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Short communication

Influenza virus emitted by naturally-infected hosts in a healthcare setting

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Background: The emergence of novel respiratory viruses such as avian influenza A(H7N9) virus and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) highlights the importance of understanding determinants of transmission to healthcare workers (HCWs) and the public.  
Objectives: We aim to determine the viral content of the air emitted by symptomatic inpatients or long-term care residents with laboratory-confirmed influenza virus infection (emitters), and in the breathing zones of healthcare workers who attend to them.

Design: A prospective pilot study of patients with laboratory-confirmed influenza virus infection was undertaken. Air within 1 m of the patient was sampled using a high volume air sampler. In addition, a lower volume air sampler was placed <1 m from the patient, with another >1 m from the patient. Viral RNA was recovered from the samplers and submitted for quantitative real time PCR. In addition, personal button samplers were provided to HCWs.

Results: The air emitted by 15 participants with laboratory-confirmed influenza virus infection was sampled. Of the patients infected with influenza A, viral RNA was recovered from the air emitted by 9/12 patients using the low-volume sampler; no viral RNA was detected from air emitted by patients with influenza B (n = 3). Influenza virus RNA was recovered from one HCW’s sampler.

Conclusions: Patients with respiratory virus infection emit virus into the air which disperses to >1 m and may reach the breathing zone of a HCW. This pilot study highlights the feasibility and importance of conducting a larger-scale study to identify determinants of exposure and transmission from patient to HCW.

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1. Background

Seasonal circulation of influenza virus is responsible for significant morbidity and mortality, despite preventative programs and practices. In addition, the emergence of novel respiratory viruses such as the Middle East Respiratory Syndrome coronavirus (MERS-CoV) [1] highlights the importance of identifying means to control potentially infectious bioaerosols to reduce risks to healthcare personnel and dissemination within healthcare facilities. Limiting spread of viral pathogens is dependent upon our understanding of key determinants for transmission. A substantial body of experimental data has been generated [2,3], including the identification of avian influenza A H5N1 viral determinants for mammalian transmission [4,5]. However, a paucity of clinical studies address outstanding questions on the risk of exposure of healthcare workers to pathogens via the respiratory route. Investigators at the National Institute for Occupational Safety and Health (NIOSH) have recovered influenza virus and respiratory syncytial virus (RSV) RNA from small (<4 μm) particles in acute care outpatient environments.

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Bischoff et al. sampled air emitted from individuals with laboratory-confirmed influenza in the hospital setting, demonstrating the presence of viral RNA in droplet nuclei recovered over 1 m from the patient [8]. These studies underscore the potential insights that may be gained from more extensive studies.

2. Objectives

We aimed to detect aerosolized viral RNA emitted by patients with laboratory-confirmed influenza virus infection, and pilot breathing zone air sampling of healthcare workers directly involved in the care of these patients.

3. Study design

3.1. Participants

Patients are routinely screened for symptoms of respiratory infection (fever and cough or sore throat) upon admission to acute care units, and a surveillance program is in place to monitor long-term care residents. Mid-turbinate (MT) swabs from symptomatic individuals are submitted to the clinical microbiology laboratory where they are run using either ResPlex II (Qiagen, Hilden, Germany) or xTAG RVP FAST v2 (Luminex, Toronto, Canada) respiratory virus panels. Between July 2013 and April 2014, a convenience sample of patients with positive MT swabs was identified through daily review of respiratory virus testing results. Inclusion criteria for emitters included positive respiratory panel PCR for influenza virus, median fluorescence intensity fluorescence intensity of >500, <48 h since the MT swab was obtained, and ability to consent. Retrospective patient chart reviews were undertaken.

Healthcare workers (HCWs) providing direct patient care to individuals with laboratory-confirmed influenza virus infection were approached beginning in 2014. HCW participants were excluded if they reported upper respiratory tract symptoms, and MT swabs were obtained to exclude contamination from asymptomatic shedding. This study was conducted with the approval of the Sunnybrook Human Research Protections Program’s Research Ethics Board.

3.2. Air sampling

Area air samples were collected on a 1.0 μm, 37 mm, polytetrafluoroethylene (PTFE) membrane filter housed in 3-piece opened cassette (SKC Inc. PA, USA) and attached to a stationary battery-powered pump at a flow rate of 4 L/min (SKC Inc. PA, USA) at a distance of 0.5–1 m and 1.1–1.5 m for 2 h from patients with laboratory-confirmed influenza virus, and in a subset of cases, outside participant rooms. Viral RNA was eluted by vortexing for 1 min from membranes in 2 mL of viral transport medium (DMEM with BSA) for storage at −80°C. In addition, the Coriolis μ (Bertin Technologies, Aix-en-Provence, France), a high volume wetted cyclone air sampler was piloted. The instrument was placed 0.5–1.0 m from the patient, and air was sampled into phosphate buffered saline with Tween at a flow rate of 250 L/min for four minutes. Samples were concentrated on Amicon centrifugal filters (Millipore, Darmstadt, Germany). Air volumes were calculated as the product of sampling time and the calibrated flow rate for both collection methods.

Personal air samples were collected in the breathing zones of HCWs via PTFE cassettes clipped to participants’ collars and attached to a portable battery-powered pump (GilAir, Sensidyne, Florida, USA). Sampling was performed at a nominal flow rate of 4 L/min for 4 h. During sampling, HCWs performed their regular duties and droplet precautions were applied according to institutional protocols.

3.3. PCR

Total RNA from 200 μL of MT and air samples was extracted using the KingFisher Flex (ThermoFisher Scientific). One-step qRT-PCR was carried out using the Superscript III Platinum One-Step qRT-PCR (Life Technologies) to quantify influenza A and influenza B, utilizing published assays developed at the CDC [9] on the ABI 7500 FAST (Applied Biosystems). Viral loads from MT swabs immersed in viral transport media and air samples were recorded as log_{10} copies/mL of media and copies/L of air sampled respectively. RNAse P was used as a reference gene for the former; absolute quantitation was used for the latter in the absence of a reference gene for air.

3.4. Statistical analysis

Statistical analysis was performed using STATA SE 10.0 (Texas, 77845 USA). Geometric mean of log viral load was used to describe patient MT and emitted viral loads and the Spearman Correlation test was used to examine the relationship between them. Spearman’s correlation coefficient factor (Rho) and p-value are reported.

4. Results

Of the 15 emitters sampled using both the Coriolis and PTFE cassettes, 12 had influenza A and 3 had influenza B. The average age was 77.0 years (range 42–98 years). Eight participants (53.3%) were female, including 1 pregnant emitter. Thirteen patients (86.7%) had co-morbidities, including diabetes, malignancy, underlying cardiopulmonary or other disorders. The average duration of admission was 6.4 (range 2–15) days. Fourteen participants (93.3%) received oseltamivir, and 13 (92.9%) of these individuals began treatment within 48 h of MT swab collection. The majority (72.2%) of air samples were obtained within 24 h of MT swab collection.

Of the 12 patients infected with influenza A, viral RNA was recovered from PTFE filters proximal to 11 patients at a distance of 0.5–1 m, 9 patients at a distance of 1.1–1.5 m distance, and in 1 of 5 cases where sampling was performed outside the room. In 2 cases, the air sampled at the 0.5–1.0 m point alone was positive; no viral RNA was recovered in one case. No viral RNA was detected from the air emitted by the 3 patients infected with influenza B. In most cases, the quantity of viral RNA found per liter of air sampled was lower for the Coriolis μ than the PTFE filter membranes (Fig. 1). Viral loads from MT swabs did not positively correlate with

![Fig. 1. Influenza virus load emitted by naturally-infected patients admitted to an acute care center.](image-url)
the amount of viral RNA recovered from air (for influenza virus at 0.5–1 m and 1.1–1.5 m, rho was 0.13 ($p = 0.68$) and −0.52 ($p = 0.08$)) respectively.

Personal sampling of HCWs’ breathing zones began midway through the influenza season. Five HCWs consented to participate, four of whom completed sampling. Influenza virus RNA was detected from one of these four HCWs’ air samples. This was not quantified since the sampling period was not equal to the exposure period, considering that HCWs continued to care for other patients. All of the baseline MT swabs from HCWs were negative.

5. Discussion

This study provides proof of principle for the detection of influenza virus from the air emitted by infected patients and the potential for HCW exposure in the workplace. Variation between emitters may be due to differences in age, symptom severity, site of replication, pre-existing immunity, and/or treatment with oseltamivir. It may also be attributable to timing of sampling and placement of the sampler relative to the bioaerosol plumes emitted. Another difference to highlight is the superior performance of PTFE membrane filters compared to the Coriolis μ in this pilot study.

This pilot work was subject to several limitations. The retrospective nature of the clinical data collection precluded accurate accounts of symptom onset and severity and of influenza vaccination status. Also, although PTFE membrane filters collect particles up to 50 μm in size at the flow rate utilized, our approach did not permit finer particle sizing, which may be informative in terms of potential of dispersion and penetration into the lower airways of uninfected bystanders. In addition, detection of viral RNA in the air is not directly representative of viral infectivity and transmission potential. Finally, the limited sample size precludes far-reaching conclusions. We have, however, demonstrated the feasibility for a larger, multi-center prospective study to identify determinants of HCW viral aerosol exposure.

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Conflict interest

The authors have no conflicts of interest to declare.

Ethical approval

Sunnybrook Research Institute Research Ethics Board approval was obtained, project identification number 129-2012.

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