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Assessment of Environmental and Surgical Mask Contamination at a Student Health Center – 2012-2013 Influenza Season

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Abstract

Increased understanding of influenza transmission is critical for pandemic planning and selecting appropriate controls for healthcare personnel safety and health. The goals of this pilot study were to assess environmental contamination in different areas and at two time periods in the influenza season and to determine the feasibility of using surgical mask contamination to evaluate potential exposure to influenza virus. Bioaerosol samples were collected over 12 days (two 6-day sessions) at 12 locations within a student health center using portable two-stage bioaerosol samplers operating 8 hours each day. Surface samples were collected each morning and afternoon from common high-contact non-porous hard surfaces from rooms and locations where bioaerosol samplers were located. Surgical masks worn by participants while in contact with patients with influenza-like illness were collected. A questionnaire administered to each of the 12 participants at the end of each workday and another at the end of each workweek assessed influenza-like illness symptoms, estimated the number of influenza-like illness patient contacts, hand hygiene, and surgical mask usage. All samples were analyzed using qPCR. Over the 12 days of the study, three of the 127 (2.4%) bioaerosol samples, two of 483 (0.41%) surface samples, and zero of 54 surgical masks were positive for influenza virus. For the duration of contact that occurred with an influenza patient on any of the 12 days, nurse practitioners and physicians reported contacts with influenza-like illness patients >60 min, medical assistants reported 15–44 minutes, and administrative staff reported <30 minutes. Given the limited number of bioaerosol and surface samples positive for influenza virus in the bioaerosol and surface samples, the absence of influenza virus on the surgical masks provides inconclusive evidence for the potential to use surgical masks to assess exposure to influenza viruses. Further studies are needed to determine feasibility of this approach in assessing healthcare personnel exposures. Information learned in this study can inform future field studies on influenza transmission.

Keywords

influenza; bioaerosol; surgical mask; healthcare; pandemic; environmental sampling

INTRODUCTION

The United States has over 18 million healthcare personnel (HCP).[1] Jones and Xia[2] estimate that during a medium-sized influenza epidemic, the mean number of occupational exposures to influenza encountered by HCP would be 81.8 million annually. A systematic review and meta-analysis of the annual incidence of influenza among healthy adults and HCP found that, compared to adults working in non-healthcare settings, HCP are at significantly higher risk of influenza.[3] Another study of 2009 pandemic H1N1 infections among 70 HCP in 22 states found that 35 (50%) HCP were most likely infected within the healthcare facility. These infections likely included patient-to-HCP and HCP-to-HCP transmissions.[4] According to the Occupational Safety and Health Administration (OSHA), HCP are at high risk for exposure to novel influenza virus during a pandemic.[5] As with many healthcare resources, HCP are in high demand during an influenza pandemic; thus, protecting HCP from acquiring illness from infected patients is critical for influenza pandemic preparedness.

Influenza can be transmitted human to human via multiple pathways. Influenza transmission may occur through contact, including direct and indirect contact with an infected individual or contaminated intermediate object (fomite), exposure to droplets from the respiratory tract (≥5 micrometers (μm) in diameter), and aerosol transmission of virus (airborne particles < 5 μm in diameter).[6-8] Aerosol and contact transmission may not require direct interactions with an infected individual. Although influenza transmission is thought to occur primarily through respiratory droplets, the relative contribution of the different modes of transmission is unclear. Additional host, influenza virus, and environmental factors further affect influenza transmission pathways and whether an exposure results in symptomatic or asymptomatic infection of susceptible hosts.[9]

Increased understanding of influenza transmission is critical for pandemic planning (e.g., selection and stockpiling of appropriate interventions targeting important transmission routes) and selecting appropriate controls (e.g., non-pharmaceutical interventions) to improve HCP safety and health. Moreover, assessing the contamination of personal protective equipment (PPE) worn by HCP treating influenza patients, for which little or no published data exists, could help determine the risk associated with extended PPE use, reuse, and cleaning during pandemic influenza when supplies are scarce.

In 2011, the Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH) initiated a 5-year research project, the Respiratory Protection Effectiveness Clinical Trial (ResPECT) study, which was a collaboration among NIOSH, CDC's Division of Healthcare Quality Promotion, Veterans Health Administration, Johns Hopkins University (JHU), the University of Houston, and the University of Colorado.[10] The ResPECT study sought to measure the magnitude of the change in incidence of laboratory confirmed influenza in healthcare practitioners wearing N95 filtering facepiece

respirators compared to surgical masks (SMs). One limitation of the ResPECT study was a lack of aerosol and surface assessment of influenza contamination.

This pilot study was designed to measure influenza virus in the environment of a healthcare facility and assess occupational exposure of HCP to influenza from patients exhibiting influenza-like illness (ILI) through a field study conducted during the 2012–2013 flu season at an urban university's student health center. The student health center was also a participant in the ResPECT study. The student health center had been randomized to use SMs as part of the ResPECT study protocol for the 2012–2013 influenza season. This pilot study aimed to evaluate whether 1) environmental (aerosol and surface) samples and HCP SM samples from exam areas where suspected influenza patients were treated contained more influenza virus than the daily control room (no patient) area, 2) samples collected during the peak influenza season contained more influenza virus than post-peak samples, and 3) if SMs could be used as a surrogate sampling device in place of environmental samples to evaluate potential exposure to influenza virus. A daily exposure survey completed by participants provided information on precautions taken during potential ILI patient encounters. Information obtained from this pilot study was used to determine the design and feasibility of a subsequent larger field investigation to assess health care worker exposure to influenza virus at Johns Hopkins University Adult Emergency Department during the 2014–2015 influenza season.

METHODS

Study Participants

The National Institute for Occupational Safety and Health (NIOSH) and Johns Hopkins Bloomberg School of Public Health (JHSPH) Institutional Review Boards reviewed and approved the protocol and procedures for this pilot study. Study participants provided written informed consent and were compensated for participating. Twelve subjects, including two physicians, five nurse practitioners, two medical assistants and three administrators participated in the study. Study participants were required to be 18 years of age or older, be employed more than 24 hours per week at the Johns Hopkins Student Health and Wellness Center, and have routine contact with patients. Participants were primarily recruited from HCPs who were enrolled in the ResPECT study. However, to obtain the necessary sample size, investigators also recruited additional HCP staff not enrolled in ResPECT but likely to have contact with patients presenting with ILI.

Study Description

The Johns Hopkins Student Health and Wellness Center (student health center) serves a combined undergraduate and graduate population of approximately 8,000 students. On a typical day, HCP see 80 to 100 students. The facility includes a self-service check-in kiosk with three computers for patient check-in, two staffed check-in/check-out desks, a "well" waiting area and separate "sick" waiting area, two triage rooms, and 19 patient exam rooms. The student health center did not do confirmation testing for influenza. The official tally of influenza cases seen each day was provided by the student health center. HCP study participants were instructed to wear SMs when in close contact with ILI patients. Data

collection occurred February 3–9, 2013, and February 24–March 2, 2013. The first week of data collection occurred one week after classes resumed following the University's winter break (December 22, 2012–January 25, 2013). The student health center was closed during winter break. The first week was selected to correspond to the anticipated peak of flu season, based on historical influenza records for the region. The timing for the second week of data collection was selected to correspond to the end of the influenza season. This pilot study included three components: 1) environmental assessment using bioaerosol and surface sampling throughout the student health center to identify the presence of influenza virus, 2) participant questionnaires to assess ILI and characterize interaction with patients having ILI, and 3) collection and analysis of SMs used by participants during interactions with patients having ILI to assess potential exposure to influenza.

Environmental Assessment

Bioaerosol Sample Collection—Bioaerosol samples were collected using portable two-stage bioaerosol samplers developed by NIOSH for influenza research.[11-14] The bioaerosol samplers segregate inhalable-sized airborne particles into three size ranges (>4.0 μm, 1.0 to 4.0 μm, and <1.0 μm) in two conical tubes (15 and 1.5 mL) and on a 37 mm polytetrafluoroethylene (PTFE) filter (Fluorophore™ PTFE, 3.0 μm, Millipore Sigma, Darmstadt, Germany) housed in a nylon cassette. Air was drawn through the bioaerosol samplers at a flow rate of 3.5 liters per minute using battery operated portable air sampling pumps (XR5000, SKC Inc., PA). Flow rates were calibrated using an electronic flow meter (TSI 4046, TSI Inc., Shoreview MN).

On each of 10 full days (during two separate weeks) of environmental sampling, bioaerosol samples were collected in 12 locations distributed across six to seven exam rooms, one to two triage rooms, check-in desk, sick waiting area, well waiting area, self-service check-in kiosk, and a control room. Except for the control area, each room or location was used by patients or by HCP administering care to patients. The control area was an unused exam room. The student health center operated under limited hours and staff on Saturdays; therefore, the number of bioaerosol samplers and duration of sampling was reduced. The bioaerosol samples were collected in two exam rooms, the check-in desk, control room (week 1), and self-service check-in kiosk (week 2). The bioaerosol samplers were placed in the rooms at a height of 102–152 centimeters (40–60 inches) above the floor and within two meters (about six feet) of where patients typically were seated. The bioaerosol samplers were placed in sampling locations shortly before 8:30 a.m. (11:00 a.m. Saturdays) when the student health center opened and removed after 5:30 p.m. (5:00 p.m. Fridays and 2:00 p.m. Saturdays) at the end of the workday. Room air temperature and relative humidity were recorded using direct reading instruments (Hobo Pro Series, Onset Corp., Bourne, MA) located with each bioaerosol sampler.

At the end of each day of bioaerosol sample collection, the PTFE filters were transferred to sterile 15 mL centrifuge tubes (Becton Dickinson, Franklin Lakes, NJ). To stabilize the bioaerosol samples, 1.0 mL of sterile Hanks balanced salt solution (HBSS) (Invitrogen, Carlsbad, CA) containing 0.1% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, Missouri) was added to the 15 and 1.5 mL centrifuge tubes from the bioaerosol samplers

and to the centrifuge tube containing the PTFE filter. All tubes were then vortexed for 60 seconds. The samples were stored at −20°C prior to laboratory analysis.

Surface Sample Collection—Surface samples were collected from rooms and locations where bioaerosol samplers were located. Samples were collected each morning before patients arrived to establish baseline contamination levels and at the same location at the end of the workday to measure daily contamination levels. Surface samples were taken on common high-contact non-porous hard surfaces such as keyboards, table surfaces, computer mice, doorknobs, patient room countertops, arms of patient chairs, and sink faucet handles. Surfaces were swabbed using sterile nylon flocked swabs (Copan Diagnostics, Corona, CA) moistened with sterile HBSS containing 0.1% BSA. For large flat surfaces, a 10 cm x 10 cm template was used to swab 100 cm^2 of surface area. For non-flat surfaces, 100 cm^2 of surface area to swab was estimated or the entire surface (e.g., doorknob, sink faucet handles, pens) was swabbed if the surface area was estimated to be less than 100 cm^2. Following surface sample collection, the swabs were placed in 15 mL centrifuge tubes containing 0.5 mL of sterile HBSS containing 0.1% BSA. The centrifuge tubes containing the swabs were vortexed for 60 seconds and stored at −20°C until ready for laboratory analysis.

Field Control Samples—Negative field controls were prepared in tandem with the field samples. Bioaerosol negative control samples were prepared by processing PTFE filters and 15 and 1.5 ml conical tubes from two-stage NIOSH samplers that did not have air drawn through them. The surface negative controls samples were prepared by processing unused sterile swabs. For positive field controls, bioaerosol sample media and the surface swab media were inoculated with reference influenza virus H1N1 strain A/WS/33 (catalog number VR-825, American Type Culture Collection, Manassas, VA) in the field. Positive and negative field control samples served as indicators of field sample integrity associated with handling and shipment of the samples, and laboratory handling and analysis.

Participant Questionnaires

Daily and Weekly Surveys—At the end of each workday, study participants completed the ResPECT daily work shift exposure surveys; participants not enrolled in the ResPECT study completed the pilot study daily work shift exposure survey. Both daily surveys asked participants about experiencing ILI symptoms, estimated total daily contact time and number of interactions with patients or coworkers having respiratory or ILI symptoms, hand hygiene practices, and usage of SMs. At the end of each week, participants also completed a survey that asked about experiencing ILI and other symptoms and absence from work because of health symptoms.

The ResPECT study defined ILI as a self-reported temperature of 37.8°C or greater plus cough and/or a sore throat with or without laboratory confirmation.[10] The ResPECT study defined acute respiratory illness, a secondary outcome measure, as the occurrence of one of the following signs or two of the following symptoms without laboratory confirmation.[10] Signs included: fever (temperature >37.8 °C), rapid breathing, inflammation of the mucous membrane in the nose, or enlarged lymph nodes. Symptoms included: vomiting/nausea,

diarrhea, cough, sputum production, fatigue, malaise, headache, sore throat, difficulty breathing, chills, sweats, body aches, and other gastrointestinal symptoms.[10]

The surveys for subjects enrolled in the ResPECT study were administered electronically as per the ResPECT study protocol and provided to the pilot study team electronically. For non-ResPECT study participants in the pilot study, the daily and weekly surveys were administered using paper forms.

Personal Protective Equipment Sample Collection

Participants were asked to provide SMs that they wore for each patient exhibiting ILI. At the beginning of each day, prior to seeing any patients, participants donned a SM, wore it for 10 minutes, and then placed it into a sealable plastic bag to serve as a baseline. After seeing a patient with ILI, the participants placed their SM into a bag, using a separate bag for each new patient. Study participants were instructed to identify collection bags containing SMs that were exposed to a direct sneeze or cough during HCP-ILI patient interactions with a colored adhesive label. All SMs were stored at −20°C until analysis. A subset of used SMs (15%) were analyzed to determine if influenza virus was present on the SM surface. All SMs that were identified as having been exposed to a direct sneeze or cough, worn during the administration of an aerosol generating procedure, or worn in a room with an influenza positive air sample were analyzed.

Sample Analyses

Prior to analysis, the bioaerosol samples containing the PTFE filters and swab samples were incubated at −4°C overnight to ensure elution of the virus. Prior to analysis of the SMs, four 25 mm diameter coupons were punched from the central portion of the SM, placed together (pooled) in 8 mL of HBSS containing 0.1% BSA, vortexed for 60 seconds, and incubated overnight at −4°C to elute virus from the coupons.

RNA Isolation and cDNA Transcription—Viral RNA was isolated from the samples using the MagMax™-96 Viral RNA Isolation Kit (Ambion) as previously described.[11] The final viral RNA volume was 30 μL. Viral RNA was immediately transcribed into complimentary DNA (cDNA) using the High Capacity RNA to cDNA Kit in accordance with the manufacturer's instructions (Applied Biosystems). The final cDNA volume was 40 μL.

Real-Time qPCR Analysis—Each collected sample was analyzed by qPCR for matrix (M1) gene copies using the following matrix-specific primers: Forward 5‘ AGATGAGTCTTCTAACCGAGGTCG 3', Reverse 5‘ TGCAAAAACATCTTCA AGTCT CTG 3‘ and probe 5’ 6FAM-TCAGGCCCCCTCAAAGCCGA-MGBNFQ 3’ Primers and probes were synthesized by Applied Biosystems and used at a final concentration of 0.8 μM and 0.2 μM, respectively.[15] Reactions containing 5 μl of cDNA as template (above) were performed and analyzed using the Applied Biosystems 7500 Fast Real-Time qPCR System under the following cycling conditions: 95°C for 20 seconds followed by 45 cycles at 95°C for 3 seconds and 60°C for 30 seconds. To determine the relative gene copy number, a standard curve was generated from 10-fold serial dilutions

of the cloned influenza H1N1 matrix gene. A negative control without template was also included in all qPCR reactions. All reactions were run in duplicate. The limit of detection (LOD) of matrix copies per qPCR reaction is 10 copies and the limit of quantitation (LOQ) of matrix copies per qPCR reaction is 15 copies.

RESULTS

Environmental Assessment

Table 1 presents the daily and weekly count of environmental samples that were positive for influenza virus each week of the pilot study. Over the 12 days (in two separate weeks) of the study, three of the 127 (2.4%) bioaerosol samples collected were positive by qPCR for influenza virus. During the first week of the study influenza virus was detected in one of the 64 (1.6%) bioaerosol samples. The positive sample, taken in a patient exam room, had detectable but not quantifiable influenza in the >4 μm size fraction. Influenza virus was detected in two of the 63 (3.2%) bioaerosol samples collected during week 2. One positive sample, taken in a patient exam room, had 621 M1 gene copies in the 1–4 μm size fraction. The other positive sample, taken in the triage room, had 612 M1 gene copies in the <1 μm size fraction.

Influenza virus was detected and quantified in two of 483 (0.004%) surface samples (Table 1). A positive surface sample (599 M1 gene copies) was detected during the first week on a shelf in the triage room. A second influenza virus positive surface sample (300 M1 gene copies) was detected on a desk surface of the computer self-service check-in kiosk during the second week. That sample was collected in the morning prior to any patients checking in to the student health center. All positive field control bioaerosol samples and surface swab samples were qPCR positive for the reference influenza virus H1N1 strain (results not shown).

Table 1 also shows the daily and weekly number of ILI patients reported by the student health center. In total, 185 ILI patients visited the student health center over the 12 days of the pilot study, as reported by the center’s director. During the first 6 days (week 1) of the pilot study, the student health center reported 16–20 patients with ILI each weekday. During the second 6 days, (week 2), 11–22 patients were reported with ILI each weekday. Four patients with ILI were reported on each Saturday of the pilot study.

The average daily air temperature in the student health center was 22°C (71±2°F), and the average daily humidity was 22±4% during the first week of sampling. Similarly, the average daily air temperature was 22°C (71±4°F), and the average daily humidity was 25±2% during the second week of sampling. A review of a test and balance report from 2011 for the air-handling units serving the student health center indicated eight units served the areas where samples were collected. The percent of recirculated air provided by the eight units ranged from 64 to 90%.

Participant Questionnaires

Daily and Weekly Surveys—Figure 1 shows a summary of HCP reported cumulative daily contact with ILI patients. Nurse practitioners and physicians reported having the most

contact with ILI patients, with one nurse practitioner and one physician reporting spending a cumulative daily duration on one or more days of more than one-hour total with ILI patients. Both medical assistants reported spending a cumulative duration of contact with ILI patients ranging from 15–44 minutes in a day. Two of the three administrative staff reported their total duration of contact with ILI patients per day was usually less than 30 minutes per day. During interactions with ILI patients, all daily survey responses (n=72) from study participants reported that they were within two meters (about six feet) of the patient.

During 12 survey days, 12 participants provided 110 daily survey responses over two separate 6-day sampling sessions separated by 15 days to the question asking how often they wore a SM each day. On the survey, three of 20 physician responses (15%), eight of 43 nurse practitioner responses (19%), and three of 17 medical assistant responses (18%) indicated the participant never wore SMs and reported zero for the number of contacts with any patients or coworkers with confirmed influenza or appearing to have respiratory illness or ILI during a survey day. Figure 2 presents the percentage of these responses received from all participants by job title reported over 12 days. The three job titles providing direct patient care (medical assistants, nurse practitioners, and physicians) most frequently reported wearing SMs with some ILI patients. The administrative HCPs use of SMs differed from HCPs providing direct patient care. They most often reported wearing SMs for all patient contacts. Only 1 (5%) physician response and 5 (12%) nurse practitioner responses reported wearing SMs during all ILI patient contacts on a day they saw patients.

Study participants reported the total duration a SM was worn during a day over the 12 days of this investigation. Figure 3 presents the responses estimating the total time HCP providing direct medical care to patients wore SMs. Medical assistants never reported wearing a SM for more than 30 total minutes on any day. Total duration of daily SM wear time varied for nurse practitioners across all time intervals, but this group most often reported wearing SMs between 30 and 59 minutes (16 of 35 responses or 46%%). Physicians' total daily SM wear time varied as well but the longest time interval of wearing a SM was most frequently reported during the 12 days of this study.

Personal Protective Equipment Samples

Study participants submitted 295 SMs worn during 381 contacts with patients presenting ILI (Table 2). The median number of SMs used per day for each study participant was 2.0 (range: 0–9) during the first week and 2.5 (range: 0–10) during the second week. The mean ratio of masks used to the number of ILI patient interactions was 0.84 (range: 0.69–1.63) during the first week and 0.71 (range: 0.51–1.00) during the second week. Four of the 295 used SMs submitted were identified by the participants as having been worn in the close proximity of a direct sneeze or cough from an ILI patient. Forty-three used SMs (15% of total used SMs), including the four identified as having been worn near a sneezing or coughing ILI patient, were analyzed for influenza virus. An additional 11 SMs donned by participants at the start of each day to serve as baseline samples were included in the SM analyses. All results for the SMs analyzed using qPCR were <LOD for influenza virus (Table 2).

DISCUSSSION

The student health center documented 185 ILI patient visits during the study and 381 self-reported study participant contacts with ILI patients. While many of the participating HCPs reported contact or indirect contact with ILI patients at this student health center, most of the air and surface samples did not detect influenza virus, and none of the SM samples were positive for the virus. Only 2.3% of the full shift air samples and 0.4% of surface samples were positive for influenza virus.

The Maryland Dept. of Health and Mental Hygiene (MDHMH) reported the 2012–2013 influenza season was the most active since the 2009 H1N1 influenza pandemic and the predominant strain was influenza A (H3N2). The 2012–2013 influenza season saw two peaks of activity. The primary peak occurred during the week ending December 29, 2012, and the secondary peak occurred during the week ending March 9, 2013.[16] Because the primary peak occurred during the university's winter break and the student health center was closed during this time, this precluded sampling during the highest level of influenza activity. The second week of sampling (February 25–March 2, 2013) occurred 8 weeks after the primary peak. Because the student health center provided only outpatient ambulatory care, the number and severity of influenza illness patients seen may not fully reflect what was happening in the community. The percentage of visits for ILI in Maryland during the weeks ending February 9, 2013 (end of the first week of sampling) and March 2, 2013 (end of the second week of sampling) were 2.0 and 2.3% respectively.[17] Note that these values are at or below the national baseline value of 2.2% of outpatient visits for ILI.[18]

Bioaerosol sampling methods were consistent with work completed by other investigators sampling for influenza virus in healthcare settings.[19-23] Liu et al. characterized the potential for short-range airborne transmission of expiratory droplets using two standing thermal manikins with one serving as the source manikin positioned 0.5 to 3.0 meters from the other.[24] Bioaerosol samplers were placed within one to two meters of ILI patients, except for the waiting areas. The average air sample volumes of almost two cubic meters over 500 minutes were larger and longer than reported in previous studies in outpatient health care settings.[11,19] Results from samples collected in rooms where aerosol-generating procedures, such as nebulization, were performed were below detectable levels for influenza virus; similar findings have been previously reported.[25] Other investigators using qPCR have reported small numbers of positive bioaerosol samples in settings where influenza patients or subjects with confirmed influenza were present.[19,,26]

Nearly all surface samples were negative for influenza virus despite the potential presence of ILI patients within the student health center each day. The surfaces evaluated were high contact, non-porous surfaces which have been shown to serve as sources of influenza virus in other studies.[19,20,27,28] Although observation of surfaces for contact was not part of the study protocol, general observations were that regular student contact with surfaces of the self-check-in kiosks and contact with most other surfaces selected was likely. Although virus viability was not assessed, other studies have shown that influenza virus can persist or remain viable on surfaces for an extended period of time ranging from hours to days.[29-31] Influenza virus on surfaces may not have been found because patients suspected to

have influenza were misclassified in the absence of point-of-care confirmation testing at the student health center. Furthermore, patients reporting ILI or upper respiratory illness were asked by front desk administrators to wear a SM, which could have prevented aerosol droplet spray from reaching nearby surfaces. No information was collected for either the number of these requests or patient compliance with these requests. In addition, HCP staff cleaned surfaces at the patient check-in kiosk and desk at the end of the day. Contract housekeeping staff cleaned all the exam rooms and service areas including tables, counter tops, and flat surfaces as well all bathroom surfaces each night. These thorough cleaning and housekeeping practices may have reduced or eliminated surface virus, if present.

Other investigators have encountered varying degrees of success with surface sampling for influenza and other viruses. Bright et al.[27] sampled classroom surfaces including student desktops, faucet handles, water fountain toggle, and entrance doorknob for influenza Type A viruses. Twenty-four percent (13/54) of the samples contained influenza virus with student desktops providing the most positive results (5 of 27 desktops). Similarly, fomite swabbing was included in a human influenza challenge study by Killingley et al.[19] to assess person-to-person transmission. Nine of 48 fomite samples (19%) were PCR positive for influenza virus. An additional study by Killingley et al.[20] included 671 surface swabs collected at 39 separate locations in houses and hospital rooms. Thirty-three (4.9%) of the surface swabs detected influenza Type A(H1N1) pandemic 2009 by PCR. However, similar to our study, Tang et al.[26] were unable to detect influenza virus RNA on surfaces that were contaminated via direct cough by subjects with confirmed influenza. The PCR primers used were specific for influenza A, not influenza B that dominated the latter part of the 2012—2013 influenza season.[16]

Because of the lack of influenza virus positive aerosol and surface samples, only a small portion of SMs were analyzed. Given the absence of influenza virus in the bioaerosol and surface samples, the absence of influenza virus on the SMs was not surprising. Further, it provides inconclusive evidence for the potential to use SMs as a means to assess exposure to influenza viruses. Unfortunately, positive control samples for SMs were not included in the sampling plan. It is possible that the negative results of the SMs samples collected from HCP are a result of loss of nucleic acid integrity during sample storage, shipment, handling, and analysis. However, in-house laboratory studies conducted to optimize methods to recover and detect influenza virus on SMs using PCR showed limited loss on nucleic acid integrity under similar storage conditions (data not shown). Other studies have detected microbial contamination on masks worn in healthcare[32,33] and demonstrated that mask contamination was correlated to aerosol contamination.[32]

The overall adherence to SM use in the presence of ILI patients in this study (78%) is similar to other studies (62–100%) where healthcare personnel recognized the need to wear respiratory protective devices or SMs and where use was monitored.[34,35] The number of SMs used per participant per day, or burn rate, was roughly 2.8. The burn rate would presumably be higher during and near the peak of the influenza season and if adherence to recommended use for contacts with ILI patients approached 100%. Carias et al. estimated respirator usage of four per worker per day throughout the pandemic in an outpatient setting when usage follows the epidemic curve.[36]

Patients presenting with ILI may, in fact, have other respiratory pathogens that are common during influenza seasons. Krosche et al. reported over 70% of acute respiratory infections of healthcare workers participating in the ResPECT study for the 2011/12 through the 2014/15 influenza seasons were characterized as Coronavirus and Rhinovirus infections. Influenza type A comprised only 12% of the acute respiratory infections.[37] Although the student and healthcare populations may have dissimilar exposure environments, it is certain some of the students with ILI had illnesses from non-influenza respiratory pathogens. Future studies could expand upon the number of pathogens detected.

Because the student health center did not perform diagnostic testing for influenza, the number of ILI patients who were confirmed positive for influenza was not known to study investigators. In addition the student health center used a definition for ILI which included signs and symptoms for acute respiratory illness not included in the CDC definition for ILI. CDC's definition is a temperature ≥37.8°C and cough and/or sore throat, in the absence of a known cause other than influenza.[38] CDC does note that not all people with influenza will have a fever.[39] As a result, more patients may have been classified as having ILI than would have occurred using the CDC definition. The WHO and National Respiratory and Enteric Virus Surveillance System collaborating laboratories in the U.S. reported 23% of specimens tested for influenza during the 2012–2013 influenza season were positive.[40] Viral surveillance by these same groups for influenza seasons 2011–2012; 2013–2014; and 2014–2015 demonstrated positive influenza test results of the specimens analyzed at 13%, 17.3%, and 18.1% respectively.[41-43] Future studies would benefit from incorporating measures to determine if potential exposures to patients suspected of having influenza did have influenza.

Although the study was originally designed to compare influenza concentration in environmental samples from exam areas and control areas, and the prevalence of influenza in environmental samples at different time periods (peak vs. off-peak), the lack of sufficient influenza positive samples negated the ability to investigate these two objectives. Factors or limitations that could contribute to the absence of positive samples for influenza include: low proportion of outpatient visits for ILI during the study weeks; misclassification of ILI patients; unpredictability of influenza season peak and prevalence levels in a community; variable virus shedding status of ILI patients; compatibility of PCR analyses with circulating influenza viruses; patient compliance with wearing SMs upon entering the health center when asked to wear a SM; housekeeping and surface cleaning practices during the day and after hours; and interaction of ILI patients with study participants in the student health center versus study non-participants also working in the student health center. Further studies are needed in settings that operate throughout the influenza season to improve the chance of catching the influenza peak. In addition, placing the bioaerosol samplers on HCP while they are administering patient care may result in increased influenza detection given the close proximity of the samplers and the patients and provide a more accurate assessment of influenza exposure.

This study presents considerations that may impact efforts to evaluate environmental contamination during yearly influenza seasons. Some factors such as when the peak of an influenza season will occur and the predominant circulating influenza virus are not

known until afterwards. The same applies to the shedding status of incoming ILI patients or how many ILI patients will visit a facility during a defined time window. Practices of participating healthcare facilities may influence the types and amounts of available information on patients coming through the doors. Voluntary practices such as requesting patients with respiratory illnesses don SMs as well as patient compliance may also influence environmental findings. Variability in the participation of study subjects completing information gathering tools influences insight into work practices and study participant exposure experience. Environmental contamination from influenza virus during annual influenza season may be influenced by numerous sources, many beyond investigators' control. The logistics of conducting observational studies to evaluate environmental contamination in attempting to evaluate the role it plays in influenza transmission should not be underestimated. The continuing variability in the influenza viruses themselves as well as route of exposure may also influence the interpretability of identified environmental viral loads as far as whether a lot or a little is needed to induce illness in vulnerable individuals.

This study provided some insight into SM adherence during influenza season at an urban campus student health center. The study sought to evaluate the presence of influenza virus in an outpatient healthcare setting during influenza season. The amount of influenza virus identified to be present would help characterize the potential for HCP exposure to influenza. Extensive air and surface sampling with procedures used by other investigators revealed minimal influenza A virus in this health center environment. The sensitivity of PCR analyses used along with the number, variety, and sample duration indicated large numbers of influenza virus were not identified as anticipated. The perennial variability of seasonal influenza presents myriad challenges for field investigations studying influenza transmission. Further studies will be needed to determine the feasibility of using SMs or N95 facepiece filtering respirators to assess HCP exposure to influenza viruses.

DISCLAIMER

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

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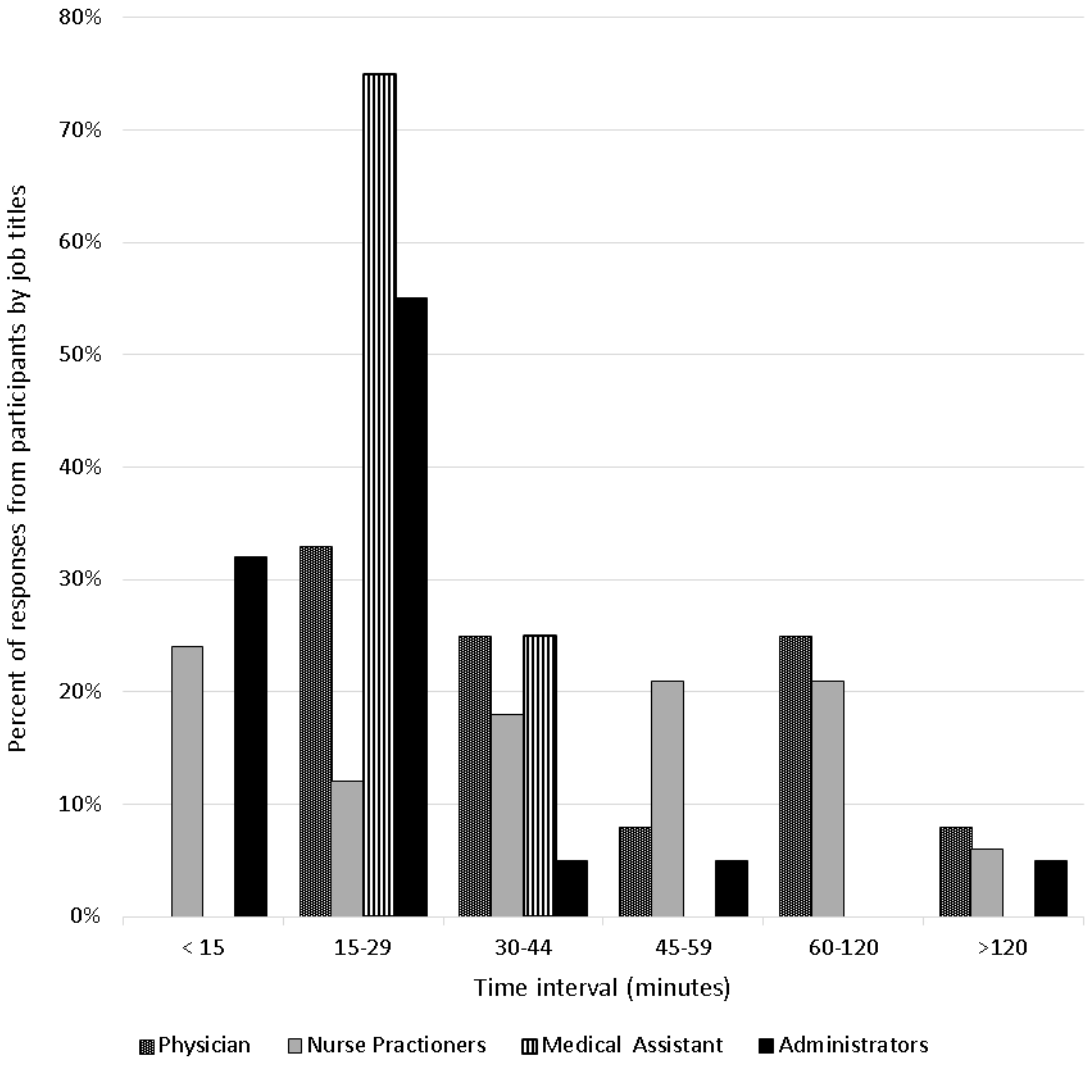

Figure 1.
Cumulative daily duration of contact with patients.

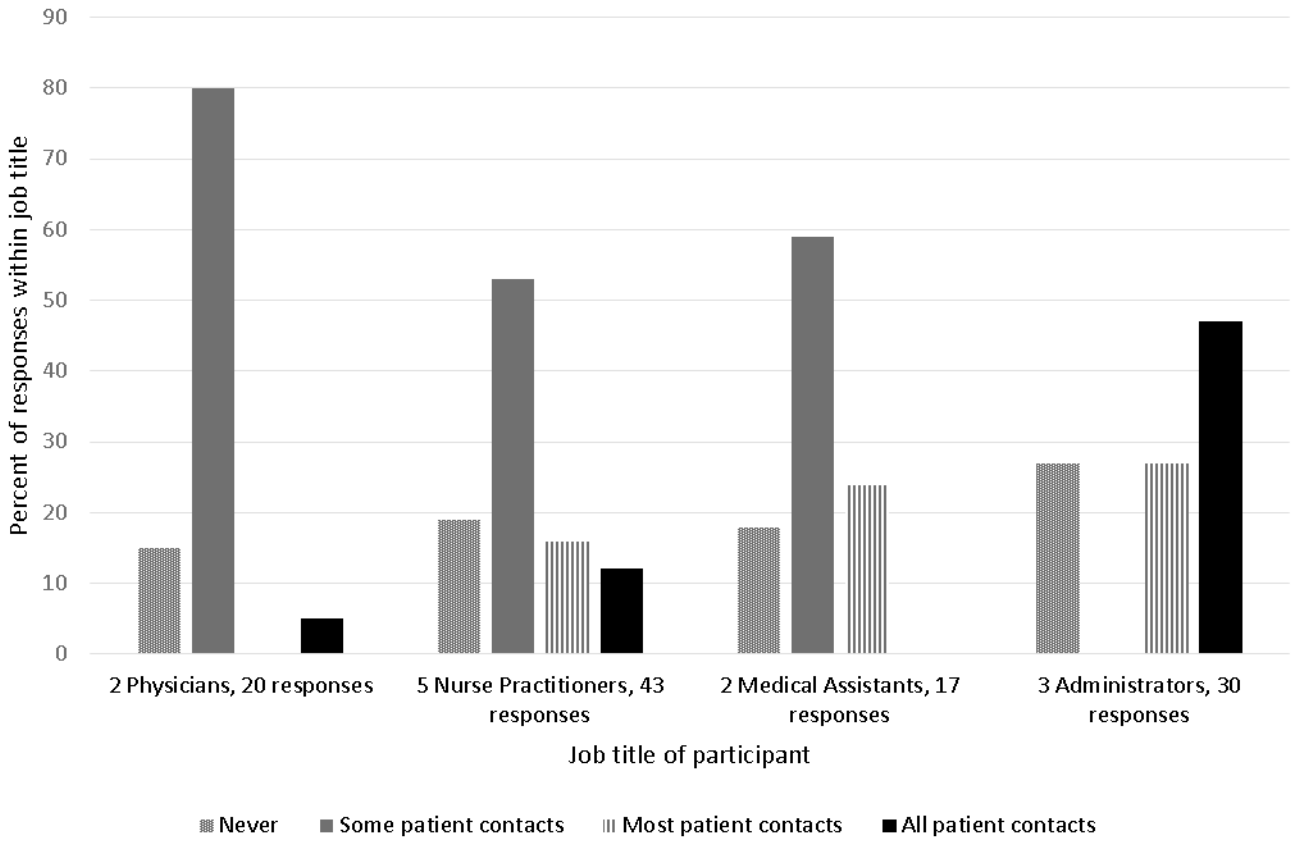

Figure 2.
Daily self-reported frequency of wearing SMs with potential ILI patients

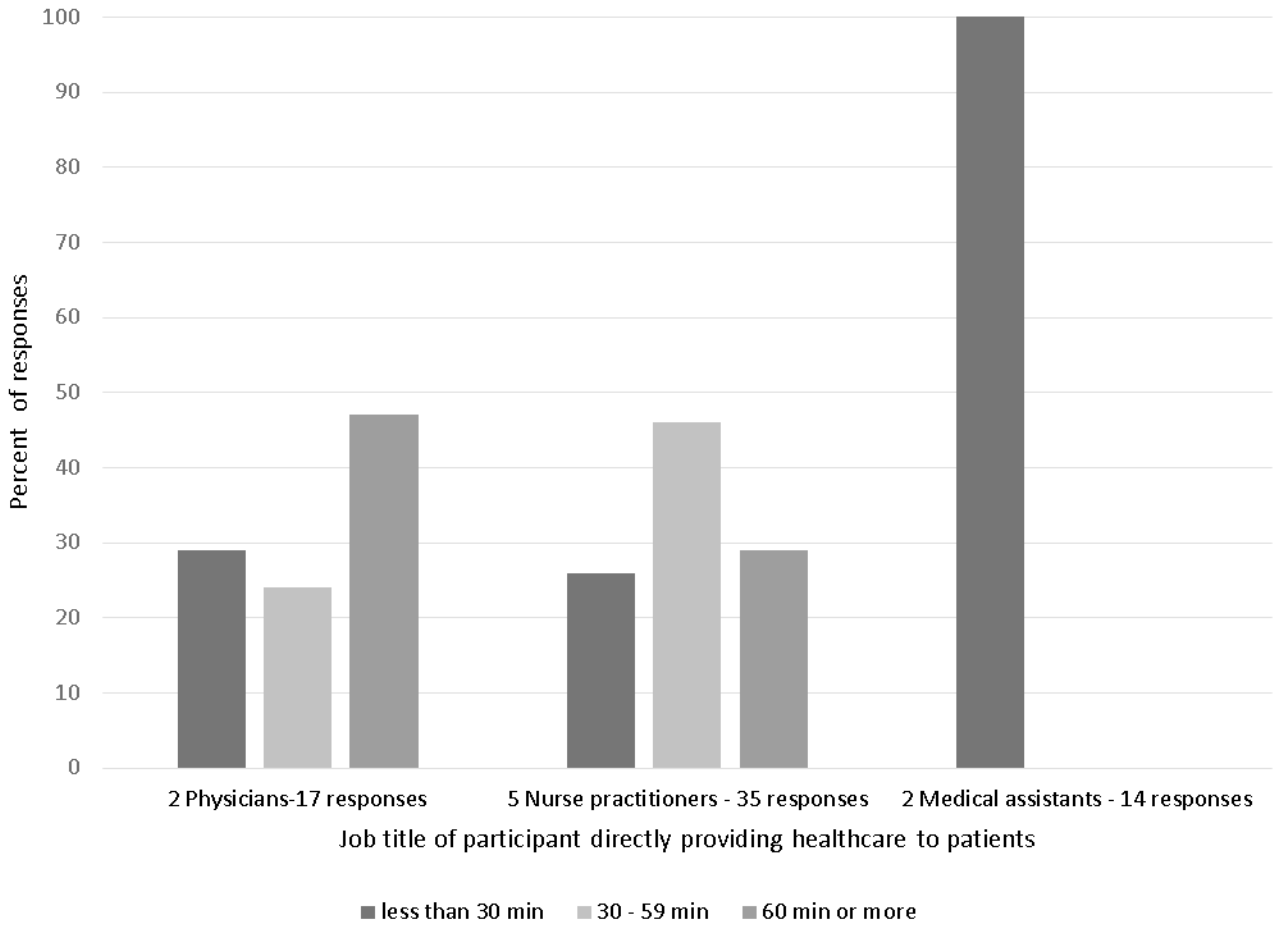

Figure 3.

Reported total duration a SM was worn over the course of a work-day by HCP providing direct patient care and reporting wearing a SM

Table 1.

Number of reported influenza-like illness (ILI) patient visits and environmental sample results, dates, setting

		Bioaerosol samples		*Surface samples*	
Date	**ILI patients reported by student health center (See note)**	**No. Samples**	**No. positive samples[A]**	**No. samples**	**No. positive samples[A]**
2/4/2013	16	12	0	51	0
2/5/2013	20	12	1[B]	45	0
2/6/2013	15	12	0	45	1[C]
2/7/2013	19	12	0	45	0
2/8/2013	16	12	0	45	0
2/9/2013	4	4	0	15	0
week 1 totals	**90**	**64**	**1**	**246**	**1**
2/25/2013	22	12	0	56	1[D]
2/26/2013	21	12	0	42	0
2/27/2013	22	12	0	45	0
2/28/2013	15	12	2[E,F]	45	0
3/1/2013	11	11	0	41	0
3/2/2013	4	4	0	8	0
week 2 totals	**95**	**63**	**2**	**237**	**1**
totals	**185**	**127**	**3**	**483**	**2**

Note: Confirmation of influenza in patients identified as cases was not available at the student health center. Numbers reported here were those provided by the student health center for each day of the study.

[A] Positive samples are the number of samples on that date that were influenza M1 qPCR positive

[B] Positive bioaerosol sample collected from exam room in the size fraction greater than 4μm

[C] Positive surface sample collected from shelf surface in triage room in the afternoon

[D] Positive surface sample collected from registration kiosk in the morning before patients arrived

[E] Positive bioaerosol sample collected from triage room in the size fraction less than 1μm

[F] Positive bioaerosol sample collected from exam room 8 in the size fraction of 1-4μm

Table 2.

Number of patient contacts and surgical masks (SMs) used

Date	Subjects	Self-reported ILI patient contacts	Used SMs Collected[A]	SMs/Subject/day	SMs/ILI patient interaction
2/4/2013	11	19	31[B]	2.8	1.63
2/5/2013	11	39	29	2.6	0.74
2/6/2013	11	33	24	2.2	0.73
2/7/2013	11	46	33	3.0	0.72
2/8/2013	10	29	20[B]	2.0	0.69
2/9/2013	2	8	10	5.0	1.25
Week 1	**56**	**174**	**147**	**2.6**	**0.84**
2/25/2013	10	45	37[B]	3.7	0.82
2/26/2013	9	40	35[B]	3.9	0.88
2/27/2013	11	53	30	2.7	0.57
2/28/2013	9	41	21	2.3	0.51
3/1/2013	9	17	14	1.6	0.82
3/2/2013	2	11	11	5.5	1.00
Week 2	**50**	**207**	**148**	**3.0**	**0.71**
Totals	**106**	**381**	**295**	**2.8**	**0.78**

[A] total of 43 masks, including the four worn in close proximity to a patient sneeze, were analyzed for influenza and determined to be nondetectable (<10 M1 gene copies)

[B] On each of these days, 1 mask (4 masks total for all days) was submitted with a sticker indicating that it was used in close proximity to a patient sneeze or cough