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Research Article

One Year Weekly Size-Resolved Air Sampling of SARS-CoV-2 in Hospital Corridors and Relations to the Indoor Environment

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Background. Airborne SARS-CoV-2 plays a prominent role in COVID-19 transmission. Numerous studies have sampled air from patient rooms, but airborne spread to other hospital areas such as corridors is less investigated. *Methods*. Size-fractionated aerosol particles were collected weekly, with 12 hours of sampling time daily, in corridors at two infectious disease wards in southern Sweden between March 2020 and May 2021. Samples were analysed with real-time reverse transcription polymerase chain reaction (RT-qPCR) for detection of SARS-CoV-2 RNA. Indoor temperature, relative humidity, and CO_2 concentration were monitored during the sampling period. *Results*. 20 of the 784 collected samples contained SARS-CoV-2 RNA, although in low concentrations. Positive air samples were found in sizes between 0.14 and 8.1 μm, but none >8.1 μm. 45% were found in submicron particles. No clear seasonal pattern was observed among the positive samples. There was no significant difference in the positivity rate of the samples between the two wards. *Conclusions*. SARS-CoV-2 was only detected in 2.6% of the aerosol samples, which indicates that the spread of airborne virus from patient rooms to the corridor was limited.

1. Introduction

The importance of airborne SARS-CoV-2 in the transmission of COVID-19 is now well established, especially in indoor environments [1, 2]. During the pandemic, numerous measurements of SARS-CoV-2 in aerosols have been carried out, and hospitals have naturally been a location of special interest [3]. Many studies on aerosol sampling have been performed in patient rooms, but other locations in hospital environments can also be of concern, such as corridors in hospital wards. Patients with COVID-19 are normally not present in these areas except for transport. However, virus-containing aerosols originating from asymptomatic healthcare workers (HCW) or from patient rooms could still be present, and the use of face masks or respirators may be less frequent outside of patient rooms, which motivates further investigations.

SARS-CoV-2 RNA has indeed been found in aerosols sampled from hospital corridors, staff rooms, and similar areas, with no patients present. According to a recent review, 21% of in total 137 air samples from corridors adjacent to patient rooms were positive for SARS-CoV-2 RNA [4]. One study even found that there was significantly more airborne SARS-CoV-2 in the investigated corridor than in the patient rooms, probably due to lower ventilation in the corridor as compared to well-ventilated patient rooms [5]. However, the small number of sampling

occasions could provide an unrepresentative picture, since an ongoing outbreak or a drop in community cases could bias the results.

Although SARS-CoV-2 has been found in aerosols of different size fractions, the sizes that are most dominant for disease spread are still unclear. Most field studies with size-separated measurements of SARS-CoV-2 in hospitals only sampled in 2-3 fractions, and the number of samples was often less than 50 [3, 4]. Larger studies, sampling from different locations in hospital areas, have detected SARS-CoV-2 in several size fractions and also found that the size distribution was related to the location where the particles were collected [6–8]. The presence of SARS-CoV-2 in submicron particles is especially important to quantify because of their ability to transport longer in air and deposit in the deeper lung when inhaled [9]. Moreover, face masks are less effective for particle sizes around 0.5-1 μm [10]. Hence, more information on the size distribution is important for understanding particle origin, transportation, and mitigation strategies.

The fate of exhaled respiratory aerosols depends on both relative humidity (RH) and temperature in the environment, as these parameters determine their equilibrium size through evaporation [11]. The chemistry inside the droplets also changes with temperature and RH, which can affect the infectivity of most respiratory viruses [12]. Although environmental parameters presumably have a strong influence on virus transmission, there is little data on their relation to the detection of airborne viral RNA. One study using multivariate analysis found an inverse correlation between both RH and temperature and the presence of virus RNA in air samples in a hospital corridor [13], but more data is needed to fully understand the complex interplay between viruses and their environment.

The aim of this study was to investigate the presence and size distribution of SARS-CoV-2-containing aerosols in hospital corridors by collecting size-fractionated aerosol samples over a long period of time. We present the most extensive data material so far from hospital corridors, sampled from over a year, starting at the very beginning of the pandemic. Temperature, RH, and carbon dioxide (CO_2) concentrations were monitored to explore any relations between indoor conditions and polymerase chain reaction-(PCR-) positive air samples. Two different wards were investigated, and these were compared with regard to sample positivity rate and environmental factors.

2. Methods

2.1. Site Description. Air samples were collected in the corridors of two infectious disease wards in the cities of Malmö and Lund in southern Sweden. Both wards cared for COVID-19 patients throughout the whole period (Figure 1(b)). The ward in Lund had 24 patient rooms, all with anterooms. The ward in Malmö had 17 patient rooms with anterooms, and a longer corridor as well as larger patient rooms compared to those in Lund. The patient rooms had air exchange rates per hour (ACH) of about 4 in Lund and 5 in Malmö. All anterooms had a positive pressure in relation to the patient room, and the corridor had a positive pressure gradient in relation to the anterooms. The corridor in Lund had an external air supply of 250 L/s for the corridor section where the sampler was placed. In Malmö, the airflow rate was 190 L/s for the corridor section. All patient rooms at both wards had outdoor access for visitors and waste handling. Schematic drawings of the corridors with indicated dimensions can be found in the Supplementary material (Figure S1-S2). The number of employees present at the wards daily was about 24-36 in Lund and 18-26 in Malmö. Face masks and respiratory masks were used by HCW during all patient care, and after the 1st of December 2020, universal masking in all healthcare buildings was implemented. Patients did not wear face masks in their own rooms, but when transported through the corridors. Information about the monthly number of COVID-19 patients at the wards was collected from the patient administration database, based on ICD-10 discharge diagnosis.

2.2. Air Sampling. Air samples were collected weekly from March 2020 to April 2021, with the exception of June, July, and August 2020 when collection was paused for practical reasons. Two 8-stage cascade impactors (Next Generation Impactor, Copley Scientific, UK) were used to collect the samples and these were run with an airflow rate of 60 L/min, 12 hours daily (from 8 a.m. to 8 p.m., turned off during the night to avoid noise), 7 days a week, representing about 300 m^3 air per week. Impactor plates were coated with a collection substrate spray (Dekati DS 515, Dekati, Finland) before use to avoid particle bounce. The impactors were placed hanging on a wall at a height of 1.2 m. The size fractions collected were >8.1 μm, 4.5–8.1 μm, 2.9–4.5 μm, 1.7–2.9 μm, 0.9–1.7 μm, 0.6–0.9 μm, 0.3–0.6 μm, and 0.1–0.3 μm.

Each size stage of the impactor sample plates (stainless steel) was swabbed with a wetted flocked nylon swab (Copan Diagnostics, USA) upon the weekly collection, and the swab was stored in 1 mL of universal transport media (UTM) as part of the Mini UTM Kit (Copan Diagnostics, USA) at -80°C until analysis. Indoor temperature, RH, and CO_2 concentration were recorded 24 hours daily with a five-minute time resolution (Model CL-11 or CP-11, Rotronic, Germany).

2.3. Sample Analysis. RNA was extracted from 200 μL of each swab sample using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Molecular Systems, Inc., Pleasanton, CA, USA) in a MagNA Pure 96 system (Roche Diagnostics Scandinavia AB, Sweden). RNA elution volume was 50 μL. The presence of SARS-CoV-2 RNA was determined by real-time reverse transcription polymerase chain reaction (RT-qPCR) using qPCRBIO Probe 1-Step Virus Detect kit (PCR Biosystems, UK) with primers and probes targeting the N-gene [14] or E-gene [15]. For positive and negative controls, quantitative synthetic SARS-CoV-2 RNA (VR-3276SD, ATCC) and nuclease-free water were used, respectively. The protocol included 50 cycles. The limit of detection was 4 copies [16] which was also confirmed in-house. All RNA samples were run in duplicates and considered positive if one or both of the duplicates had a Ct value

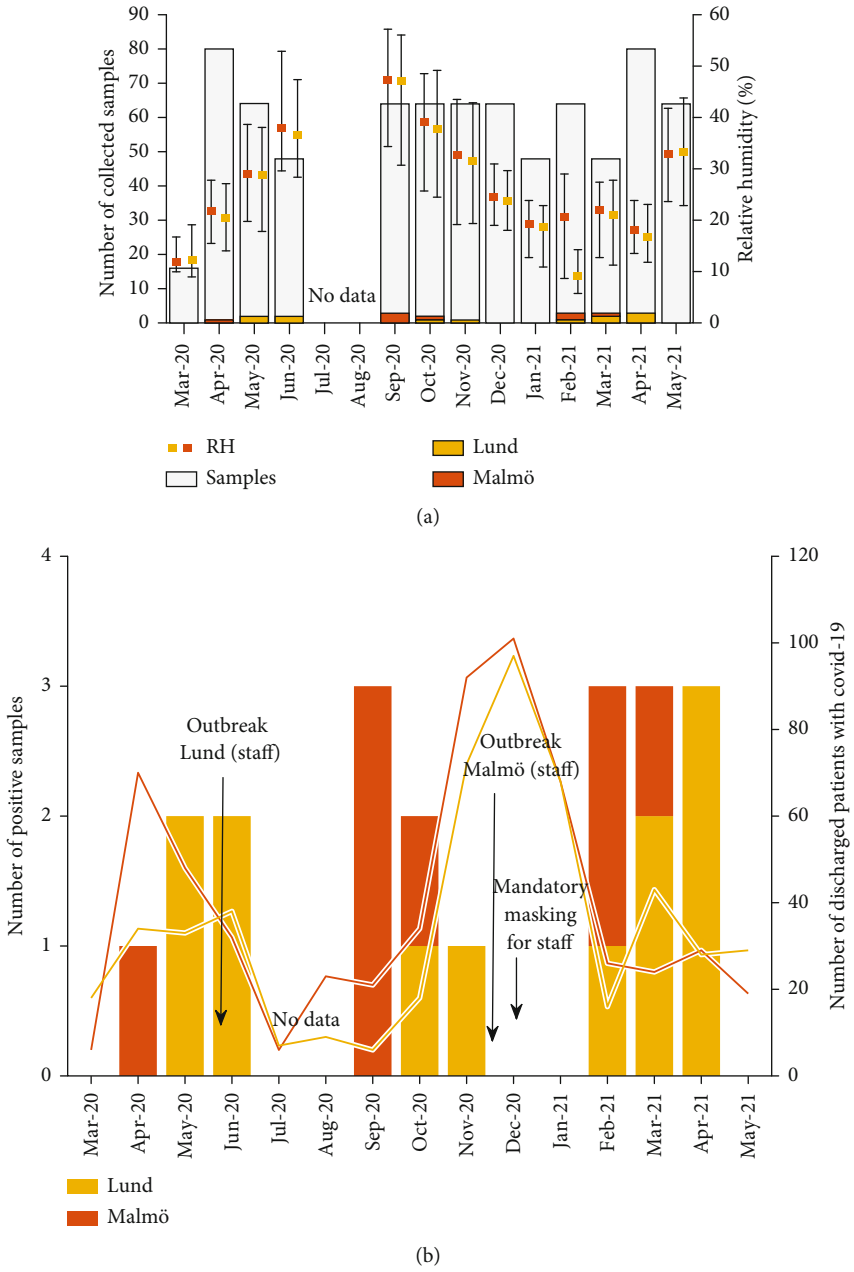

Figure 1: (a) Bars show the total number of samples collected, with positive samples indicated in color for each month (left axis) Points show the median measured RH (right axis) per month during the entire sampling period, and error bars show 10% and 90% percentile values. No samples were collected in July-August 2020. The same number of samples were collected each calendar week (Monday-Monday), resulting in different numbers of samples per calendar month. (b) Bars show the number of samples that were positive for SARS-CoV-2 by RT-qPCR per month, for Malmö in red and Lund in yellow (left axis), and the line shows the number of discharged patients per month diagnosed with COVID-19 in the sampled wards per month of the measurement period (red and yellow) on the right axis.

of <40.5. Ct values are presented as single values (for single positives) or mean of the duplicates. Negative controls were also run in duplicate for each RT-qPCR run, in total 40 negative controls.

3. Results

In total, 784 size-fractionated air samples (392 samples from each site) were collected from two infectious disease ward corridors over a period of 49 weeks, and 20 (2.6%) of these samples were positive (Figure 1(a)). The positive air samples were found from 15 weeks. From 4 of those weeks, more than one size fraction was positive, and in one of the four, positive samples were found in adjacent size fractions. The mean Ct value of positive samples was 39.8 (range 37.4-40.4), indicating that the concentration of SARS-CoV-2 RNA in the air samples was very low (close to the detection limit).

No clear seasonal pattern was observed as positive samples were found during all months except for March 2020, December-January 2020-2021, and May 2021 (Figures 1(a)

and 1(b)). At the ward in Malmö, 7 fractions from 5 different weeks were positive, while in Lund, 13 fractions were positive from 10 different weeks, but the difference was not significant ($p = 0.17$ using the chi-square test).

The mean temperature in the corridors was 23.4 ± 0.4°C. RH was more variable with a median of 27% and a range between 6 and 67% (Figure 1(a)). The lowest RH was observed in February (Lund) and March (Malmö), whereas the highest RH was measured in June for both sites (Figure 1(a)). CO_2 concentrations were low both at the ward in Lund (451 ± 43 ppm) and Malmö (448 ± 35 ppm), which is close to normal outdoor levels.

During the study period, the number of people with COVID-19 per month in the region (Region Skåne), where both hospitals were located, varied from around 1000 to 35000 confirmed cases, with a major peak in December 2020 [17]. The number of patients with confirmed COVID-19 per month at the wards where air sampling was performed was up to around 100 when community cases peaked, in December 2020 (Figure 1(b)). During our study, a COVID-19 outbreak was observed among the HCW in Lund at the end of May and beginning of June 2020, and the ward in Malmö was partly affected by an outbreak in November/December 2020 (Figure 1(b)).

Positive samples were found in all size fractions except the very largest (>8.1 μm) (Figure 2). Interestingly, 9 of 20 positive samples (45%) were found in size fractions below 0.9 μm. We found no relation between particle sizes and RH when RNA was detected (Figure 3).

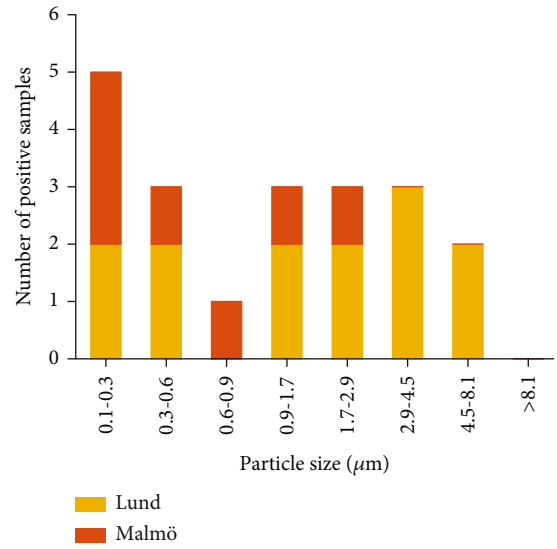

Figure 2: The total number of sample fractions positive for SARS-CoV-2 in RT-qPCR for each size fraction at each of the measurement sites.

4. Discussion

In this study, we collected weekly size-fractionated air samples from hospital corridors at two infectious disease wards over more than one year during the COVID-19 pandemic. The fraction of samples positive for SARS-CoV-2 RNA was low, 2.6%, and all positive samples were close to the detection limit. No relation was found between detected RNA and measured indoor parameters such as temperature or relative humidity. The low proportion of positive samples has been observed in our earlier study in a similar setting where sampling was not size-fractionated, and where we found 3 positive of 51 samples (5.9%) in corridors, compared to 26 of 231 (11.2%) in patient rooms [18]. Other studies collecting air from corridors also report low SARS-CoV-2 positivity rates (~3-10% in most cases) [4, 6–8, 19]. Besides corridors, air samples collected from other hospital areas, mostly from patient rooms, have had a positivity rate of about 10%, but most measurements were performed closer to patients [3]. The low amount of positive samples in corridors indicates that ventilation, as well as routines regarding anterooms and transport of infectious patients, are in place and work well, which prevents the majority of SARS-CoV-2-containing aerosols from leaking from patient rooms into the corridors.

A likely contributing source of airborne SARS-CoV-2 in corridors is nonsymptomatic HCW. An outbreak mainly among HCW in Lund during May/June 2020 coincided with a slight elevation in societal cases, and during this period, we observed four positive air samples in the corridor in Lund, but none in Malmö. The HCW outbreak in Malmö in early Dec 2020, when community case numbers were soaring, was not visible in our data. A possible explanation could be more efficient ventilation than in Lund. Another explanation is the increased capacity of diagnostic testing of patients and staff by PCR, which was improved and more sensitive compared to the very early phase of the pandemic. As a consequence, all staff were tested regularly for COVID-19 by PCR, and thus, infected HCW were generally identified at an early stage and stayed home from work. Another noteworthy event is the introduction of mandatory mask use in common areas, for both HCW and visitors, at both wards in Dec 2020. However, we still observed some positive samples after the routine was implemented.

The low positivity rate is expected and can be attributed to several factors: usually, no patients were present in the corridors, HCW wore face masks in the area, and effective ventilation and anterooms are designed to prevent airborne particles in patient rooms from entering the corridor. We estimated the risk of false positives as low since negative controls were negative for all runs. The low concentrations, presenting as high Ct values, push the performance of the RT-qPCR method as detection is close to stochastic in this Ct region. Thus, it is possible that we underestimated the number of positive samples as some samples may have contained concentrations below the detection limit in the PCR. For example, during the weeks with more than one positive size fraction, we would have expected to find positive samples in adjacent size fractions, but only did so in one of the four occasions. It is possible that other sampling and analysis methods, such as other targets for RT-qPCR, would have yielded slightly different results. The field in general needs to address the challenges of low concentrations of airborne pathogens and the sequent importance of improved sensitivity of detection methods.

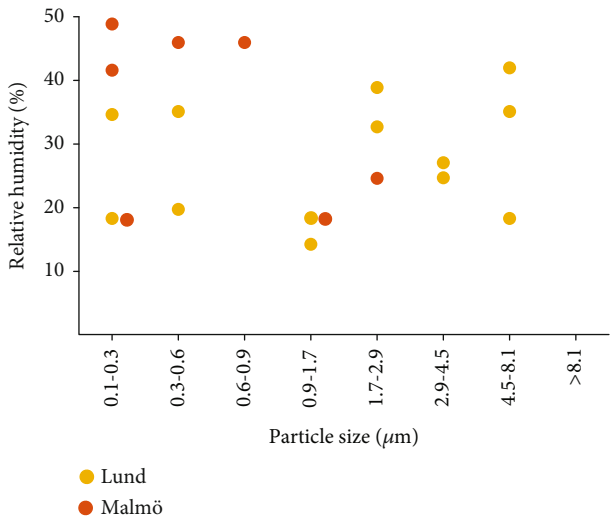

Figure 3: Mean relative humidity (during the week of collection) for each SARS-CoV-2-positive size fraction in Lund and Malmö. For two of the positive samples, RH was nearly identical; hence, points are shown side by side.

Positive samples were found in all size fractions except the largest (>8.1 μm), and interestingly, almost half of the positive samples were found in particle sizes below 1 μm. It is reasonable that we find more small particles in the corridor because these can travel far from their source, because of longer residence time in the air, and no direct sources are expected to be present in the corridor. In a study of directly exhaled breath aerosols containing SARS-CoV-2 RNA, around 25% was found in submicron particles [20]. In studies with size-fractionated samples collected from indoor environments, SARS-CoV-2 RNA has been detected in submicron particles, both in homes, cars, and hospital areas [21–23]. These small virus-containing particles are of extra concern as they can transport further in the air, extending the risk of transmission, and also deposit and infect the lower respiratory tract when inhaled, potentially causing more severe disease [24].

RH determines the water evaporation or condensation rate and thereby affects the particle size. In this study, we were not able to find any relations between the indoor environment parameters and positive air samples (Figure 1), or to size distribution of the positive samples (Figure 3). This was mainly due to the low number of positive samples, which limited statistical analysis, but also the fact that temperature and CO_2 levels were very stable. The RH displayed more variability, ranging from 6 to 67%, and although RH could affect emission rates or residence time of airborne viruses, no association with positive samples was found here. As the air samples were analysed per week, connections to transient RH values were difficult to explore. Moreover, within this RH range, aerosol droplets will have reached their final size by evaporation within 20 s; for significant effects on size and residence time, RH would need to be around 90% [25]. In conclusion, it seems that the indoor environment in the investigated hospital corridors was sufficiently ventilated to mitigate airborne virus transmission.

Many studies have investigated the effect of temperature and RH on SARS-CoV-2 survival on surfaces or in liquids, with disinfection as a motive [26–28]. Airborne SARS-CoV-2 viability also seems to be sensitive to microphysical processes, such as pH change, which arise from changes in environmental parameters, e.g., RH [29]. We did not investigate the infectivity of the collected RNA as the low RNA concentrations in collected samples did not allow for cell culturing, and the employed air sampling technique was not optimal for culturing the collected virus.

The collection method also affects the possibility of detecting viral airborne RNA. In this study, the NGI impactor was chosen for obtaining size resolution and collecting a large volume over a long time, as the longitudinal aspect was also an aim. However, sample plates were collected only once a week, raising the question of RNA stability on the stainless steel plates for this period of time. Studies have found infectious viruses up to day 7 on stainless steel [26] and remaining RNA stability on dry swabs up to 26 days [30], indicating that recovering RNA on stainless steel plates after 7 days should be possible. The same impactor was used in a previous study by our research group but for the collection of norovirus in hospital corridors during outbreaks among patients [31]. Interestingly, norovirus was then detected in three of four patient outbreaks. One reason for this higher positivity rate could be that the present study investigated infection ward corridors, where there were anterooms in all patient rooms, compared to medicine and geriatric wards in the norovirus study where there were no anterooms.

Despite these limitations, the present study contributes to the existing literature with large sample material over a long time in hospital corridors, an environment that is less investigated than the areas closer to patients. It is also a step towards gaining more detailed information about which sizes of aerosols contain SARS-CoV-2.

Further research is needed to investigate the origin of SARS-CoV-2-containing aerosol particles in hospital corridors, especially the smallest aerosols that may have travelled longer. This could be achieved by comparing RNA sequences of the sampled virus with virus sampled from patients at the ward. Another unanswered question is that of SARS-CoV-2 viability in real-life conditions over long sampling times. As we look past the receding COVID-19 pandemic, the presence of other respiratory viruses in common areas of the hospital also needs to be further investigated.

5. Conclusions

Sampled air from hospital corridors contained low concentrations of SARS-CoV-2 RNA, indicating a low risk of transmission of COVID-19 in this area. Nearly half of the detected RNA was found in aerosol particles below 1 μm that can travel far due to their long residence time in the air, possibly extending transmission risks. No association in positive samples or size fractions to environmental factors (e.g., RH, temperature, or CO_2) was observed. Neither could an evident association to an outbreak or mandatory mask use be

established, but any conclusion should be avoided due to the low number of positive weekly samples. Additional research on the size of SARS-CoV-2 aerosol vehicles is needed to increase understanding of virus-laden particle transport, behavior, and origin.

Data Availability

Data is available on reasonable request to the corresponding author.

Disclosure

Parts of this work were presented as a meeting abstract at the 11th International Aerosol Conference (IAC) 2022. Since the submission of this abstract, Dr. Sviataslau Sasinovich has made an important contribution to the scientific and practical work with RT-qPCR analysis of all samples, interpretation of these results, and writing of the manuscript. Hence, he was added to the author list of the final manuscript.

Conflicts of Interest

The authors report no conflict of interest.

Acknowledgments

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Supplementary Materials

Figure S1: drawing of the corridor of the infectious disease ward in Lund. Each patient room has one anteroom towards the corridor and one towards the outside of the building. Each patient room also has a toilet. Staff areas and points of external air supply are indicated. All air extraction points were placed in patient rooms and toilets. The air sampler was placed in the red box. Figure S2: drawing of the entire corridor of the infectious disease ward in Malmö. Each patient room has one anteroom towards the corridor and one towards the outside of the building. Each patient room also has a toilet. Staff areas, entrances, and points of external air supply are indicated. The instrument was placed in the red box. All air extraction points were placed in anterooms to patient rooms and in toilets. There was a negative pressure in the anterooms compared to the corridor, and then a negative pressure in the patient room compared to the anteroom. Figure S2: the layout of the infectious disease ward in Malmö, with 17 patient rooms, staff rooms, and a circle-shaped corridor. *(Supplementary Materials)*

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