MAJOR ARTICLE

Bioaerosol Sampling in Clinical Settings: A Promising, Noninvasive Approach for Detecting Respiratory Viruses

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Background. Seeking a noninvasive method to conduct surveillance for respiratory pathogens, we sought to examine the usefulness of 2 types of off-the-shelf aerosol samplers to detect respiratory viruses in Singapore.

Methods. In this pilot study, we ran the aerosol samplers several times each week with patients present in the patient waiting areas at 3 primary health clinics during the months of April and May 2016. We used a SKC BioSampler with a BioLite Air Sampling Pump (run for 60 min at 8 L/min) and SKC AirChek TOUCH personal air samplers with polytetrafluoroethylene Teflon filter cassettes (run for 180 min at 5 L/min). The aerosol specimens and controls were studied with molecular assays for influenza A virus, influenza B virus, adenoviruses, and coronaviruses.

Results. Overall, 16 (33.3%) of the 48 specimens indicated evidence of at least 1 respiratory pathogen, with 1 (2%) positive for influenza A virus, 3 (6%) positive for influenza B virus, and 12 (25%) positive for adenovirus.

Conclusions. Although we were not able to correlate molecular detection with individual patient illness, patients with common acute respiratory illnesses were present during the samplings. Combined with molecular assays, it would suggest that aerosol sampling has potential as a noninvasive method for novel respiratory virus detection in clinical settings.

Keywords. adenoviruses; bioaerosol; epidemiology; influenza viruses; respiratory viruses.

Although much of human-to-human respiratory virus transmission occurs through direct contact (personal or fomite), there is increasing evidence that respiratory viruses may be transmitted in air via large or small respiratory droplets at distances greater than previous anticipated [1–6]. In recent studies, researchers at the University of Minnesota, Ohio State University, University of Georgia, and Wake Forest University have pioneered the use of novel bioaerosol sampling techniques in US agricultural and clinical settings [5–8]. We too have found these techniques useful in detecting respiratory viruses in agricultural settings in China [9, 10]. In combination with molecular techniques, bioaerosol sampling can advance the detection efforts of novel airborne respiratory viruses soon after they are introduced into a new population. Our overall goal is to adapt bioaerosol sampling methods as a noninvasive measure for detecting novel emerging respiratory pathogen (human and animal-reservoired coronaviruses, influenza viruses, adenoviruses, and enteroviruses). In this pilot study, we sought to evaluate the use of 2 off-the-shelf aerosol samplers in various clinical settings in Singapore, a major transportation hub in Southeast Asia.

METHODS

Sampling Site Selection

In this pilot study, we gained permission from collaborating institutions to study patient waiting areas in the emergency department of a busy pediatric hospital, a busy public primary care clinic, and a private primary care clinic. Study approval was obtained from the Centralized Institutional Review Board (CIRB Ref: 2015/3044). Following the advice of host medical staff at each medical facility, we selected sampling locations where patient congregation would be most dense and prolonged. This often involved triage areas, consultation waiting areas, or pharmacy waiting areas. We ran 2 types of aerosol samplers during busy patient activity periods (morning and afternoon sessions) 1 day each week for 4 weeks.

Bioaerosol Sampling

We compared 2 types of aerosol samplers in these clinical settings: (1) The SKC BioSampler is an efficient airborne particle collection device that is able to collect viable viruses over a 20–30 minute period; (2) The SKC AirChek TOUCH Personal Sampler is a small, portable sampling device that can be used to collect nonviable viral particles over a several hour period.

SKC BioSampler

We followed our previous published methods [9], using a SKC BioSampler (modified liquid impinger) with a BioLite Air Sampling Pump set to a calibrated flow rate of 8 L/min. Each BioSampler collection vessel was prefilled with 15 mL sterile virus collection medium (phosphate-buffered saline [PBS] with 0.5% w/v bovine serum albumin [BSA] fraction V) and placed at a height of 1.5 meters from the floor. BioSamplers were run for 60 minutes to achieve a total sampled air volume of 480 liters.

Personal Sampler

Two portable AirChek TOUCH personal air samplers (SKC Inc., Eighty Four, PA) were concomitantly run with the BioSampler (separate areas) using preloaded 3-part polytetrafluoroethylene (PTFE) Teflon filter cassettes (SKC Inc.). The filters were prewetted with 100 µL isopropanol to reduce hydrophobicity before use. The filter cassette holder was set at the position of 1.5-m height from the floor. The pump was set at a flow rate of 5 L/min for 180 minutes to allow approximately 900 liters of total air collection for each sample. After the sampling period was completed, each filter was removed from the cassette and swabbed with a flocked swab prewetted with sterile virus collection medium (PBS with 0.5% w/v BSA fraction V). Swabs from 2 personal samplers were then extruded into one 15-mL of sterile virus collection medium and kept on ice prior to transportation back to the laboratory.

HOBO Data Logger

A HOBO U12 Data Logger (Onset Computer Corp., Bourne, MA) was used to collect air temperature and relative humidity data at each sampling site. The devices were set to take readings at 1-minute intervals during each bioaerosol sampling period.

Sample Processing

All bioaerosol samples were transported on wet ice to the Duke-NUS Laboratory of One Health at the end of each sampling day for processing. At the laboratory, samples were concentrated to 500 µL by ultrafiltration using Amicon Ultra-15 filter units (Merck, Darmstadt, Germany), aliquoted and stored at −80°C until molecular testing and virus isolation could be conducted.

Molecular Assays

In this pilot work, we adapted previously published primers and probes to previously recognized human viruses. Later, we will expand this work to also detect similar animal reservoired viruses.

Influenza A/B Virus

Viral ribonucleic acid (RNA) was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Inc., Valencia, CA) and then assessed with real-time, reverse-transcription polymerase chain reaction (qRT-PCR) using the SuperScript III Platinum One-Step RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Inc., Waltham, MA) with World Health Organization primers and probes for influenza A [11] and the published primers and probe sequences for influenza B detection [12].

For influenza A- and influenza B-positive specimens, 200 µL of concentrated positive samples were inoculated into MDCK cells with Dulbecco's modified Eagle's medium (DMEM) containing 100 U/mL penicillin, 100 µg/mL streptomycin, 0.2% (w/v) BSA, 25 mM HEPES buffer, and 1 µg/mL TPCK-trypsin, and incubated at 37°C for 7 days (daily checks for Cytopathic effect [CPE] were made).

Coronavirus

Extracted viral RNA was tested for coronavirus by RT-PCR using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Inc.) and pan-coronavirus universal primers that target the polymerase gene [13].

Adenovirus

Viral deoxyribonucleic acid (DNA) was extracted using a QIAamp DNA Blood Mini Kit (QIAGEN, Inc.) and tested with a real-time PCR (qPCR) adenovirus molecular assay [14] using a QuantiNova Probe PCR kit (QIAGEN, Inc.). Adenovirus-positive specimens were subtyped using gel-based screening PCR targeting the hexon gene [15].

Positive PCR bands were purified using a QIAquick PCR Purification kit (QIAGEN, Inc.) before being submitted to a local sequencing company (AITbiotech Pte Ltd, Singapore) for Sanger sequencing. For adenovirus-positive samples, 200 µL of concentrated positive samples were inoculated into A549 cells with DMEM 2% (v/v) fetal bovine serum and incubated at 37°C for 7 days (daily checks were made for CPE).

RESULTS

During the sampling period, a total of 24 BioSampler and 24 PTFE filter-based cassette air samplings were collected (Table 1). Overall, 16 (33.3%) of the 48 specimens indicated evidence of at least 1 respiratory pathogen (Table 1), with 1 (2%) positive for influenza A virus, 3 (6%) positive for influenza B virus, and 12 (25%) positive for adenovirus. One of the 12 adenovirus-positive specimens was successfully studied for partial hexon sequencing and found to be a human adenovirus type B3. Based on the top BLASTN hit (http://blast.ncbi.nlm.nih.gov), the partial hexon sequence of this adenovirus-positive specimen was most similar (100% nucleotide identity) to human adenovirus 3 isolate GZ_31_2011 (accession no. KR090803). None of the 48 samples were positive for coronavirus.

The public hospital yielded the most positive specimens. Among 16 samples collected, 1 (6%) was positive for influenza A virus, 2 (13%) were positive for influenza B virus, and 7 (44%)

Table 1.   Aerosol Sampling Data and Molecular Assays Results at Three Medical Sites in Singapore, April–May 2016

Sample ID	Sample Type	Location	Temp (°C)	RH (%)	FluA	FluB	AdV	CoV
Hosp-week1	Bio Sampler A	Emergency ward	24.72	57.51	–	–	–	–
Hosp-week1	Personal Sampler A	Emergency ward	24.53	56.86	–	+	–	–
Hosp-week1	Bio Sampler B	Emergency ward	25.65	55.44	–	–	–	–
Hosp-week1	Personal Sampler B	Emergency ward	25.52	55.71	–	+	–	–
Hosp-week2	Bio Sampler A	Emergency ward	23.87	61.78	–	–	–	–
Hosp-week2	Personal Sampler A	Emergency ward	23.94	61.42	–	–	+	–
Hosp-week2	Bio Sampler B	Emergency ward	24.45	57.73	–	–	+	–
Hosp-week2	Personal Sampler B	Emergency ward	24.38	58.55	–	–	+	–
Hosp-week3	Bio Sampler A	Emergency ward	24.04	60.40	–	–	+	–
Hosp-week3	Personal Sampler A	Emergency ward	23.98	59.92	–	–	+	–
Hosp-week3	Bio Sampler B	Emergency ward	25.08	58.56	–	–	+	–
Hosp-week3	Personal Sampler B	Emergency ward	24.67	58.57	+	–	+	–
Hosp-week4	Bio Sampler A	Emergency ward	23.39	60.10	–	–	–	–
Hosp-week4	Personal Sampler A	Emergency ward	23.29	59.54	–	–	–	–
Hosp-week4	Bio Sampler B	Emergency ward	24.57	60.08	–	–	–	–
Hosp-week4	Personal Sampler B	Emergency ward	23.99	59.38	–	–	–	–
Public clinic-week1	Bio Sampler A	Registration	27.46	53.75	–	–	–	–
Public clinic-week1	Personal Sampler A	Registration	26.37	55.18	–	–	–	–
Public clinic-week1	Bio Sampler B	Registration	25.62	57.08	–	–	–	–
Public clinic-week1	Personal Sampler B	Registration	25.65	57.20	–	–	–	–
Public clinic-week2	Bio Sampler A	Pharmacy	24.66	63.21	–	–	–	–
Public clinic-week2	Personal Sampler A	Pharmacy	24.78	62.88	–	–	–	–
Public clinic-week2	Bio Sampler B	Pharmacy	25.30	67.06	–	–	–	–
Public clinic-week2	Personal Sampler B	Pharmacy	25.21	67.73	–	–	–	–
Public clinic-week3	Bio Sampler A	Registration	25.70	57.92	–	–	–	–
Public clinic-week3	Personal Sampler A	Registration	25.68	58.20	–	–	–	–
Public clinic-week3	Bio Sampler B	Registration	25.82	57.17	–	–	–	–
Public clinic-week3	Personal Sampler B	Registration	25.65	57.25	–	–	+	–
Public clinic-week4	Bio Sampler A	Pharmacy	25.13	63.90	–	–	–	–
Public clinic-week4	Personal Sampler A	Pharmacy	25.16	64.44	–	–	–	–
Public clinic-week4	Bio Sampler B	Pharmacy	25.19	65.73	–	–	–	–
Public clinic-week4	Personal Sampler B	Pharmacy	25.27	66.06	–	–	–	–
Private clinic-week1	Bio Sampler A	Waiting area	25.52	55.14	–	–	–	–
Private clinic-week1	Personal Sampler A	Waiting area	25.54	55.36	–	–	–	–
Private clinic-week1	Bio Sampler B	Waiting area	25.42	55.02	–	–	–	–
Private clinic-week1	Personal Sampler B	Waiting area	25.40	54.60	–	–	–	–
Private clinic-week2	Bio Sampler A	Waiting area	26.11	54.09	–	–	–	–
Private clinic-week2	Personal Sampler A	Waiting area	26.26	55.33	–	–	–	–
Private clinic-week2	Bio Sampler B	Waiting area	26.33	55.85	–	–	–	–
Private clinic-week2	Personal Sampler B	Waiting area	26.12	54.77	–	–	–	–
Private clinic-week3	Bio Sampler A	Waiting area	25.88	56.33	–	–	–	–
Private clinic-week3	Personal Sampler A	Waiting area	25.85	56.66	–	–	+	–
Private clinic-week3	Bio Sampler B	Waiting area	25.46	54.15	–	–	–	–
Private clinic-week3	Personal Sampler B	Waiting area	25.57	54.56	–	–	+	–
Private clinic-week4	Bio Sampler A	Waiting area	25.69	56.48	–	–	+	–
Private clinic-week4	Personal Sampler A	Waiting area	25.85	57.09	–	+	–	–
Private clinic-week4	Bio Sampler B	Waiting area	25.20	56.01	–	–	–	–
Private clinic-week4	Personal Sampler B	Waiting area	25.27	54.65	–	–	+	–

Abbreviations: AdV, adenovirus; CoV, coronavirus; Hosp; hospital; Flu A, influenza A virus; Flu B, influenza B virus; ID, identification; Temp, average temperature; RH, relative humidity.

were positive for adenovirus. The public primary care clinic yielded only 1 (6%) positive adenovirus specimen. The private primary care clinic yielded 1 (6%) influenza B-positive and 3 (18%) adenovirus-positive specimens. None of the specimens cultured on MDCK cells were observed to have amplified viable virus. Among the 12 adenovirus-positive specimens, 1 yielded a hexon gene band, which when sequenced was identified as adenovirus type B3. No viable virus was observed for specimens cultured on A549 cells.

Among the 24 samples collected using the BioSampler, 4 (16.67%) were positive for adenovirus. The Personal Sampler demonstrated higher rates of molecular detection with 1 (4%)

influenza A-positive, 3 (12.5%) influenza B-positive, and 8 (33.33%) adenovirus-positive specimens identified.

During the sampling periods, air temperatures ranged from 23.29°C to 27.46°C and the relative humidity (RH) ranged from 53.75% to 67.73% (Table 1). Although the positive samples were sparse, there was no statistically significant difference between the mean air temperature or mean RH between groups of samples that were test-positive or test-negative for any virus, influenza A virus, influenza B virus, or adenovirus.

DISCUSSION

Although the study of individual patient swab specimens is considered the gold standard for clinical respiratory virus detection and characterization, several recent publications have demonstrated that aerosol sampling may also play an important role in understanding viral transmission in a number of field settings. Aerosol studies have clearly documented the presence of influenza A and other viruses in swine and poultry farms [5, 9, 16]. Additional studies have documented the value of aerosol sampling in various human settings. Some of these aerosol sampling data imply that close human-human contact may not be necessary for transmission. In a 2009 report, Blachere et al [17] found 53% of air samples collected in the US hospital's emergency department were positive for influenza virus. In a 2012 report, Wan et al [1] documented molecular evidence of adenovirus (18%) or *Mycoplasma pneumoniae* (46%) in aerosol samples obtained in a Taiwanese pediatric hospital. In a 2013 report, Bischoff et al [6] found molecular evidence of influenza within 1.8 meters of hospitalized patients, suggesting that healthcare professionals might be exposed to infectious small-particle aerosols. In 2016 reports, Grayson [7], Lednicky [8], and Coleman [18] have similarly found molecular evidence of respiratory viruses in medical, apartment, or school settings with some samples containing viable virus [8]. Aerosol sampling has a number of advantages. In animal husbandry it can be used to explain transmission between barns or between farms [19, 20]. In the human setting, aerosol sampling involves minimal or no intrusions to patient flow and disruption of clinical care, no direct patient contact, and no requirement for informed consent. In contrast to individual patient studies, a single aerosol specimen collected over 30 minutes to 3 hours can represent a low-cost way to study multiple patients with a goal of informing healthcare officials when a novel respiratory virus has entered or become established in a healthcare setting. This approach could be particularly useful in settings where large numbers of people congregate or are in transit. Modern examples of novel respiratory virus introductions include novel zoonotic influenza A viruses and Middle East Respiratory Syndrome virus. Aerosol sampling has already been demonstrated in detecting novel H5 influenza A viruses in live animal markets in both China and the United States [10, 21].

In general, our findings were biologically understandable. We found a higher prevalence of positive samples in the public health hospital, which arguably had the most patients, more acutely ill patients with symptoms, and longer patient wait times. We also observed a higher respiratory virus detection rate using the Personal Sampler. It is possible this increased rate was due to the longer run period or that specimens were combined, resulting in a higher concentration of target nucleic acid. Finally, our finding of a higher prevalence of adenovirus compared with the other 3 viruses tested for could be explained by the relative environmental stability of adenoviruses (double-stranded DNA viruses) compared with influenza viruses and coronaviruses (RNA viruses).

This pilot study was limited in that we could not link specific aerosol results with individual patient illness, patient densities, or populations studies, which will likely be the focus of future research. However, the 3 sites did each report patients with respiratory illness signs and symptoms during the sampling periods, suggesting that the sick or accompanying asymptomatic shedders might be the source of aerosolized virus. The site with the most virus was in an emergency department where patients are more likely to manifest acute respiratory or enteric diseases. The study is also limited in that we did not have strong culture-positive evidence of viable virus in the positive specimens. Furthermore, although not the focus of our study, we are uncertain that if viable virus was present, and if it was in high enough titer, if it could cause human-to-human infection. However, in a number of animal husbandry and human medicine settings, the detection of molecular evidence of pathogens was strongly correlated with viable virus and animal-to-animal, human-to-human, or animal-to-human respiratory transmission [9, 16, 17, 21, 22, 23].

CONCLUSIONS

In summary, we conducted a pilot bioaerosol sampling study in 3 medical facilities in Singapore with a goal of screening for influenza A and B viruses, adenoviruses, and coronaviruses among waiting patients. Over the 2-month period, a total of 48 bioaerosol samples were collected from the 3 sites. Our finding of molecular evidence of influenza A virus, influenza B virus, or adenovirus in up to 33.3% of collected samples and no such evidence in the control samples suggests that at least some of the patients were shedding virus in aerosolized respiratory droplets. As such, with aerosol sampling being noninvasive and relatively low in cost with respect to manpower and laboratory assays, one can see a role for such sampling in the surveillance for respiratory pathogens in clinical settings.

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