment (approximately 200 m3) with two infected subjects with mild symptoms of COVID-19 including fever, coughing and sneezing. The SARS-CoV-2 infection was confirmed by quantitative polymerase chain reaction (qPCR) analysis from nasal swab. The 48-h sampling of the airborne particles was conducted 5–7 days after the first symptoms onset in March 2021. The sampler was placed in the living room and both subjects carried out their daily activities normally.

Hospital environment

Investigation in hospital environment was conducted in the University hospital Dubrava in Zagreb, Croatia. The University hospital Dubrava was one of the first hospitals which was focused on the treatment of severe symptom COVID-19 patients and it was chosen as a Primary respiratory – intensivist centre (PRIC) for northwest of Croatia because of the high expertise of the intensivists, the equipment and the transport connections with other hospitals. Furthermore, significant organizational and infrastructural changes have been made within the hospital, such as formation of 2 hospital wards specialized in the treatment of COVID-19 patients: COVID-19 intensive care unit (ICU) and respiratory centre (RC), in order to provide a top-quality health care for COVID-19 patients while limiting the transmission of this extremely contagious disease during the pandemic outbreak. The air exchange rate (AER) of the PRIC hospital was 1.6–2 h-1 as a part of HVAC settings. Hospital policy during the sampling period included: restricted visitation and FFP2/3 masking and wearing protective equipment (gloves, specialized suites) for all hospital staff. Hospital room floors were disinfected on a daily basis. From September 2021 to January 2022, investigation was conducted at three different sampling sites within the hospital: patient room in ICU (ICUR), patient room (RCR) and medical staff workstation (MSW) in the RC.

 Sampling in the ICUR was conducted in a room (34 m3 in size) with 2 patients in terminal stage of COVID-19 or suffering from general organ failure, including respiratory failure what requires invasive (IV) or non-invasive (NIV) positive pressure ventilation. Patients were not able to perform any speaking activities.

 Respiratory centre is used for treatment of the COVID-19 patient with mild to severe symptoms. The samplings in RC were conducted at two different sites: in the room (43 m3 in size) with 2 patients (unless stated otherwise) (RCR) and at the medical staff workstation (MSW, the room of 15 m3 in size) located in the centre of the RC. Medical staff workstation was accessed only by the medical staff in order to prepare therapy for the patients in the RC, representing the only sampling site in this study without a direct emission of respiratory particles from the infected subjects. During sampling in both, ICUR and RCR, the sampler was located between two hospital beds, approximately 1.5 m from each patient. At all three sampling sites in the hospital environment, the room door was always kept open in order to facilitate the work of the medical staff allowing free circulation of air from the entire ICU or RC ward.

2.2. Sampling of size-segregated particles containing SARS-CoV-2 genetic material

The airborne particles were collected by a nanoMOUDI R122 cascade impactor (TSI, USA). On the cascade impactor, by successively diminishing the nozzle sizes, particles are categorized by their inertia and are collected in different stages by their size. The nanoMOUDI R122 was operating at the air flow of 30 L min-1 simultaneously separating particles in the 14 size fractions (0.010 ̶ 0.018, 0.018 ̶ 0.032, 0.032 ̶ 0.056, 0.056 ̶ 0.100, 0.100 ̶ 0.180, 0.180 ̶ 0.320, 0.320 ̶ 0.560, 0.560 ̶ 1.0, 1.0 ̶ 1.8, 1.8 ̶ 3.2, 3.2 ̶ 5.6, 5.6 ̶ 10, 10 ̶ 18, 18 ̶ 32 µm). The samples were collected on aluminium foils as substrates. We initially tested the quartz filters and aluminium foil and since a small volume (500 L) of buffer was used to extract the viral RNA, aluminium foil was better suited, since quartz absorbed most of the buffer. Before sampling, the aluminium foils were sterilized by 70% ethanol and exposure to UV radiation for 20 min in a laminar flow hood under sterile conditions in clean bench. Note that silicone spray was not used to minimize particle bounce, as it was particularly important to avoid any potential contamination of the sterilized filters during filter manipulation. Between each sampling experiment, all stages of the sampler were thoroughly cleaned with 70% ethanol. The cascade inlet was at the height of 1.5 m above the floor and the summary of the sampling conditions conducted within this study are shown in Table 1.

**Table 1.** Summary of airborne particle samplings at three different locations in the hospital (Intensive care unit (ICU) patient room, ICUR; Respiratory centre (RC) patient room, RCR; Respiratory centre medical staff workstation, MSW) and home care environment. Details about the patients can be found in SI Table 1.

|  |  |  |  |
| --- | --- | --- | --- |
| **sampling site** | **sample**  | **sampling time (days)** | **subjects**  |
| **bed A** | **bed B** |
| **patient** | **respiratory support** | **patient** | **respiratory support** |
| ICU | ICUR 1 | 3 | PA | IV | PB | NIV |
| ICUR 2 | 7 | PC | IV | PD | NIV |
| RC | RCRL 1 | 3 | PE  | LF | PF | LF |
| RCRL 2 | 4 | PG  | LF | PH | LF |
| RCRL 3 | 7 | PI  | LF | PJ | LF |
| RCRL 4 | 5 | PI  | LF | PK  | / |
| RCRH 5 | 3 | PG  | HF | / |
| RCRH 6 | 3 | PL  | HF | / |
| RCRH 7 | 6 | PE  | HF/LF | PM  | / |
| PF | LF |
| MSW 1 | 7 | accessed only by medical staff |
| MSW 2 | 4 |
| home care | home care | 2 | PN | PO |

\* IV, invasive ventilation; NIV, non-invasive ventilation;

 RCRH samples collected during high flow nasal oxygen (HF) therapy;

 RCRL samples collected during low flow nasal oxygen (LF) therapy.

2.3. Extraction and quantification of SARS-CoV-2 genetic material in airborne particles

2.3.1. RNA isolation

Upon sampling, the aluminium foils containing the collected particles were packed into sterile Petri dishes and were immediately sent to the laboratory for processing. The entire sample handling process was carried out in a laminar flow hood under sterile conditions to avoid cross-contamination and all instruments used were sterilized. Foils were folded with sterile tweezers and put into 1.5 mL Eppendorf tubes. 500 μL of DNA/RNA Shield™ (Zymo, USA) was added and the samples were incubated overnight at +4 °C for extraction. Total RNA was isolated using Quick-RNA™ Viral Kit (Zymo, USA) according to the manufacturers’ protocol. Briefly. 400 μL of total RNA in DNA/RNA Shield was incubated with 800 μL of Viral RNA Buffer and mixed. The 700 μL of the mixture was transferred into a Zymo-Spin™ IC Column placed in a collection tube. The sample was centrifuged for 2 min at 12 000 ×g. Afterwards, the flow-through was discarded and the rest of the mixture was transferred on the column and centrifuged. The column was transferred into a new collection tube. 500 μL of Viral Wash Buffer was added to the column and centrifuged for 1 min and the flow-through was discarded. The washing step was repeated once more. Then, 500 μL of 96% ethanol was added to the column and centrifuged for 1 min at 12 000 ×g. Flow-through was discarded and the column was placed into a new tube. 30 μL of DNase/RNase-free water was added to the column matrix, incubated for 1 min at room temperature and centrifuged for 1 min at 12 000 ×g.

2.3.2. Reverse transcription and quantitative PCR

For reverse transcription and quantitative PCR (qPCR) analysis, the reaction consisted of 5 μL of 2× Luna Universal Probe One-Step Reaction Mix (NEB, SAD), 0.5 μL 20× Luna WarmStart® RT Enzyme Mix (NEB, SAD), 0.5 μL of TaqMan probe and 4 μL of total RNA. The list of TaqMan probes and its sequences is listed in SI Table 2. Orf1 is SARS specific, while S is Cov-19 specific primer. Actin is a control primer for RNA isolation control, as in the air human cells are present and were collected during sampling step. Reverse transcription and qPCR were performed on StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The following program was performed: first a reverse transcription step was performed for 10 min at 55°C which was immediately followed by amplification and detection part which consisted of denaturing step for 20 s at 95 °C, followed by 40 cycles of 1 s at 95 °C and 20 s at 60 °C. Samples were considered positive if amplification occurred and the cycle threshold value (Ct) for quantification less than 40. The negative control reaction consisted of non-template control, while as positive control a previously tested positive sample was used. In cases of absence of amplification (absence of desired target) after 40 cycles, the result was reported as undetermined. The quantification of RNA copies in each sample was done according to calibration curves obtained for Orf1 and S probe shown in SI Fig. 1.

 The molecular test performed on the collected samples resulted in Ct values < 40 for the two target genes, Orf1 and S for 93.45% of the positive samples. In rest of the positive samples analysed (6.54%), we have detected only one of the probes. Since the detection of at least one of the Orf1 or S genes proves the presence of the SARS-CoV-2 RNA and the aim of this study is detection and quantification of SARS-CoV-2 genetic material in size-segregated particles, in case when both probes were determined as positive, the higher concentration obtained in qPCR aliquot was considered for further quantification in air samples. Details can be found in SI Table 3.

2.4. Quantification of the SARS-CoV-2 genetic material in collected size-segregated particles

The concentrations of SARS-CoV-2 RNA copies (c) are calculated by Eq. (1):

 c= caliq \* 7.5/Vair (RNA copies m-3 ) (Eq. 1)

where caliq is copies/4 μL cDNA estimated by qPCR, Vair is volume of air collected (m3). The caliq of cDNA from qPCR were estimated using a standard calibration curve. Adjustment by the factor of 7.5 was used to account for the fact that only 4 μL of cDNA were used for qPCR reaction out of the total 30 μL elution. We calculated the percent of positive samples by dividing the number of positive samples by the number of total samples collected for each location and/or size range.

2.5. Evaluation of the dose received and the assessment of the individual infection risk

The dose of SARS-CoV-2 genetic material (D, expressed as RNA copies) received by a susceptible subject exposed to a certain SARS-CoV-2 genetic material concentration over time, *c*(*t*), for a certain time interval, *T*, can be evaluated by integrating the SARS-CoV-2 genetic material concentration over time as in Eq. 2:

 (RNA copies) (Eq. 2)

The dose of SARS-CoV-2 genetic material received by a susceptible subject is affected by the inhalation rate (IR), subsequently by their activity level inhalation fraction (IF) and time of exposure. The IR was determined to be 0.96 m3 h-1 which is average value between standing (0.54 m3 h-1) and doing light exercise (1.38 m3 h-1) (Adams, 1993; Buonanno et al., 2020a). In case that all particles are inhaled by susceptible subject, the IF is 1.

 The simulation of airborne transmission of SARS-CoV-2 was performed adopting the infection risk assessment proposed by Buonanno et al., (2020b), typically implemented to evaluate the transmission dynamics of infectious diseases and to predict the risk of these diseases to the public. Even though the viral load, air flow, local flow direction and velocity of the emission act synergistically and need to be considered, the use of a homogenous exposure-point concentration is standard practice in environmental risk assessment. The approach presented in this work is based on the assumptions that susceptible person is equally exposed to a uniform, average concentration within the exposure unit. Once the dose of infection for a given exposure conditions was obtained at each sampling environment, the individual infection risk was calculated. Thus, in order to evaluate the individual infection risk (R) of an exposed subject for a given exposure scenarios, as a function of the exposure time (*t*) of susceptible subject, the concentration will be integrated over time through the Wells–Riley equation (Riley et al., 1978) as shown in Eq.3:

 (%) (Eq. 3)

where HID63 represents the human infectious dose for 63% of susceptible subjects, i.e. the number of RNA copies needed to initiate the infection with a probability of 63%. For SARS-CoV-2, a HID63 value of 7 × 102 RNA copies was adopted based on the thermodynamic-equilibrium dose–response model developed by Gale (2020). In order to show the contribution of super- and sub-micron airborne particles to the risk of infection, in this work we have evaluated the individual infection risk for three exposure scenarios for a person staying in the room exposed to SARS-CoV-2 genetic material: a) without a mask; b) wearing a surgical and c) wearing a FFP2 mask protection. The IF varied in order to simulate the above scenarios: it was determined according to the literature as 1, 0.75 and 0.2 for a), b) and c), respectively (Bagheri et al., 2021; Howard et al., 2021; Sommerstein et al., 2020). We point out that the risk of infection is strongly influenced by the virus viability (and not just by the SARS-CoV-2 genetic material concentration); nonetheless, our measurements did not provide information regarding the virus viability, thus, in our model, as conservative approach, we considered the SARS-CoV-2 genetic material remaining viable over the entire exposure time, then obtaining high-end values of risk of infection.

3. Results

3.1. Detection and quantification of SARS-CoV-2 genetic material

SARS-CoV-2 genetic material was detected at all investigated sampling sites (three hospital sites and one home care site). Positive qPCR results were detected in 10 out of 14 overall inspected particle size fractions (14), nine in 0.180–32 µm and one in 0.0180–0.032 µm size range. The genetic material was not found in the four (0.010 ̶ 0.018, 0.032 ̶ 0.180 µm) size fractions (Table 2). The broadest size distribution of SARS-CoV-2 genetic material was found in the MSW, where the genetic material was detected in 71.4% of particle size ranges, followed by the RCR and ICUR where SARS-CoV-2 genetic material was detected in 57.1% and 35.7% of investigated size ranges, respectively (Table 2). In home care environment, SARS-CoV-2 genetic material was detected in 2 out of 14 size fractions (14.3%). Considering super- and sub-micrometric size ranges, the SARS-CoV-2 genetic material was detected in both particle size ranges at all sampling sites. More specifically, it was detected in all super-micrometric particle size fractions at the MSW and RCR while in the ICUR and home care environment positive qPCR results were detected in 50% and 16.7% of super-micrometric size range fractions, respectively (Table 2). The broadest distribution of genetic material in super-micrometric fraction was found in the MSW (50% of the size ranges), followed by RCR with positive results in 25% of the sub-micrometric size ranges. In ICUR and home care only one super-micrometric size range (12.5%) showed positive qPCR results.

**Table 2.** Detection of SARS-CoV-2 genetic material in fine resolution of size distribution and summarized with respect to sub- and super-micrometric size fractions analysed in hospital (Intensive care unit patient room, ICUR; Respiratory centre patient room, RCR; Respiratory centre medical staff workstation, MSW) and home care environment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **particle size****range (µm)** | **MSW** | **RCR** | **ICUR** | **home care** |
| 18 ̶ 32 | + | + | + |  |
| 10 ̶ 18 | + | + |   |  |
| 5.6 ̶ 10 | + | + | + |  |
| 3.2 ̶ 5.6 | + | + | + |  |
| 1.8 ̶ 3.2 | + | + |   | + |
| 1.0 ̶ 1.8 | + | + | + |  |
| 0.560 ̶ 1.0 | + | + | + | + |
| 0.320 ̶ 0.560 | + | + |   |  |
| 0.180 ̶ 0.320 | + |   |   |  |
| 0.100 ̶ 0.180 |   |   |   |  |
| 0.056 ̶ 0.100 |  |  |  |  |
| 0.032 ̶ 0.056 |  |  |  |  |
| 0.018 ̶ 0.032 | + |   |   |  |
| 0.010 ̶ 0.018 |  |  |  |  |
| **all stages (%)** | **71.4** | **57.1** | **35.7** | **14.3** |
| > 1 µm | 100 | 100 | 50.0 | 16.7 |
| < 1 µm | 50.0 | 25.0 | 12.5 | 12.5 |

In total, 168 samples were collected, of which 45.2% were determined positive for SARS-CoV-2 (76/168). More precisely, 81.9% (59/72) of super-, and 17.7% (17/96) of sub-micrometric samples were positive for SARS-CoV-2 (SI Table 3). Table 3. shows the number of positive results forSARS-CoV-2 detected in each size fraction relative to total size fractions collected (TSF; N=14), as well as the contribution of positive results in the total super- (N=8) and sub-micrometric (N=6) particle size fractions in all sample sets analysed. Overall, the highest share of positive samples was detected in MSW and RCR.  In MSW, RCRH, and RCRL 2, 3, 4 positive samples were detected in all super-micrometric fractions. The highest contribution to sub-micrometric particle size range was detected in MSW, RCRL 3, RCRH 6 and 7. In some samples (ICUR 1 and RCRL 1) genetic material was not detected in sub-micrometric range (Fig. 1, Table 3). The contribution of SARS-CoV-2 genetic material was in favour of super-micrometric compared to sub-micrometric particle size range (Fig. 1, Table 3).

**Table 3.** Number of positive results forSARS-CoV-2 detected in each size fraction relative to total size fractions collected (TSF; N=14), as well as the contribution (CON %) of positive results in the total super- (N=8) and sub-micrometric (N=6) particle size fractions in all sample sets analysed from different environments (Intensive care unit patient room, ICUR; Respiratory centre patient room, RCR; Respiratory centre medical staff workstation, MSW; home care).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | size range | **ICUR** **1** | **ICUR 2** | **RCRL****1** | **RCRL 2** | **RCRL 3** | **RCRL 4** | **RCRH 5** | **RCRH 6** | **RCRH 7** | **MWS 1** | **MWS** **2** | **home care** |
|  CON(%) | TSF | 2/14 | 4/14 | 5/14 | 7/14 | 8/14 | 7/14 | 7/14 | 8/14 | 8/14 | 8/14 | 10/14 | 2/14 |
| >1 µm | 33.3 | 50.0 | 83.3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 16.7 |
| <1 µm | 0 | 12.5 | 0 | 12.5 | 25.0 | 12.5 | 12.5 | 25.0 | 25.0 | 25.0 | 50.0 | 12.5 |

\* RCRH samples collected during high flow nasal oxygen (HF) therapy;

\*\*RCRL samples collected during low flow nasal oxygen (LF) therapy

The overall concentration range of detected SARS-CoV-2 genetic material in TSF ranged from 0.1 to 9.5 RNA copies m-3 in different studied environments. The lowest concentration was detected in the ICUR (0.1 RNA copies m-3). Generally, higher concentration of SARS-CoV-2 genetic material in TSF was detected in the RCRH when patients with more severe COVID-19 symptoms were treated by high flow nasal oxygen (HF) (3.0–9.5 RNA copies m-3) compared to the samples collected in the RCRL when treating patients with mild COVID-19 symptoms by low flow nasal oxygen (LF) or without respiratory support (0.6–1.8 RNA copies m-3). The concentration detected in home environment was 0.7 RNA copies m-3. Concentrations detected in the MSW were higher than concentrations obtained for ICUR, RCRL and home care environment.

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**Fig. 1.** Concentrations of SARS-CoV-2 genetic material detected in total suspended fractions (TSF), and super- and sub-micrometric particle size fractions in different hospital (Intensive care unit patient room, ICUR; Respiratory centre patient room, RCR; Respiratory centre medical staff workstation, MSW) and home care environment. RCRH samples are collected during high flow nasal oxygen therapy while RCRL samples during low flow nasal oxygen therapy.

3.2 Size distribution of particles containing SARS-CoV-2 genetic material in different environments

Concentrations of SARS-COV-2 genetic material for each size range of 12 samplings conducted in this study are shown in detail in SI Table 3. Figure 2 shows size distributions of one representative sampling from each investigated environment with COVID-19 positive subject(s).



Fig. 2. Size distribution of one representative sampling from each investigated environment occupied with COVID-19 positive subject(s): Intensive care unit patient room, ICUR; Respiratory centre patient room where samples were collected during low flow nasal oxygen therapy, RCRL; Respiratory centre patient room where samples were collected during high flow nasal oxygen therapy, RCRH; Respiratory centre medical staff workstation, MSW; home care.

Average maximal concentrations obtained considering the maximum concentration values detected in each sample set from specific environment studied followed the order: RCRH (5.6 ± 3.4 RNA copies m-3), MSW (4.5 ± 0.4 RNA copies m-3), RCRL (1.3 ± 0.5 RNA copies m-3), home care environment (0.4 ± 0.3 RNA copies m-3) and ICUR (0.1 ± 0.0 RNA copies m-3) (SI Table 3). Sampling sites considered to be under the influence of direct emission of respiratory particles include ICU and RC rooms as well as home care environment, even though the infected subjects in the home care environment were not continously in the close proximity of the sampler. In ICUR 1 (sampled for 3 days) the SARS CoV-2 genetic material was detected in two fractions of super-micrometric size range (3.2–5.6 µm and 18–32 µm). Prolongation of the sampling up to seven days (ICUR 2), apart from the detection of SARS-CoV-2 in three super-micrometric size ranges (1–1.8, 3.2–5.6, 5.6–10 µm), enabled the detection of SARS-CoV-2 genetic material in sub-micrometric range (0.56–1 µm). The highest concentration of SARS-CoV-2 genetic material was detected in 3.2–5.6 µm size range. The SARS-CoV-2 genetic material in RCRL samples was distributed through whole super-micrometric size range and in two sub-micrometric size ranges covering 0.32–1 µm. The maximum concentration in RCRL samples was detected in 5.6–10 µm size fraction. Furthermore, the concentrations in RCRH, were relatively higher in comparison to RCRL, contributing also to sub-micrometric ranges. The results obtained during both samplings (RCRH5 and RCRH6), when only one patient supported by HF was present in the room, confirmed two maximums, one in the super-micrometric mode (18–32 um) and one in the sub-micrometric mode (0.56–1 um) (details in SI Table 3.). The room in the centre of the hospital ward, MSW, was the only sampling site which was not under the influence of direct emission of respiratory particles. The highest concentration of SARS-CoV-2 genetic material in MSW was detected in size range 1.8–3.2 μm and 0.56–1 μm. Considering the size of a SARS-CoV-2, the genetic material found in the 18-32 nm size range in MWS 2 points to the presence of fragmented viral RNA, excluding the possibility of an entire virus particle being present. During the home care sampling, the SARS-CoV-2 genetic material was detected in super-micrometric (1.8–3.2 µm) and sub-micrometric (0.56–1 μm) size range.

3.3. Risk assessment considering obtained size distribution of particles containing SARS-CoV-2 genetic material in different environments

The estimation of individual infectious risk obtained from the Eq. 3. for super- and sub-micrometric particle size ranges as a function of the time of exposure to SARS-CoV-2 during one representative sampling in each studied environment are presented in Table 4. Since van Doremalen et al., (2020) reported that SARS-CoV-2 remains viable in the air for up to 3 h, our hypothetical exposure scenario was evaluated for exposure time from 15 min up to 3 h.

**Table 4.** Individual risk of infection (R, %) as a function of the exposure time for super-micrometric and sub-micrometric particles containing SARS-CoV-2 genetic material received by a susceptible subject during stay in one representative studied environment with COVID-19 positive subject(s) (Intensive care unit patient room, ICUR 2; Respiratory centre patient room, RCRL 4, RCRH 7; Respiratory centre medical staff workstation, MSW 1, home care). The unacceptable R (> 0.1%) as suggested by Buonanno et al. (2020b) is shown in red.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| environment | size mode | **15 min** | **30 min** | **1 h** | **2 h** | **3 h** |
| ICUR 2 | > 1 µm | 0.00 | 0.01 | 0.01 | 0.03 | 0.04 |
| RCRL 4 | 0.06 | **0.11** | **0.23** | **0.45** | **0.67** |
| RCRH 7 | **0.31** | **0.62** | **1.23** | **2.44** | **3.64** |
| MWS 1 | **0.15** | **0.30** | **0.59** | **1.18** | **1.76** |
| home care | 0.02 | 0.04 | 0.08 | **0.15** | **0.23** |
| ICUR 2 |  < 1 µm | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |
| RC 4 | 0.00 | 0.01 | 0.02 | 0.03 | 0.05 |
| RC 7 | 0.02 | 0.03 | 0.06 | **0.13** | **0.19** |
| MWS 1 | 0.02 | 0.03 | 0.06 | **0.13** | **0.19** |
| home care | 0.01 | 0.01 | 0.02 | 0.04 | 0.06 |

The obtained results show generally higher infectious risk for super- in comparison to sub-micrometric particles. The highest risk of super-micrometric particles containing SARS-CoV-2 is obtained for exposure in RCRH, following the MSW 1, RCRL and home care environment. Table 5 shows the exposure-risk relationship for three scenarios with regard to the level of protection (no mask, surgical and FFP2 mask) in investigated environments. In scenario shown on Table 5, the exposure of a susceptible subject will not reach an unacceptable risk of 0.1% within 3 h in the ICUR. In home environment, a susceptible subject can stay up to 1 h without protection or up to 2 h with a surgical mask and up to 3 h with a FFP2 mask avoiding the unacceptable risk of infection. Furthermore, a susceptible subject is in risk staying in the RCRH or in the MSW without a mask protection. Surgical mask does not provide sufficient protection in these environments under investigated conditions, therefore the FFP2 masks are necessary. The susceptible subject can stay in the RCR maximum up to 30 min with FFP2 mask, while in the MSW up to 1 h in order to avoid unacceptable risk. A well-mixed volume assumption is a prerequisite for all risk calculations.

**Table 5.** Individual risk of infection (R, %) as a function of the exposure time and the effect of different types of mask protection wearing by a susceptible subject during stay in one representative studied environment with COVID-19 positive subject(s) (Intensive care unit patient room, ICUR 2; Respiratory centre patient room, RCRL 4, RCRH 7; Respiratory centre medical staff workstation, MSW 1, home care). The unacceptable R (> 0.1%) as suggested by Buonanno et al. (2020b) is shown in red.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| protection | environment | **15 min** | **30 min** | **1 h** | **2 h** | **3 h** |
| no mask | ICUR 2 | 0.00 | 0.01 | 0.02 | 0.03 | 0.05 |
| RCRL 4 | 0.06 | **0.12** | **0.24** | **0.48** | **0.72** |
| RCRH 7 | **0.32** | **0.65** | **1.29** | **2.57** | **3.82** |
| MWS 1 | **0.16** | **0.33** | **0.65** | **1.30** | **1.94** |
| home care | 0.02 | 0.05 | **0.10** | **0.20** | **0.29** |
| surgical mask | ICUR 2 | 0.00 | 0.01 | 0.01 | 0.03 | 0.04 |
| RCRL 4 | 0.05 | 0.09 | **0.18** | **0.36** | **0.54** |
| RCRH 7 | **0.24** | **0.49** | **0.97** | **1.93** | **2.88** |
| MWS 1 | **0.12** | **0.24** | **0.49** | **0.98** | **1.46** |
| home care | 0.02 | 0.04 | 0.07 | **0.15** | **0.22** |
| FFP2 mask | ICUR 2 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |
| RCRL 4 | 0.01 | 0.02 | 0.05 | 0.10 | **0.14** |
| RCRH 7 | 0.06 | **0.13** | **0.26** | **0.52** | **0.78** |
| MWS 1 | 0.03 | 0.07 | **0.13** | **0.26** | **0.39** |
| home care | 0.00 | 0.01 | 0.02 | 0.04 | 0.06 |

4. Discussion

4.1. Size distribution of SARS-CoV-2 genetic material in hospital and home care environments

The determination of the size of particles carrying viral RNA is critical to understanding their respiratory tract deposition, health impact, residence time in ambient air, and the potential for longer distance transport. Thus, the importance of different particle size contribution to the spread of COVID-19 is widely debated during the pandemic (e. g. Tang et al., 2021, Wang et al., 2021). However, the existing data are still scarce. A limited number of studies have presented size-resolved information on particle containing SARS-CoV-2 RNA and most of them generally made only a rough distinction between particle size ranges (Chia et al., 2020; del Real et al., 2022; Lednicky et al., 2021; Liu et al., 2020; Nannu Shankar et al., 2022; Oksanen et al., 2022; Santarpia et al., 2022; Stern et al., 2021b; Stern et al., 2022) depending on the type of the sampler, rarely reporting on sub-micrometric sizes which could be of interest due to the size of the SARS-CoV-2 virus. Among them, only few reporting on size-segregated aerosol samples from hospital air and detected SARS- CoV-2 RNA (Chia et al., 2020, del Real et al., 2022; Liu et al., 2020; Oksanen et al., 2022; Santarpia et al., 2022; Stern et al., 2021b; Stern et al., 2022). Among mentioned studies, only some detected SARS-CoV-2 in sub-micrometric size fractions: Liu et al. (2020) in 0.25–0.5 μm and 0.5–1 μm, Nannu Shankar et al., 2022 in 0.5–1 μm and 0.25–0.5 μm, Oksanen et al., (2022) in 0.65–1.1 µm and Santarpia et al., (2022) in <1 μm and all of them reported relatively higher concentrations in sub-micrometric in comparison to super-micrometric size fractions. Therefore, the presence of SARS-CoV-2 in sub-micrometric should be investigated further on a finer size resolution. Even though we generally detected relatively higher concentration of genetic material in super-micrometric size fractions in comparison to sub-micrometric, however, which is due to the sampled size fractions up to the 32 µm, the presented fine size resolution in sub-micrometric size fractions emphasizes the importance of the presence of viral material in particles that can remain airborne for a longer time and be transmitted over greater distances compared to larger particles. Therefore, this study provides one of the largest datasets of size-fractionated airborne SARS-CoV-2 RNA to date, showing that SARS-CoV-2 RNA is present in super- as well as in super-micrometric airborne particles, with a special emphasis on the sub-micrometric particles. To our knowledge, this study is the only study conducted in Europe that has detected the SARS-CoV-2 genetic material in fine resolution of size-segregated particles in the hospital and home care environments.

Some authors claim that cascade impactors and common PM samplers can damage the viral particles (Bhardwaj et al., 2021, Pan et al., 2019, Walls et al., 2016), thus, the fragmentation of SARS-CoV-2 RNA material detected in this and in studies using similar devices should be accounted. Furthermore, the fragmentation of the SARS-CoV-2 RNA could be a result of a spontaneous decomposition of its genetic material over time in the airborne particles or after the impaction on the substrate during the sampling due to an unavoidable presence of ribonucleases (RNases) in the sampling environment (Deutscher 2006, Jaag et al., 2011). Indeed, the SARS-CoV-2 RNA material in this work detected in size ranges (as 0.018–0.032 μm fraction) smaller than the size of the viral particle (100 nm, Lee, 2020; Laue et al., 2021) likely corresponds to fragmented SARS-CoV-2 genetic material originating from a damaged or decayed viral particle. Considering all of the above, fragmentation of RNA molecule could disable the synthesis of the complementary chain during the PCR reactions, disabling the amplification of genetic material of interest and underestimating the viral loads. Therefore, the detection of genetic material in aerosol samples in this work was done by specific approach using two gene probes, Orf1 and S, to account for the fragmentation of the collected genetic material.

Of the all samples collected in this study, 45.2% (76/168) had a positive qPCR result for SARS-CoV-2, which is within the range previously found in hospital environment from 0-77% (Chia et al., 2020; Ding et al., 2020; Liu et al., 2020; Ong et al. 2020; Santarpia et al., 2020; Santarpia et al., 2022; Stern et al., 2021a; Stern et al., 2021b; Stern et al., 2022). It is important to note that obtained percentage is related to the number of size fractions studied, which are in some studies substantially lower (3–5 size fractions) in comparison to our study (14 size fractions). Besides the environmental viral concentration, the percentage of positive samples also depends on the AER and/or methodological differences applied, especially sampling time, flow rate, and sampler position. Furthermore, differences in extraction protocol from the collection substrate, variability in RNA degradation rates, or differences in PCR sensitivity among studies should also be considered when comparing different studies, as this could lead to differences in results.

 In this study, very low or undetectable concentrations of airborne RNA copies were measured in the ICUR. Since the patients in the ICUR are generally in the late or terminal stage of the COVID-19 disease, it is expected that their viral load is significantly lower in comparison with the patients in the initial, or early stage of the COVID-19. Furthermore, the patients in the ICUR were receiving mechanical ventilation via endotracheal tube with a cuff (IV) or via tight seal total face mask (NIV), meaning that their breathing is completely controlled by the medical equipment, minimizing the spreading of the exhaled, potentially virus containing, particles. Compared to ICUR, the concentrations of SARS-CoV-2 genetic material in TSF in RCR were higher and comparable with those of airborne SARS-CoV-2 (0–9 copies m-3) found in most of the patient areas in hospital reported by Liu et al. (2020). This suggests that the negative pressure and air exchange rate inside the ICUR and RCR were generally effective in limiting the airborne transmission of already low concentration SARS-CoV-2. Moreover, studies in both ICUR and RCR showed that the prolongation of the sampling time enabled the accumulation of particles, increasing the possibility of accumulation of the detectable concentrations of virus containing particles. Higher concentration of SARS-CoV-2 genetic material was detected in the RCRH samples when patients with more severe COVID-19 symptoms were treated by high flow nasal oxygen (3.0–9.5 RNA copies m-3) compared to the RCRL samples collected when patients with mild COVID-19 symptoms were supported by LF nasal oxygen or without respiratory support (0.6–1.8 RNA copies m-3). Namely, HF oxygen therapy through a nasal cannula is a technique whereby a mixture of heated and humidified oxygen and air are delivered to the nose at high flow rates. The contribution of a patient supported by HF could strongly contribute to the super-micrometric, especially; size ranges 1.8–3.2 μm considering a large number of fresh respiratory particles emitted due to the above mentioned respiratory system support. When emitted, respiratory particles are drawn into the sampler and they could deposit before they are dried, contributing to the highest particle size ranges. However, particles emitted by a subject can also experience water loss by evaporation in couple of seconds, up to minutes, and since the collection of the particles was continuous, there is a possibility that both, relatively fresh as well as dried respiratory particles were distributed in lower size ranges. As a result, in all RCRH the genetic material was detected in sub-micrometric size ranges which correspond to already aged and dried particles.

The concentration of SARS-CoV-2 detected in the MSW is comparable to the concentrations detected in MSW in one of the hospitals studied (6 copies m-3) but lower than in the other hospital studied (11–42 copies m-3) by Liu et al. (2020). In general, in this study, MSW was characterized by higher concentration of SARS-CoV-2 genetic material in comparison to sampling sites occupied by patients, the same as it was found by Liu et al., (2020). This could be due to the fact that air circulation in medical staff areas is isolated by hospital design from the air circulation in the rooms occupied by patients. The broad size distribution of genetic material of interest determined in the MSW is showing the possibility that particles containing SARS-CoV-2 genetic material may be transported over longer distances than expected. The sampling of genetic material in MSW is specific because there was no direct emission of respiratory particles in close proximity of the sampler. Therefore, the results from MSW are the most representative in terms of concentration and size distribution of SARS-CoV-2 genetic material and could be indicative for size-segregated distribution of particles containing infectious material for indoor environment.

 Considering that the doors of all patient rooms were kept open, there was a free air circulating from every patient’s room through the whole ICU as well as RC ward during the sampling time. Therefore, the obtained concentrations as well as the size distribution reflect the synergistic influence of direct emission (dominating when infected subjects were staying in the investigated room close to the sampler) and transmission of airborne particles containing viable viruses and/or inactivated viruses and/or fragments of their genetic material. Furthermore, respiratory particle nuclei experience water loss by evaporation while non-volatile constituents such as virus particles remain floating in the air. For bioaerosols with an initial diameter of less than 20 μm, desiccation at a relative humidity of 50% occurs in less than 1 s (Nicas et al, 2005; Tellier, 2022; Xia et al., 2007) and the infectivity of aerosolized viruses could persist for hours. Since the dryer was not used during this study, we can assume that detected super-micrometric particles could be freshly emitted, sampled and deposited at certain impactor stage, while sub-micrometric particles could be also a result of drying and aging of larger respiratory particles after the emission, becoming small enough, with minimal contribution to the aerosol mass, to be further transported by the air circulation depending on their aerodynamic diameter. On the other hand, small, as well as large particles, can easily be resuspended from the surfaces. This corresponds to the realistic conditions in the indoor environment and could serve as an explanation for detection of SARS-CoV-2 in home care environment.

4.2. Estimation of the risk of SARS-CoV-2 infection

It is necessary to understand the characteristics and mechanisms behind the generation of respiratory particles (Ai and Melikov, 2018; Holmgren et al., 2010), the survival of viruses in emitted particles (van Doremalen et al., 2020), their airborne transmission and final effect of human exposure to them (Ai et al., 2019; Buonanno et al., 2020a; Buonanno et al., 2020b, Buonanno et al, 2022; Cortellessa et al., 2021; Mikszewski et al., 2022). The consequent contagion risk assessment is a complex issue that requires multidisciplinary approach and according to our knowledge, no risk assessment has been done so far on real size-segregated samples data. This work presents a study conducted in real conditions, providing a data set which can be used for understanding which particle size fraction could be estimated as the one with the highest concentration of pathogen genetic material. These data can be used for risk assessment of susceptible person directly breathing the respiratory jet in close proximity to an infected host, and the information on the size distribution of airborne particles carrying SARS-CoV-2 is useful for modelling the expected transmission distance and establishing the maximum coverage by airborne precautions. This property of airborne SARS-CoV-2 is essential for predicting the spread of the disease so it becomes imperative to understand the role of airborne particles that serve as vectors for SARS-CoV-2 in the hospital workplace in which conventional infection control strategies may fall short. It is important to note that, given the sampling method, the present study did not measure viability of the virus. Previous studies have found SARS-CoV-2 to be viable in hospital air, including in particles < 1 μm (Lednicky et al., 2020; Santarpia et al., 2022). However, in this work we estimated the individual infection risk based on the concentrations detected in different environments occupied with infected subjects as a continuous emission source. Further, the concentrations obtained in this study were not time-weighted averages over sampling periods and it is possible that the emissions of the virus occurred as isolated events (e.g., a sneeze, cough, or speaking) from infected people rather than as a continuous flux over sampling period. The obtained results indicate the necessity of the protective masks in presence of infected subjects, especially while staying for longer period of time in the hospital environments. Furthermore, hospital staff could benefit from these results considering location specific features – including airflow, cleaning frequency, and patient status –when designing airborne prevention protocols. Airborne precautions, including those targeting fine and ultrafine particles, seem to be a convincing strategy to protect healthcare workers from the risk of COVID-19.

5. Conclusions

The conducted study detected SARS-CoV-2 genetic material and determined its size distribution in airborne particles generated by infected subjects in hospital and home environments in order to carry out the assessment of the risk of infection, and generate data for future research. The SARS-CoV-2 genetic material was detected in all investigated sampling sites (three hospital sites and one home care site) throughout 71.4% of investigated particle size fractions, nine ranging from 0.180 to 32 µm and one in from 0.0180 to 0.032 µm. The broadest size distribution of SARS-CoV-2 genetic material was found in the MSW, followed by the rooms with patients, RCR and ICUR. There is a clear variability of the concentration and contribution of genetic material to different size ranges considering different sampling environments.

 This study provides one of the largest datasets of size-fractionated airborne SARS-CoV-2 RNA to date, where we showed that SARS-CoV-2 RNA is present in super- as well as in super-micrometric airborne particles, with a special emphasis on the sub-micrometric particles larger than 180 nm in size. This study is, to our knowledge, the only which detected the SARS-CoV-2 genetic material in fine resolution of size-segregated particles in hospital environment conducted in the Europe. On the basis of obtained SARS-CoV-2 genetic material concentrations, the individual infectious risk was evaluated for each sampling site suggesting that unaccepted risk of individual infection by SARS-CoV-2 could occur even in areas that are not occupied by infected subjects. Thus, this study supports the role of fine and ultrafine particle size modes in the transmission of SARS-CoV-2, and emphasizes the importance of efficient respiratory protection and airborne isolation precautions to protect from exposure to fine and ultrafine SARS-CoV-2 containing aerosol when interacting with infected subjects, regardless of symptoms or medical procedure being performed.

 Since recent works are highlighting the relative importance of airborne transmission of COVID-19, it is crucial that experimental results of sampling in real conditions are promoted and implemented to limit the transmission of SARS-CoV-2 in healthcare, and that efficient respiratory protection and hygiene practices are used in public settings. Although we are aware that transmission and infection by SARS-CoV-2 in the healthcare settings can differ from everyday situations where transmission and infection is possible, the obtained results could have important implications for the prevention of infection of the public and protection of medical staff by ensuring optimal personal protection for medical staff, as well as people sharing household with infected person.

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