Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Journal of Hospital Infection 108 (2021) 33–42

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

# Detection of influenza virus in air samples of patient rooms

A. Chamseddine [a], N. Soudani [b,c], Z. Kanafani [d], I. Alameddine [a], G. Dbaibo [e], H. Zaraket [b], M. El-Fadel [a,*]

[a] Department of Civil and Environmental Engineering, American University of Beirut, Beirut, Lebanon
[b] Department of Experimental Pathology, Immunology and Microbiology, American University of Beirut, Beirut, Lebanon
[c] Doctoral School of Science and Technology, Faculty of Sciences, Lebanese University, Beirut, Lebanon
[d] Department of Internal Medicine, American University of Beirut, Beirut, Lebanon
[e] Department of Paediatric and Adolescent Medicine, American University of Beirut, Beirut, Lebanon

ARTICLE INFO

*Article history:*
Received 18 August 2020
Accepted 27 October 2020
Available online 2 November 2020

*Keywords:*
Hospital environment
Indoor air quality
Influenza virus
Respiratory syncytial virus
Real-time PCR (RT-PCR)

SUMMARY

***Background:*** Understanding the transmission and dispersal of influenza virus and respiratory syncytial virus (RSV) via aerosols is essential for the development of preventative measures in hospital environments and healthcare facilities.

***Methods:*** During the 2017–2018 influenza season, patients with confirmed influenza or RSV infections were enrolled. Room air samples were collected close (0.30 m) to and distant (2.20 m) from patients' heads. Real-time polymerase chain reaction was used to detect and quantify viral particles in the air samples. The plaque assay was used to determine the infectiousness of the detected viruses.

***Findings:*** Fifty-one air samples were collected from the rooms of 29 patients with laboratory-confirmed influenza; 51% of the samples tested positive for influenza A virus (IAV). Among the IAV-positive patients, 65% were emitters (had at least one positive air sample), reflecting a higher risk of nosocomial transmission compared with non-emitters. The majority (61.5%) of the IAV-positive air samples were collected 0.3 m from a patient's head, while the remaining IAV-positive air samples were collected 2.2 m from a patient's head. The positivity rate of IAV in air samples was influenced by distance from the patient's head and day of sample collection after hospital admission. Only three patients with RSV infection were recruited and none of them were emitters.

***Conclusion:*** Influenza virus can be aerosolized beyond 1 m in patient rooms, which is the distance considered to be safe by infection control practices. Further investigations are needed to determine the extent of infectivity of aerosolized virus particles.

© 2020 The Healthcare Infection Society. Published by Elsevier Ltd. All rights reserved.

## Introduction

Monitoring and controlling microbiological contaminants, including respiratory viruses, in the indoor air of hospitals has become an integral part of infection prevention strategies in hospitals [1,2]. In recent years, debate and concerns regarding the potential for airborne transmission of respiratory viruses

* Corresponding author. Address: Department of Civil and Environmental Engineering, American University of Beirut, PO Box 11-0236, Riad El Solh 1107 2020, Beirut, Lebanon. Tel.: +961 1 350000x3470.
*E-mail address:* mfadel@aub.edu.lb (M. El-Fadel).

https://doi.org/10.1016/j.jhin.2020.10.020
0195-6701/© 2020 The Healthcare Infection Society. Published by Elsevier Ltd. All rights reserved.

have intensified with the emergence of avian influenza virus, severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus [3—5]. More recently, concerns over airborne infections have resurfaced with the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It has been reported that bioaerosols carrying SARS-CoV-2 can remain suspended in the air for a few hours [6]. Several groups have detected SARS-CoV-2 RNA in air samples collected in hospital rooms, supporting the potential transmission of SARS-CoV-2 via aerosols in indoor environments [7]. The World Health Organization (WHO) has highlighted the risk of airborne transmission during procedures that generate bioaerosols, such as intubation, tracheostomy, open suction and manual ventilation [8]. The COVID-19 pandemic has raised many questions regarding how much is known about airborne infections.

Influenza and respiratory syncytial virus (RSV) are the main causes of respiratory infections that require hospitalization. Most of the severe and life-threatening complications associated with influenza and RSV infections are observed in infants, elderly people and immunocompromised patients [9,10]. Influenza is a highly contagious respiratory virus estimated to infect approximately 5—10% of adults and 20—30% of children each year, resulting in up to 650,000 deaths globally each year [11,12]. Influenza A virus (IAV) has numerous subtypes and a broad host range. IAV is of particular concern as this virus has historically caused several pandemics [13,14]. Annual influenza outbreaks are mainly caused by IAV (H1N1 and H3N2 subtypes) and influenza B virus (IBV) [14,15]. RSV, on the other hand, is a major cause of moderate-to-severe respiratory infections [16—18]. In 2015, it was estimated that 33.1 million episodes of RSV resulted in approximately 3.2 million hospitalizations and around 60,000 in-hospital deaths among children aged <5 years [17]. Vulnerable patients infected with RSV are likely to experience extended hospital stays with increased risk of morbidity and mortality [18—20]. Both the influenza virus and RSV spread indirectly through contact with contaminated surfaces or via respiratory droplets and aerosols, particularly in crowded areas [12,21—26]. These particles cause infections in people nearby, as they settle in the upper respiratory tract. Airborne transmission occurs via droplet nuclei <5 μm, which remain suspended in the air and can be disseminated long distances by the air current. Although these aerosolized particles contain a low concentration of infectious particles, they are likely to cause lower respiratory tract infections because of their small size [3,27,28]. Several international organizations, such as the Institute of Medicine, the European Centre for Disease and Control, the US Centers for Disease Control and Prevention (CDC) and WHO, have highlighted the need for further research regarding the transmission routes of influenza virus and other respiratory viruses [3,11,12,14,29—32].

Nosocomial transmission of influenza and RSV has been well documented, and constitutes a major concern for hospitalized patients given their vulnerability to developing severe diseases [21,30,33—37]. The rate of healthcare-associated influenza infections ranges between 2% and 7% [38—40]. Data from the USA revealed that the median duration of hospital stay was significantly longer for nosocomial influenza cases compared with community-acquired cases (7.5 vs 3 days, respectively) [40]. Nosocomial infections were also associated with poor clinical outcomes, including intensive care unit admission and death. Therefore, it is essential to follow and enhance infection control guidelines to minimize the occurrence of these infections in healthcare settings. Healthcare providers are requested to adhere to droplet precautions when dealing with patients infected with influenza or RSV [29,41]. Droplet precautions include wearing a surgical mask, maintaining a 1-m distance from the patient, and placing patients in single rooms (if possible) or physically separated (>1 m between beds) [42]. Also, patients should use masks, follow cough and sneezing etiquette, and perform hand hygiene. However, these guidelines remain controversial, with more evidence to support the role of aerosols in influenza and RSV transmission. As such, this study assessed the presence of airborne influenza virus and RSV in patient rooms.

## Methods

### *Study design*

Air sampling was conducted during the influenza season from January to March 2018 at a major tertiary care hospital in Beirut, Lebanon. Air samples were collected from the rooms of patients with laboratory-confirmed influenza or RSV infections. The Coriolis μ Biological Air Sampler (Bertin Instruments, Montigny-le-Bretonneux, France) was used to collect air samples at two locations within each room. One sample was taken 0.3 m from the patient's head (A), simulating the typical distance between a patient and visitors or healthcare providers performing near-bed procedures, while the other sample was taken 0.5 m from the door and 2.2 m from the patient's head (B) (Figure 1), simulating the distance that physicians usually keep from patients with influenza or RSV infection. For smaller rooms, only one air sample was collected, 0.3 m from the patient's head. The air collected from patient rooms was aspirated for 10 min at a flow rate of 300 L/min, and drawn into a collection tube containing 15 mL of sterile collection media. The numbers of coughs and sneezes during air sampling were recorded. None of the air sampling procedures were performed in the presence of aerosol-generating procedures, such as bronchoscopy, intubation, open suction of airways and nebulizer therapy. The air sampler and the collection tubes were decontaminated with 70% ethanol to prevent potential carryover contamination.

Temperature and relative humidity were monitored and recorded in patient rooms using VELOCICALC ® Air Velocity Meter Model 9545 (TSI Corp., Shoreview, MN, USA).

### *Virological analysis*

The samples were immediately transported on ice to a laboratory, where they were aliquoted and stored at -80°C. Within 1 week of sample collection, RNA was extracted from a 1-mL sample aliquot using PureLink Viral RNA/DNA Mini Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer's specifications. The specimens were screened for IAV, IBV and RSV by real-time polymerase chain reaction (RT-PCR) using primers and probes specific for the M gene, NS gene and G gene, respectively [43—46]. The sequences of primers and probes were obtained from CDC, and the experimental procedure was performed as described previously [46]. In brief, One-Step RT-PCR AgPath-ID (Thermo Fisher Scientific, Waltham, MA,

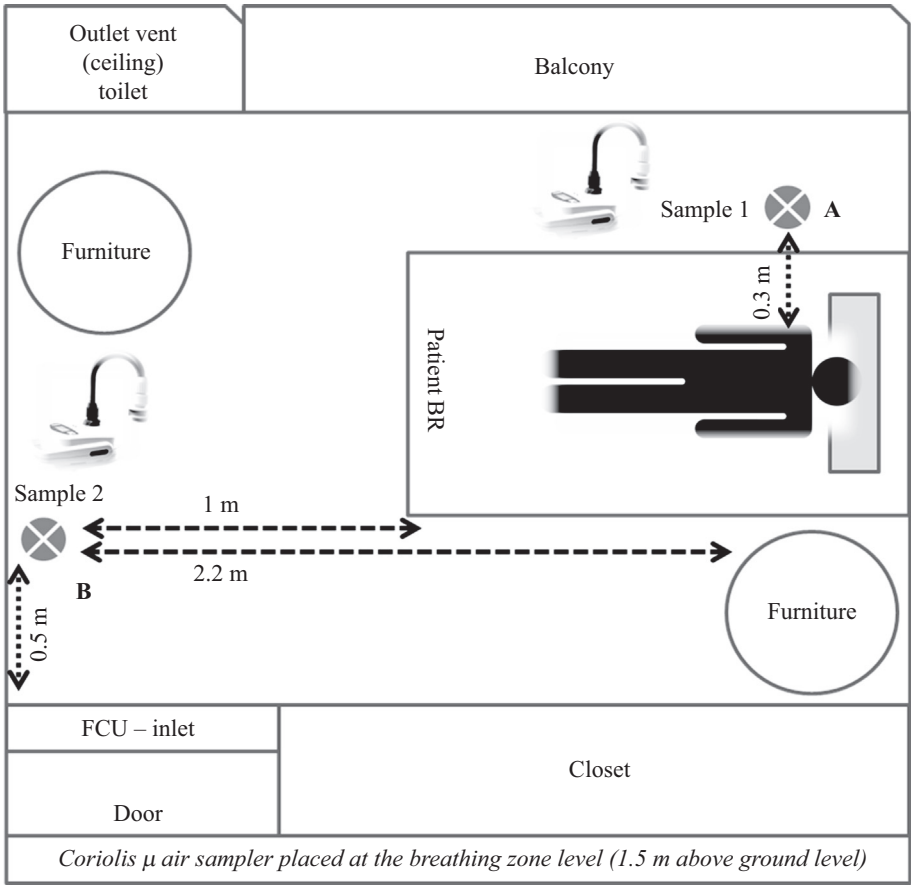


**Figure 1.** Dimensions and sampling locations of patient rooms (Coriolis μ Biological Air Sampler was placed 1.5 m above ground, i.e. breathing zone level). Two samples (whenever tolerated by the patient) were collected from patient rooms: samples that were taken close (~0.3 m) to a patient's head were labelled as 'A', and samples collected distant (~2.2 m) from a patient's head were labelled as 'B'.

USA) was used to amplify the target sequences on a CFX96 RT-PCR system (Bio-Rad, Hercules, CA, USA). IAV and IBV were multiplexed, whereas RSV was screened independently according to the following protocol: 45°C for 10 min, 95°C for 10 min, and 45 cycles at 95°C for 15 s and 55°C for 1 min. All runs were performed in the presence of a no-template control and positive control for each target. Extraction controls were screened to exclude cross-contamination during extraction. IAV-positive samples were further subtyped via RT-PCR.

The viral copy number of influenza (M gene) in each sample was quantified relative to a standard curve obtained from a serial dilution of a known concentration of purified complete genome containing the gene of interest (MBC028; Vircell, Granada, Spain). The cycle threshold obtained by RT-PCR from each sample was plotted to determine the equivalent M gene copy number (copies/$m^3$) according to the following formula:

$$\text{M gene}\frac{\text{copies}}{m^3} = \frac{\text{M gene copies PCR} \times \left(\frac{V_{extract}}{V_{PCR}}\right)}{U \times t}$$

where the M gene copy number was calculated relative to the standard curve (copies/μL), $V_{extract}$ is the eluted volume from the extraction (μL), $V_{PCR}$ is the volume analysed by RT-PCR (μL), $U$ is the airflow rate ($m^3$/min), and $t$ is the sampling duration (min).

Detection and quantification of the infectious virus in the air samples was performed using the plaque assay as described previously [46]. Briefly, at the end of the influenza season, an aliquot of each sample was thawed and serially diluted (10-fold). MDCK cells seeded in six-well plates were inoculated with 200 μL of each dilution and incubated at 37°C for 1 h with shaking every 15 min. Next, the cells were covered with 3 mL of 0.5% nutritive overlay. After 72 h, the cells were stained with crystal violet solution.

## Statistical analysis

Statistical analysis was performed using Prism 7. Pearson's Chi-squared analysis with 95% confidence intervals ($P<0.05$) was used to assess the association between virus shedding and various factors such as age group and distance. Welch's unequal variance $t$-test was used to assess the relationship between viral copy number and days since hospital admission, and to determine the influence of coughing and sneezing on IAV emission. For continuous variables, means and standard deviations (SD) were calculated. $P<0.05$ was considered to indicate statistical significance.

## Ethical approval

This study was approved by the institutional review board at the American University of Beirut and the hospital's

administration. All patients provided written informed consent before sample collection. The sampling procedures performed did not replace or obstruct routine medical care procedures or institutional protocols at the study hospital.

## Results

In total, 29 subjects (11 males and 18 females) with confirmed IAV ($N$=26) or RSV ($N$=3) infections were recruited. All patients infected with IAV were administered antiviral treatment upon hospitalization. Seventeen of 26 patients infected with IAV were considered to be 'emitters', defined as having at least one positive air sample (Table I). The rate of detection did not differ significantly between the different age groups in this study ($P$>0.05) (Table I). Details on the air samples collected are presented in Appendix A.

The results showed that 51% (26/51) of the collected air samples were positive for IAV. Of the IAV-positive samples, 61.5 % ($N$=16) were collected 0.3 m from a patient's head (close) and 38.5 % ($N$=10) were collected 2.2 m from a patient's head (distant) (Table II). None of the distant air samples were IAV-positive when the corresponding close sample was negative. The majority (95%, $N$=21) of air samples that were collected on the first day of hospital admission were positive for IAV, unlike the samples collected on the second (24%, $N$=4) and third (8%, $N$=1) days of hospital admission (Figure 2). IAV was detected in 100% ($N$=12) of the close samples and 90% ($N$=9) of the distant samples collected on the first day of hospital admission. The percentage of IAV detected in the close samples decreased to 33% ($N$=3) and 13% ($N$=1) on the second and third days of hospital admission, respectively, compared with 13% ($N$=1) and 0% ($N$=0), respectively, for the distant samples (Figure 2). None of the air samples (collected on the second and third days of hospital admission) from the rooms of the three patients infected with RSV were positive for RSV on PCR.

Next, the IAV viral load (M gene copy numbers) in the collected air samples was quantified using quantitative RT-PCR. The mean viral load in the 16 IAV-positive close air samples was 3681.25 copies/m$^3$ (700–12,800 copies/m$^3$), compared with 2616.67 copies/m$^3$ (650–15,900 copies/m$^3$) in the 10 distant samples (Figure 2). The viral loads did not differ significantly between the close and distant air samples. Also, virus yields from the air samples collected on the first day of hospital admission (mean 4281 copies/m$^3$) were higher compared with the samples collected on the second day of hospital admission (mean 1690 copies/m$^3$) ($P$=0.07). None of the collected air samples yielded infectious virus using the plaque assay. The detection of IAV RNA in the air samples obtained from patient rooms was mainly associated with patients who were coughing and/or sneezing. During air sampling, 65% (17/26) of the patients were coughing and sneezing, whereas 23% (6/26) were only coughing, 4% (1/26) were only sneezing, and 8% (2/26) were neither coughing nor sneezing (asymptomatic). The air samples that were collected from rooms where the patients were both coughing and sneezing had significantly higher viral loads compared with rooms where the patients only coughed or sneezed, or did not cough or sneeze ($P$=0.048) (Figure 3).

Temperature and relative humidity measurements inside patient rooms were within the accepted guidelines of the American Society of Heating, Refrigerating and Air-Conditioning Engineers and the thermal comfort standards defined for healthcare facilities (i.e. relative humidity of 30–60%, temperature of 20–24°C). Temperatures recorded in patient rooms ranged from 21.5 to 23.5°C [mean 22.5 (SD 0.60) °C], while relative humidity ranged from 40% to 45% [mean 43.3 (SD 1.34) %]. There was no significant association between the detection of IAV in air samples and room temperature ($P$>0.05) or relative humidity ($P$>0.05).

**Table I**
Characteristics of patients with influenza classified as emitters

| Age group (years) | $N$[a] | Males | Females | IAV-positive air samples (%, $N$) | $t$-test $P$-value[b] |
|---|---|---|---|---|---|
| Children (0–<18) | 3 | 2 | 1 | 33 (1) | >0.05 |
| Adults (18–64) | 17 | 4 | 13 | 71 (12) | >0.05 |
| Elderly (65) | 9 | 5 | 4 | 44 (4) | >0.05 |
| Total | 29 | 11 | 18 | 59 (17) | |

IAV, influenza A virus.
[a] This table shows the characteristics of patients that yielded at least one influenza-positive air sample on real-time polymerase chain reaction. None of the patients with respiratory syncytial virus infection (age 1–4 months) had positive air samples, and thus their information is not included in this table.
[b] The $t$-test was performed to compare the positivity rate by age group. While there were differences in emission by age, the differences were not significant ($P$>0.05), particularly given the unequal sample size in the three age groups.

**Table II**
Distribution of influenza-positive air samples collected from different locations

| Location | $N$[c] | IAV-positive air samples (%, $N$) |
|---|---|---|
| Close samples[a] | 29 | 55 (16) |
| Distant samples[b] | 22 | 45 (10) |
| Total samples | 51 | 51 (26) |

IAV, influenza A virus.
[a] Air samples collected 0.30 m from a patient's head.
[b] Air samples collected 2.20 m from a patient's head and 0.50 m from the door.
[c] Air samples that were collected from the rooms of patients infected with respiratory syncytial virus were negative by real-time polymerase chain reaction and were therefore not included.

## Discussion

Respiratory viruses are mainly transmitted via droplets and aerosols and, to a lesser extent, via contact with fomites [47]. The COVID-19 pandemic revived the debate regarding the potential for airborne transmission of respiratory viruses, and highlighted the knowledge gaps regarding this route of transmission and its consequences on infection control guidelines [48,49]. The present study investigated the aerosolization of influenza and RSV in patient rooms in order to assess the appropriateness of the recommended infection control procedures.

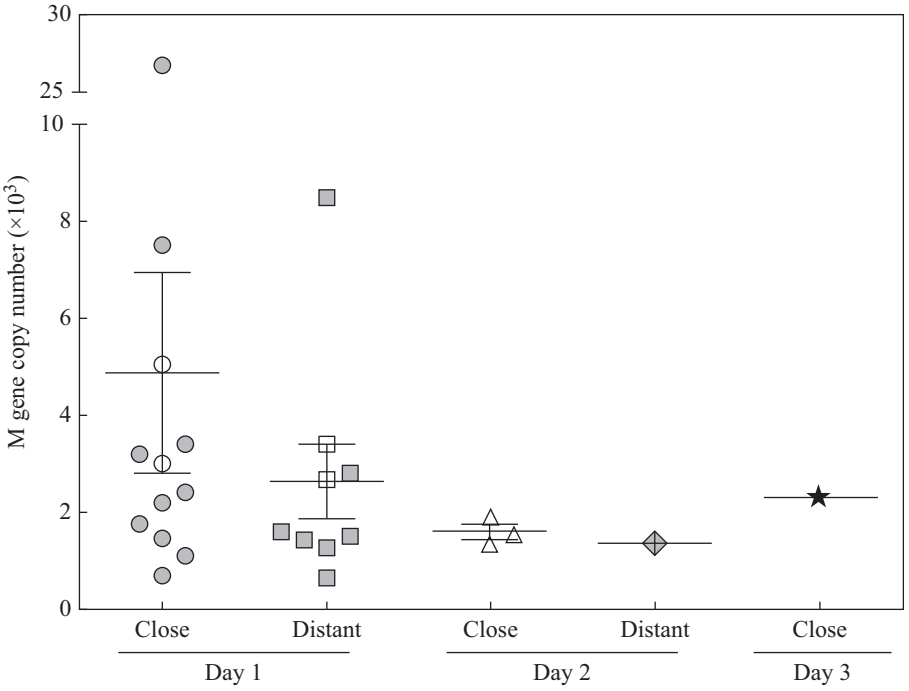


**Figure 2.** Copy number of influenza A virus (M gene) detected in air samples relative to day of hospital admission. Samples designated as 'close' were collected 0.3 m from a patient's head, and samples designated as 'distant' were collected 2.2 m from a patient's head.

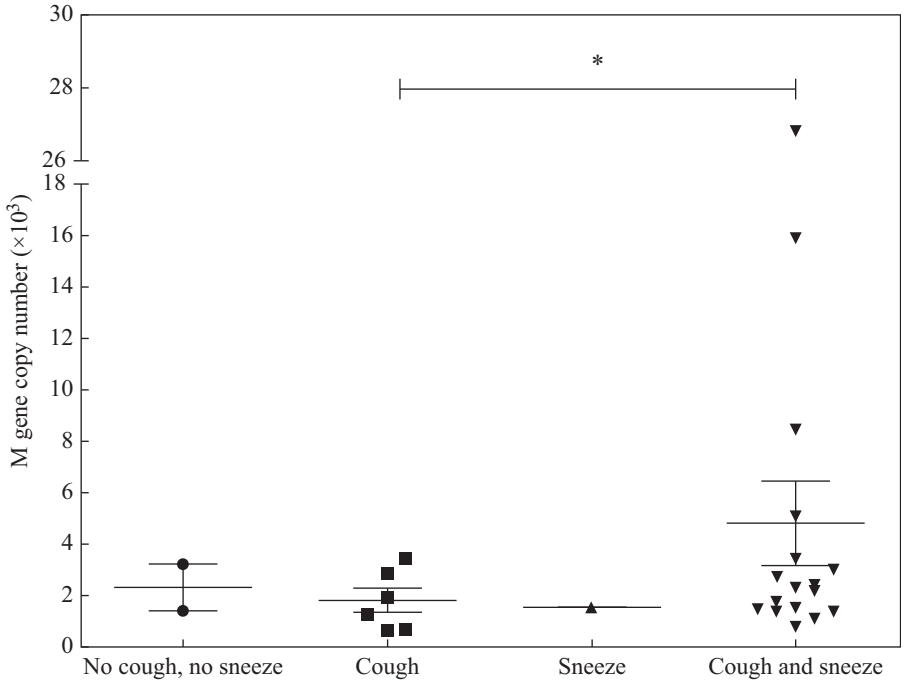


**Figure 3.** Influence of coughing and/or sneezing on influenza A virus emission in the collected air samples. Statistical significance between variables was determined using *t*-test (*$P<0.05$).

Influenza viral RNA was detected in 51% of the air samples collected from the rooms of patients with influenza. These patients were classified as emitters with a higher risk for nosocomial transmission via the airborne route than non-emitters. These results support previous findings indicating that sneezing and coughing are significantly associated with

higher detection of influenza virus in air samples [30]. However, sneezing alone and coughing alone were not associated with higher levels of influenza emission compared with the asymptomatic group. This is consistent with previous studies showing that simple breathing (exhalation) is sufficient for aerosolization of the virus [50]. Also, the generation of very fine aerosols (1 μm) by mouth breathing was found to exceed that for nose breathing, talking and coughing [51]. However, contrary to previous studies, the present study was not able to demonstrate that the aerosolized virus retained its infectivity [48—50]. This may be attributed to the harsh conditions generated inside the air sampling machine [52], or the sensitivity of the plaque assay method, which has a detection limit of 500 plaque-forming units/mL [53]. Current guidelines for epidemic- and pandemic-prone acute respiratory infections, including those for COVID-19, recommend airborne precautions when aerosol-generating procedures, such as intubation and ventilation, are being performed [54—56]. However, studies, including the present study, have demonstrated that even in the absence of such procedures, the virus can be detected in the air of patient rooms, suggesting aerosolization; as such, reconsideration of existing guidelines is warranted [7,31,33,50,57].

Influenza virus transmission is reported primarily by large droplets traveling up to 1 m from the source [14,29,58]. The present study found that the majority of air samples collected 0.3 m from infected patients were positive, particularly on the first day of hospital admission, which increases the exposure risk of healthcare providers and visitors if proper infection control measures are not followed. However, albeit to a lesser extent, healthcare providers and visitors could still be exposed to aerosolized virus up to 2.2 m away from patients with symptomatic influenza. These findings raise concerns beyond the current WHO and CDC recommendations (i.e. spacing of 1 m) regarding the adequacy of protection of visitors and healthcare providers during routine care operations in hospitals and similar healthcare facilities.

Kulkarni *et al.* [59] showed that RSV-infected children hospitalized for bronchiolitis produce aerosols that contain infectious virus particles, whereas Wan *et al.* [60] reported that RSV was detected in only one of 13 samples collected at a close distance (0.6—1.8 m) and in none of the samples collected further away. In the present study, none of the air samples collected from the rooms of three RSV-infected patients were positive by RT-PCR. The lack of RSV detection in the air samples may be due to the small number of patients infected with RSV included in this study. Furthermore, the respiratory symptoms in RSV-infected patients usually appear approximately 3 days prior to hospital admission [61,62]. Therefore, the lack of sampling on the first day of admission from the rooms of patients with RSV may also have reduced the ability to detect the virus in the air. Moreover, the shorter duration (10 min) of air sampling in the present study compared with 30 min in the study by Kulkarni *et al.* [59] may have influenced RSV detection in the collected air samples. Another factor could be the degradation of the RSV virus during sampling, and/or the differences in sampling efficiencies of various air samplers [52].

Adults shed influenza virus for an average of 1 week, with a peak on the second day of the illness, whereas children can shed the virus for a longer period with a noticeable decline in RNA detection in samples collected on subsequent days [27,63,64]. A study in Germany showed that the mean duration of symptoms before admission to the emergency department was 5.2 days, and this did not differ significantly between IAV and IBV [65]. Similarly, a study in China estimated the duration from symptom onset to hospitalization for patients with IAV infection to be 5.9 days; this period was shorter for patients with IBV infection (3.7 days). In the present study, a higher influenza viral load was detected in samples collected on the first day of hospital admission compared with samples collected on the second and third days of hospital admission. Therefore, it is likely that these patients were already clearing the virus, particularly as they were on antiviral therapy. These findings are consistent with previous studies showing that the highest influenza viral load is detected on the first day of admission in oseltamivir-treated patients [66,67]. The present findings are also in line with studies undertaken in college communities and multi-centre areas which revealed a negative association between the detection of aerosolized IAV and the time elapsed since illness onset [50,57]. The present study also supports previous findings that all patients do not emit influenza virus similarly [30—32,68]. Hence, the identification of 'super-emitters' and factors associated with this may improve our understanding of the transmission of influenza virus.

The limitations of the study include the sample size and the lack of clinical data (e.g. vaccination history, body mass index, etc.), which precludes the analysis of more complex variables that might affect the presence of airborne viruses. Also, the samples were collected solely from the rooms of symptomatic patients and in departments that were accessible for this type of work. Thus, the risk of asymptomatic virus emitters was not addressed. The authors were unable to access the emergency department, which may be a hub for the dissemination of respiratory viruses. Regardless of these limitations, this study offers valuable information for infection prevention and control practices in hospitals, and questions the notion of a safe distance of 1 m.

## Acknowledgements

The authors wish to thank the hospital administration, Dr. Yolla Youssef and nurse managers for facilitating the sampling programme.

**Conflict of interest statement**
None declared.

**Funding sources**
This study was supported through joint funding from the Lebanese National Council for Scientific Research and the American University of Beirut. Additional funding for equipment was obtained through the Dar Al-Handasah (Shair & Partners) Endowed Chair Fund.

## Appendix A

*Summary of air-quality data collected from patient rooms.*

| | Date | Time | Sample ID[a] | Age | Sex | RT-PCR | Emitters[b] | Day of admission[c] | Coughing[d] | Sneezing[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 24/1/2018 | 9:40 AM | 1A | 1 m | M | ND | No | 2 | 0 | 0 |
| 2 | 24/1/2018 | 9:55 AM | 1B | 1 m[g] | M | ND | No | 2 | 0 | 0 |
| 2 | 24/1/2018 | 10:55AM | 2A | 50 y | F | Influenza A | Yes | 3 | 2 | 1 |
| 3 | 30/1/2018 | 11:35 AM | 3A | 4 m[g] | F | ND | No | 3 | 0 | 0 |
| 4 | 7/2/2018 | 11:10 AM | 4A | 8 m[g] | M | Influenza A | Yes | 1 | 0 | 0 |
| 6 | 7/2/2018 | 11:25 AM | 4B | 8 m | M | Influenza A | Yes | 1 | 0 | 0 |
| 5 | 7/2/2018 | 11:50 AM | 5A | 38 y | F | Influenza A | Yes | 2 | 3 | 1 |
| 6 | 7/2/2018 | 12:10 PM | 6A | 40 y | F | Influenza A | Yes | 1 | 1 | 0 |
| 9 | 7/2/2018 | 12:25 PM | 6B | 40 y | F | Influenza A | Yes | 1 | 1 | 1 |
| 7 | 9/2/2018 | 9:00 AM | 7A | 45 y | M | Influenza A | Yes | 1 | 1 | 1 |
| 1 | 9/2/2018 | 9:15 AM | 7 B | 45 y | M | Influenza A | Yes | 1 | 1 | 0 |
| 8 | 9/2/2018 | 9:40 AM | 8A | 31 y | F | Influenza A | Yes | 1 | 1 | 0 |
| 9 | 9/2/2018 | 10:00 AM | 9A | 23 y | M | Influenza A | Yes | 1 | 2 | 1 |
| 1 | 9/2/2018 | 10:15 AM | 9B | 23 y | M | Influenza A | Yes | 1 | 1 | 1 |
| 10 | 12/2/2018 | 10:30 AM | 10A | 51 y | F | Influenza A | Yes | 1 | 2 | 1 |
| 1 | 12/2/2018 | 10:45 AM | 10B | 51 y | F | Influenza A | Yes | 1 | 1 | 1 |
| 11 | 12/2/2018 | 11:10 AM | 11A | 38 y | F | Influenza A | Yes | 1 | 1 | 1 |
| 1 | 12/2/2018 | 11:25 AM | 11B | 38 y | F | Influenza A | Yes | 1 | 1 | 0 |
| 12 | 12/2/2018 | 11:40 AM | 12A | 91 y | F | Influenza A | Yes | 1 | 2 | 1 |
| 13 | 15/2/2018 | 9:25 AM | 13A | 55 y | F | Influenza A | Yes | 1 | 2 | 1 |
| 2 | 15/2/2018 | 9:40 AM | 13B | 55 y | F | ND | No | 1 | 0 | 0 |
| 14 | 15/2/2018 | 10:00 AM | 14A | 80 y | M | Influenza A | Yes | 1 | 2 | 2 |
| 2 | 15/2/2018 | 10:15 AM | 14 B | 80 y | M | Influenza A | Yes | 1 | 1 | 1 |
| 15 | 21/2/2018 | 10:15 AM | 15A | 70 y | F | Influenza A | Yes | 1 | 1 | 2 |
| 2 | 21/2/2018 | 10:30 AM | 15B | 70 y | F | Influenza A | Yes | 1 | 1 | 1 |
| 16 | 21/2/2018 | 10:45 AM | 16A | 39 y | F | Influenza A | Yes | 1 | 1 | 2 |
| 2 | 21/2/2018 | 11:00 AM | 16B | 39 y | F | Influenza A | Yes | 1 | 1 | 0 |
| 17 | 21/2/2018 | 11:15 AM | 17A | 31 y | F | Influenza A | Yes | 2 | 1 | 0 |
| 2 | 21/2/2018 | 11:25 AM | 17B | 31 y | F | ND | No | 2 | 1 | 0 |
| 18 | 21/2/2018 | 11:45 AM | 18A | 53 y | F | ND | No | 2 | 1 | 0 |
| 3 | 21/2/2018 | 11:55 AM | 18B | 53 y | F | ND | No | 2 | 0 | 0 |
| 19 | 2/3/2018 | 9:00 AM | 19A | 23 y | F | ND | No | 2 | 1 | 0 |
| 3 | 2/3/2018 | 9:10 AM | 19B | 23 y | F | Influenza A | Yes | 2 | 1 | 1 |
| 20 | 2/3/2018 | 9:25 AM | 20A | 77 y | M | Influenza A | Yes | 2 | 0 | 2 |
| 3 | 2/3/2018 | 9:40 AM | 20B | 77 y | M | ND | No | 2 | 1 | 0 |
| 21 | 2/3/2018 | 9:55 AM | 21A | 50 y | M | ND | No | 2 | 1 | 1 |
| 3 | 2/3/2018 | 10:05 AM | 21B | 50 y | M | ND | No | 2 | 0 | 0 |
| 22 | 2/3/2018 | 10:20 AM | 22A | 18 y | F | ND | No | 3 | 1 | 0 |
| 3 | 2/3/2018 | 10:30 AM | 22B | 18 y | F | ND | No | 3 | 0 | 0 |
| 23 | 8/3/2018 | 10:55 AM | 23A | 85 y | F | ND | No | 2 | 1 | 1 |
| 4 | 8/3/2018 | 11:10 AM | 23B | 85 y | F | ND | No | 2 | 0 | 1 |
| 24 | 8/3/2018 | 11:25 AM | 24A | 45 y | F | ND | No | 2 | 1 | 0 |
| 4 | 8/3/2018 | 11:40 AM | 24B | 45 y | F | ND | No | 2 | 0 | 0 |
| 25 | 8/3/2018 | 11:55 AM | 25A | 86 y | F | ND | No | 3 | 1 | 1 |
| 4 | 8/3/2018 | 12:10 PM | 25B | 86 y | F | ND | No | 3 | 1 | 0 |
| 26 | 12/3/2018 | 10:00 AM | 26A | 70 y | M | ND | No | 3 | 0 | 1 |
| 4 | 12/3/2018 | 10:10 AM | 26B | 70 y | M | ND | No | 3 | 0 | 0 |

(*continued on next page*)

(*continued*)

| | Date | Time | Sample ID[a] | Age | Sex | RT-PCR | Emitters[b] | Day of admission[c] | Coughing[d] | Sneezing[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 12/3/2018 | 10:25 AM | 27A | 88 y | M | ND | No | 3 | 1 | 1 |
| 4 | 12/3/2018 | 10:40 AM | 27B | 88 y | M | ND | No | 3 | 0 | 1 |
| 28 | 12/3/2018 | 10:55 AM | 28A | 70 y | M | ND | No | 3 | 0 | 0 |
| 29 | 12/3/2018 | 11:10 AM | 29A | 36 y | M | ND | No | 3 | 0 | 0 |

RT-PCR, real-time polymerase chain reaction; m, months; y, years; M, male; F, female; ND, not determined.
[a]Two samples were collected from patient rooms: samples that were taken close (0.3 m) to a patient's head were labelled as 'A' and those collected distant (2.2 m) from a patient's head were labelled as 'B'.
[b]Emitters were defined as patients with an air sample positive for influenza by RT-PCR.
[c]Only three patients with respiratory syncytial virus infection were recruited in this study, and their air samples were collected on admission days 2, 3 and 1, respectively. None of these samples yielded positive results.
[d]All patients with influenza were receiving antiviral medication, and no visitors, relatives or healthcare providers were inside the patient rooms during sampling.

## References


[1] Zahar JR, Jolivet S, Adam H, Dananché C, Lizon J, Alfandari S, et al. [French recommendations on control measures to reduce the infectious risk in immunocompromised patients]. J Mycol Medicale 2017;27:449–56.
[2] Baurès E, Blanchard O, Mercier F, Surget E, le Cann P, Rivier A, et al. Indoor air quality in two French hospitals: measurement of chemical and microbiological contaminants. Sci Total Environ 2018;642:168–79.
[3] Hall CB. The spread of influenza and other respiratory viruses: complexities and conjectures. Clin Infect Dis Off Publ Infect Dis Soc Am 2007;45:353–9.
[4] Edwards DA, Man JC, Brand P, Katstra JP, Sommerer K, Stone HA, et al. Inhaling to mitigate exhaled bioaerosols. Proc Natl Acad Sci USA 2004;101:17383–8.
[5] World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) – The Kingdom of Saudi Arabia. Geneva: WHO; n.d. Available at: http://www.who.int/csr/don/24-february-2020-mers-saudi-arabia/en/ [last accessed June 2020].
[6] Ningthoujam R. COVID 19 can spread through breathing, talking, study estimates. Curr Med Res Pract 2020;10:132–3.
[7] Morawska L, Tang JW, Bahnfleth W, Bluyssen PM, Boerstra A, Buonanno G, et al. How can airborne transmission of COVID-19 indoors be minimised? Environ Int 2020;142:105832.
[8] World Health Organization. Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations. Geneva: WHO; n.d. Available at: https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations [last accessed May 2020].
[9] Mullooly JP, Bridges CB, Thompson WW, Chen J, Weintraub E, Jackson LA, et al. Influenza- and RSV-associated hospitalizations among adults. Vaccine 2007;25:846–55.
[10] Jansen AGSC, Sanders EaM, Hoes AW, Loon AM van, Hak E. Influenza- and respiratory syncytial virus-associated mortality and hospitalisations. Eur Respir J 2007;30:1158–66.
[11] World Health Organization. Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. Geneva: WHO; n.d. Available at: https://www.who.int/csr/bioriskreduction/infection_control/publication/en/ [last accessed April 2019].
[12] World Health Organization. Influenza. Geneva: WHO; n.d. Available at: http://www.who.int/biologicals/vaccines/influenza/en/ [last accessed April 2019].
[13] Freitas ARR, Donalisio MR. Excess of mortality in adults and elderly and circulation of subtypes of influenza virus in southern Brazil. Front Immunol 2017;8:1903.
[14] Centers for Disease Prevention and Control. Disease burden of influenza. Atlanta, GA: CDC; 2019. Available at: https://www.cdc.gov/flu/about/burden/index.html [last accessed April 2019].
[15] Centers for Disease Prevention and Control. 2009 H1N1 flu. Atlanta, GA: CDC; n.d. Available at: https://www.cdc.gov/h1n1flu/ [last accessed April 2019].
[16] Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010;375:1545–55.
[17] Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017;390:946–58.
[18] Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005;352:1749–59.
[19] Lee N, Lui GCY, Wong KT, Li TCM, Tse ECM, Chan JYC, et al. High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. Clin Infect Dis Off Publ Infect Dis Soc Am 2013;57:1069–77.
[20] Volling C, Hassan K, Mazzulli T, Green K, Al-Den A, Hunter P, et al. Respiratory syncytial virus infection-associated hospitalization in adults: a retrospective cohort study. BMC Infect Dis 2014;14:665.
[21] Álvarez-Lerma F, Marín-Corral J, Vilà C, Masclans JR, Loeches IM, Barbadillo S, et al. Characteristics of patients with hospital-acquired influenza A (H1N1)pdm09 virus admitted to the intensive care unit. J Hosp Infect 2017;95:200–6.
[22] Pan Y, Wang Q, Yang P, Zhang L, Wu S, Zhang Y, et al. Influenza vaccination in preventing outbreaks in schools: a long-term ecological overview. Vaccine 2017;35:7133–8.
[23] Wang Y, Chen L, Yu J, Pang Y, Zhang J, Zhang T, et al. The effectiveness of influenza vaccination among nursery school children in China during the 2016/17 influenza season. Vaccine 2018;36:2456–61.
[24] Haber N. Respiratory syncytial virus infection in elderly adults. Med Mal Infect 2018;48:377–82.
[25] Gerretsen HE, Sande CJ. Development of respiratory syncytial virus (RSV) vaccines for infants. J Infect 2017;74(Suppl. 1):S143–6.
[26] Kestler M, Muñoz P, Mateos M, Adrados D, Bouza E. Respiratory syncytial virus burden among adults during flu season: an underestimated pathology. J Hosp Infect 2018;100:463–8.
[27] Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. Clin Infect Dis Off Publ Infect Dis Soc Am 2003;37:1094–101.

[28] Fabian P, McDevitt JJ, DeHaan WH, Fung ROP, Cowling BJ, Chan KH, et al. Influenza virus in human exhaled breath: an observational study. PLoS One 2008;3:e2691.
[29] Centers for Disease Control and Prevention. Prevention strategies for seasonal influenza in healthcare settings. Atlanta, GA: CDC; 2018. Available at: https://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm [last accessed April 2019].
[30] Bischoff WE, Swett K, Leng I, Peters TR. Exposure to influenza virus aerosols during routine patient care. J Infect Dis 2013;207:1037–46.
[31] Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis 2006;12:1657–62.
[32] Belser JA, Maines TR, Tumpey TM, Katz JM. Influenza A virus transmission: contributing factors and clinical implications. Expert Rev Mol Med 2010;12:e39.
[33] Blachere FM, Lindsley WG, Pearce TA, Anderson SE, Fisher M, Khakoo R, et al. Measurement of airborne influenza virus in a hospital emergency department. Clin Infect Dis Off Publ Infect Dis Soc Am 2009;48:438–40.
[34] Oguma T, Saito R, Masaki H, Hoshino K, Zaraket H, Suzuki Y, et al. Molecular characteristics of outbreaks of nosocomial infection with influenza A/H3N2 virus variants. Infect Control Hosp Epidemiol 2011;32:267–75.
[35] Cunha BA. Viral influenza-like illnesses: dynamic interrelationships during the 2015–2016 influenza season in hospitalized patients. J Hosp Infect 2017;95:275–9.
[36] Leung NHL, Zhou J, Chu DKW, Yu H, Lindsley WG, Beezhold DH, et al. Quantification of influenza virus RNA in aerosols in patient rooms. PLoS One 2016;11:e0148669.
[37] Bont L. Nosocomial RSV infection control and outbreak management. Paediatr Respir Rev 2009;10(Suppl. 1):16–7.
[38] Enstone JE, Myles PR, Openshaw PJM, Gadd EM, Lim WS, Semple MG, et al. Nosocomial pandemic (H1N1) 2009, United Kingdom, 2009–2010. Emerg Infect Dis 2011;17:592–8.
[39] Wilkinson K, Mitchell R, Taylor G, Amihod B, Frenette C, Gravel D, et al. Laboratory-confirmed pandemic H1N1 influenza in hospitalized adults: findings from the Canadian Nosocomial Infections Surveillance Program, 2009–2010. Infect Control Hosp Epidemiol 2012;33:1043–6.
[40] Jhung MA, D'Mello T, Pérez A, Aragon D, Bennett NM, Cooper T, et al. Hospital-onset influenza hospitalizations — United States, 2010–2011. Am J Infect Control 2014;42:7–11.
[41] Bennett JV, Jarvis WR, Brachman PS. Bennett & Brachman's hospital infections. Philadelphia, PA: Lippincott Williams & Wilkins; 2007.
[42] Centers for Disease Control and Prevention. Isolation precautions. Atlanta, GA: CDC; 2020. Available at: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html [last accessed June 2020].
[43] World Health Organization. CDC protocol of real-time RTPCR for influenza A (H1N1). Geneva: WHO; n.d. Available at: https://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/ [last accessed June 2020].
[44] Wangchuk S, Thapa B, Zangmo S, Jarman RG, Bhoomiboonchoo P, Gibbons RV. Influenza surveillance from November 2008 to 2011; including pandemic influenza A(H1N1)pdm09 in Bhutan. Influenza Other Respir Viruses 2013;7:426–30.
[45] Caserta MT, Yang H, Gill SR, Holden-Wiltse J, Pryhuber G. Viral respiratory infections in preterm infants during and after hospitalization. J Pediatr 2017;182. 53–8.e3.
[46] Soudani N, Hage-Sleiman R, Karam W, Dbaibo G, Zaraket H. Ceramide suppresses influenza A virus replication in vitro. J Virol 2019;93. e00053-19.
[47] Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. Appl Environ Microbiol 2007;73:1687–96.
[48] Fennelly KP. Particle sizes of infectious aerosols: implications for infection control. Lancet Respir Med 2020;8:914–24.
[49] Morawska L, Milton DK. It is time to address airborne transmission of COVID-19. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa939.
[50] Yan J, Grantham M, Pantelic J, Mesquita PJB de, Albert B, Liu F, et al. Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. Proc Natl Acad Sci 2018;115:1081–6.
[51] Papineni RS, Rosenthal FS. The size distribution of droplets in the exhaled breath of healthy human subjects. J Aerosol Med 1997;10:105–16.
[52] Dybwad M, Skogan G, Blatny JM. Comparative testing and evaluation of nine different air samplers: end-to-end sampling efficiencies as specific performance measurements for bioaerosol applications. Aerosol Sci Technol 2014;48:282–95.
[53] Xue J, Chambers BS, Hensley SE, López CB. Propagation and characterization of influenza virus stocks that lack high levels of defective viral genomes and hemagglutinin mutations. Front Microbiol 2016;7:326.
[54] World Health Organization. Rational use of personal protective equipment for coronavirus disease (COVID-19) and considerations during severe shortages. Geneva: WHO; 2020. Available at: https://www.who.int/publications-detail-redirect/rational-use-of-personal-protective-equipment-for-coronavirus-disease-(covid-19)-and-considerations-during-severe-shortages [last accessed October 2020].
[55] World Health Organization. Infection prevention and control of epidemic- and pandemic prone acute respiratory infections in health care. Geneva: WHO; n.d. Available at: https://www.who.int/csr/bioriskreduction/infection_control/publication/en/ [last accessed October 2020].
[56] Centers for Disease Control and Prevention. Coronavirus disease 2019 (COVID-19). Atlanta, GA: CDC; 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/specific-groups/people-at-higher-risk.html [last accessed March 2020].
[57] Killingley B, Greatorex J, Digard P, Wise H, Garcia F, Varsani H, et al. The environmental deposition of influenza virus from patients infected with influenza A(H1N1)pdm09: implications for infection prevention and control. J Infect Public Health 2016;9:278–88.
[58] Centers for Disease Control and Prevention. H1N1 flu. Swine flu: general information. Atlanta, GA: CDC; n.d. Available at: https://www.cdc.gov/h1n1flu/general_info.htm. [last accessed April 2019].
[59] Kulkarni H, Smith CM, Lee DDH, Hirst RA, Easton AJ, O'Callaghan C. Evidence of respiratory syncytial virus spread by aerosol: time to revisit infection control strategies? Am J Respir Crit Care Med 2016;194:308–16.
[60] Wan G-H, Huang C-G, Chung F-F, Lin T-Y, Tsao K-C, Huang Y-C. Detection of common respiratory viruses and *Mycoplasma pneumoniae* in patient-occupied rooms in pediatric wards. Medicine 2016;95:e3014.
[61] Maksić H, Heljić S, Skokić F, Šumanović-Glamuzina D, Milošević V, Zlatanović A, et al. Predictors and incidence of hospitalization due to respiratory syncytial virus (RSV)-associated lower respiratory tract infection (LRTI) in non-prophylaxed moderate-to-late preterm infants in Bosnia and Herzegovina. Bosn J Basic Med Sci 2018;18:279–88.
[62] Rodríguez DA, Rodriguez-Martinez CE, Cárdenas AC, Quilaguy IE, Mayorga LY, Falla LM, et al. Predictors of severity and mortality in children hospitalized with respiratory syncytial virus infection in a tropical region. Pediatr Pulmonol 2014;49:269–76.
[63] Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, Lipsitch M, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 2010;201:1509–16.
[64] Ng S, Lopez R, Kuan G, Gresh L, Balmaseda A, Harris E, et al. The timeline of influenza virus shedding in children and adults in a household transmission study of influenza in Managua, Nicaragua. Pediatr Infect Dis J 2016;35:583–6.

[65] Mohammad S, Korn K, Schellhaas B, Neurath MF, Goertz RS. Clinical characteristics of influenza in season 2017/2018 in a German emergency department: a retrospective analysis. Microbiol Insights 2019;12:1178636119890302.

[66] Rodrigues Guimarães Alves V, Perosa AH, de Souza Luna LK, Cruz JS, Conte DD, Bellei N. Influenza A(H1N1)pdm09 infection and viral load analysis in patients with different clinical presentations. Mem Inst Oswaldo Cruz 2020;115:e200009.

[67] Giannella M, Alonso M, Garcia de Viedma D, Lopez Roa P, Catalán P, Padilla B, et al. Prolonged viral shedding in pandemic influenza A(H1N1): clinical significance and viral load analysis in hospitalized patients. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2011;17:1160—5.

[68] Park J-E, Ryu Y. Transmissibility and severity of influenza virus by subtype. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 2018;65:288—92.