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Assessment of influenza virus exposure and recovery from contaminated surgical masks and N95 respirators

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ABSTRACT

Healthcare workers (HCWs) are at significantly higher risk of exposure to influenza virus during seasonal epidemics and global pandemics. During the 2009 influenza pandemic, some healthcare organizations recommended that HCWs wear respiratory protection such as filtering facepiece respirators, while others indicated that facemasks such as surgical masks (SMs) were sufficient. To assess the level of exposure a HCW may possibly encounter, the aim of this study was to (1.) evaluate if SMs and N95 respirators can serve as "personal bioaerosol samplers" for influenza virus and (2.) determine if SMs and N95 respirators contaminated by influenza laden aerosols can serve as a source of infectious virus for indirect contact transmission. This effort is part of a National Institute for Occupational Safety and Health 5-year multidisciplinary study to determine the routes of influenza transmission in healthcare settings. A coughing simulator was programmed to cough aerosol particles containing influenza virus over a wide concentration range into an aerosol exposure simulation chamber virus/L of exam room air), and a breathing simulator was used to collect virus on either a SM or N95 respirator. Extraction buffers containing nonionic and anionic detergents as well as various protein additives were used to recover influenza virus from the masks and respirators. The inclusion of 0.1% SDS resulted in maximal influenza RNA recovery (41.3%) but with a complete loss of infectivity whereas inclusion of 0.1% bovine serum albumin resulted in reduced RNA recovery (6.8%) but maximal retention of virus infectivity (17.9%). Our results show that a HCW's potential exposure to airborne influenza virus can be assessed in part through analysis of their SMs and N95 respirators, which can effectively serve as personal bioaerosol samplers.

1. Introduction

Genetic and environmental factors are constantly influencing the transmissibility and infectivity of influenza viruses. As a result, millions of people worldwide are at risk of developing an acute viral infection, and seasonal epidemics as well as global pandemics continue to cause significant morbidity and mortality. The CDC estimates that 9.2–35.6 million influenza illnesses and 12,000–15,000 deaths in the United States have occurred annually since 2010 (CDC, 2017. While vaccination is considered one of the first lines of defense against influenza virus, vaccines may not be immediately available during an outbreak of a novel influenza virus. A better understanding of influenza exposure and transmission is needed to determine the best interventions to avoid the spread of this virus.

Current literature shows that transmission occurs through direct and indirect contact with infectious respiratory secretions (Brankston et al., 2007; Killingley and Nguyen-Van-Tam, 2013; Tellier, 2009; Weber and Stilianakis, 2008) and growing experimental evidence indicates that influenza viruses are transmitted through airborne respiratory particles (Bischoff et al., 2013; Blachere et al., 2009; Lednicky and Loeb, 2013; Leung et al., 2016; Lindsley et al., 2010a; Thompson et al., 2013; Tseng et al., 2010; Yang et al., 2011). Engineering and administrative controls are important in mitigating the spread of infectious diseases. However, transmission-based precautions such as hand washing and the use of personal protective equipment (PPE) including gloves, gowns and masks, also play a major role in protecting healthcare workers and

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preventing healthcare-associated infections. Although respiratory PPE greatly limits exposure to airborne particles, recommendations for PPE usage vary and depend on the application. To reduce exposure to seasonal influenza, the Centers for Disease Control and Prevention (CDC) recommends that HCWs wear SMs during routine patient care and respiratory protection such as N95 respirators while performing aerosol-generating procedures (CDC, 2009). Surgical masks offer limited protection against infectious bioaerosols, yet effectively protect healthcare workers from contact with large particles and are frequently worn to prevent contamination of sterile environments. In comparison, filtering facepiece respirators such as N95 respirators are designed to filter infectious airborne contaminants but healthcare workers often find them to be less comfortable than facemasks, and they must be fit-tested to ensure effective protection. Several laboratory studies have shown that N95 respirators are nearly completely effective at blocking infectious influenza bioaerosols but SMs are not (Bischoff et al., 2013; Harnish et al., 2013; Janssen et al., 2013; Makison Booth et al., 2013; Noti et al., 2012).

Studies investigating the incidence of influenza among HCWs suggest that healthcare employees are at high risk for exposure particularly during an influenza pandemic (Kuster et al., 2011; OSHA, 2015; Peterson et al., 2016; Santos et al., 2010; Wise et al., 2011). Given the elevated demand for HCWs during a pandemic, in 2011 NIOSH initiated a 5-year multidisciplinary study entitled “Why Healthcare Staff Catch the Flu” (WHSCF) to improve our understanding of how influenza is transmitted including the potential for both aerosol and contact transmission routes. To monitor exposure to influenza aerosols within healthcare settings, studies have used stationary or personal aerosol samplers (Bischoff et al., 2013; Blachere et al., 2009; Lindsley et al., 2010a). Stationary aerosol samplers are usually placed in a patient room and the aerosol is collected on a filter over a period of time. Unfortunately with stationary aerosol samplers, sample collection often occurs away from the patient and is not indicative of direct exposure to the healthcare worker. Personal aerosol samplers have been placed on healthcare workers while conducting patient care activities and analyzed for influenza virus. The use of personal aerosol samplers permits the collection of bioaerosols that are more representative of a healthcare employee’s exposure when in close contact with a patient. Nonetheless, personal aerosol samplers can be cumbersome to wear, are expensive to purchase, and are often available for a limited number of study participants. As part of the WHSCF study, PPE from HCWs and aerosol samples from the Johns Hopkins Student Health Facility and the Adult Emergency Department were collected and analyzed for influenza to determine the relationship between levels of airborne influenza virus and PPE contamination. In particular, SMs and N95 respirators were assessed to determine whether these PPE can serve as “personal bioaerosol samplers” to evaluate potential airborne exposure to influenza virus and also determine if contaminated PPE could serve as a source for infectious virus. Surgical masks and respirators are ubiquitous in a healthcare setting during influenza season and may serve as a tool to assess HCW exposure during specific patient encounters and care activities which may increase exposure potential, such as aerosol generating procedures. However, collection and subsequent detection of influenza from PPE can be difficult. Experimental challenges such as the effect of storage conditions on virus infectivity and nucleic acid stability, low virus recovery efficiency from porous PPE materials, and potentially low virus concentrations of virus expected on respirator and masks used in the field are a few of the concerns this study aimed to address. To establish whether contaminated PPE could be used to assess levels of airborne influenza exposure within a healthcare setting, laboratory studies were performed utilizing a previously described aerosol exposure simulation chamber, with coughing and breathing simulators (Lindsley et al., 2013; Noti et al., 2013; Noti et al., 2012). Aerosol samples along with SMs and N95 respirators placed on the breathing simulator, were analyzed to determine the lowest concentration of influenza virus that could be detected both in the air and on respiratory PPE.

2. Materials and methods

2.1. Cell and virus stock

Madin-Darby canine kidney (MDCK) cells (ATCC CCL-34) and influenza A(H1N1) strain A/WS/33 (ATCC VR-825) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Complete growth medium for MDCK cells consisted of Eagle’s Minimum Essential Medium (EMEM) (ATCC) containing 10% fetal bovine serum (Hyclone Laboratories Inc, Logan, UT), 200 units/ml penicillin G, 200 µg/ml streptomycin (Invitrogen, Carlsbad, CA). MDCK cells were incubated at 35 °C in a humidified 5% CO_2 incubator until approximately 80% confluent. Propagation of influenza A(H1N1) [1.0×10^7 $TCID_{50}$] and dilution in Viral Transport Media (VTM) consisting of Hank’s Balanced Salt Solution (1X HBSS; ThermoFisher Scientific) supplemented with 0.1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), 100 units/ml penicillin G and 100 units/ml streptomycin (ThermoFisher Scientific), was performed as previously described (Blachere et al., 2011).

2.2. RNA Isolation/cDNA transcription/Quantitative PCR

Viral RNA was isolated using the MagMAX™-96 viral RNA Isolation Kit (ThermoFisher Scientific, Waltham, MA) as described previously (Blachere et al., 2011). The entire volume of eluted RNA (32 µl) was transcribed into 40 µl cDNA using the High Capacity RNA to cDNA Transcription Kit (ThermoFisher Scientific) in accordance with the manufacturer’s instructions. Quantitative PCR (qPCR) of the influenza matrix (M1) gene expression was performed as described previously (Blachere et al., 2011).

2.3. Virus infectivity following storage

To assess the effects of temperature and length of storage on influenza A(H1N1) infectivity, viral suspensions with a tissue culture infectious dose $(TCID)_{50}$ of 10^6 (high concentration), $TCID_{50}$ 10^4 (medium concentration) and $TCID_{50}$ 10^2 (lowest concentration), were prepared by directly inoculating virus into VTM and storing at either 4 °C, −20 °C or −80 °C for 1, 2, 4, 6, 14 or 18 days. Following storage, viral infectivity (as measured by plaque forming units per milliliter (pfu/mL) of virus solution) was determined by viral plaque assay (Blachere et al., 2011).

2.4. Aerosol exposure simulation chamber

To simulate exposure of a healthcare worker to airborne infectious influenza, a SM or N95 respirator was sealed to the breathing manikin’s face and a coughing simulator was programmed to cough influenza virus. All aerosol studies were conducted within a 3.2 m × 3.2 m × 2.3 m high environmental chamber that was set up to simulate a patient examination room (Lindsley et al., 2013). A schematic diagram of the aerosol exposure simulation chamber can be found in publications by Noti et al. (Noti et al., 2013; Noti et al., 2012) The room included a HEPA filtration system to remove airborne particles before/after testing, an ultraviolet germicidal irradiation system to disinfect the room between experiments, NIOSH BC 251 two-stage cyclone samplers (Lindsley et al., 2006), and coughing and breathing simulators to mimic a coughing patient and breathing healthcare worker. The coughing simulator was programmed to cough a size range of 0.1–30 µm aerosol particles containing influenza virus (0.1 µm is the approximate size on a single influenza virion) over a wide range of concentrations (Lindsley et al. 2013). Influenza A(H1N1) was aerosolized with an Aeroneb 2.5–4-µm volume median diameter micropump nebulizer (Aerogen, Galway, Ireland), as described previously (Noti