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Major article

Environmental sampling for respiratory pathogens in Jeddah airport during the 2013 Hajj season

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Background: Respiratory tract infections (RTIs) are common during the Hajj season and are caused by a variety of organisms, which can be transmitted via the air or contaminated surfaces. We conducted a study aimed at sampling the environment in the King Abdul Aziz International (KAAI) Airport, Pilgrims City, Jeddah, during Hajj season to detect respiratory pathogens.

Methods: Active air sampling was conducted using air biosamplers, and swabs were used to sample frequently touched surfaces. A respiratory multiplex array was used to detect bacterial and viral respiratory pathogens.

Results: Of the 58 environmental samples, 8 were positive for at least 1 pathogen. One air sample (1 of 18 samples, 5.5%) tested positive for influenza B virus. Of the 40 surface samples, 7 (17.5%) were positive for pathogens. These were human adenovirus (3 out of 7, 42.8%), human coronavirus OC43/HKU1 (3 out of 7, 42.8%), *Haemophilus influenzae* (1 out of 7, 14.2%), and *Moraxella catarrhalis* (1 out of 7, 14.2%). Chair handles were the most commonly contaminated surfaces. The handles of 1 chair were cocontaminated with coronavirus OC43/HKU1 and *H influenzae*.

Conclusion: Respiratory pathogens were detected in the air and on surfaces in the KAAI Airport in Pilgrims City. Larger-scale studies based on our study are warranted to determine the role of the environment in transmission of respiratory pathogens during mass gathering events (eg, Hajj) such that public health preventative measures might be better targeted.

Mass gatherings, such as sporting or musical events, political rallies, or those of spiritual nature (eg, Hajj), bring large numbers of people to a specific site for a defined period of time. Such gatherings increase the likelihood of the spread of infectious diseases. The Kingdom of Saudi Arabia annually hosts >2 million Muslim pilgrims from around 184 countries during the Hajj pilgrimage, making it one of the largest and most culturally and geographically diverse mass gatherings in the world.[1] Respiratory tract infections (RTIs) are the most common infection transmitted between pilgrims during Hajj, and most pilgrims develop RTIs during their few weeks stay in Makkah and Madinah.[2] In one study, 60.8% of pilgrims attending primary care centers during the Hajj had a respiratory disease.[3] Among a cohort of 154 French pilgrims participating in the 2012 Hajj, 83.4% had respiratory symptoms, including 41.0% with influenza-like illness. Pneumonia is also a leading cause of hospitalization of pilgrims in Saudi hospitals during Hajj, including intensive care units.[4,5] In 1986, pneumonia was the second most common cause of hospitalization with an incidence of 4.8 per 100,000 and a case fatality rate of 34%.[6]

Viruses are a common cause of RTIs during Hajj. In one study, nasopharyngeal and throat swabs were taken from 3,218 pilgrims during the 2009 Hajj season and tested for 18 respiratory viruses. The main viruses detected among pilgrims were rhinovirus-enterovirus (12.9%), coronaviruses (0.8%), respiratory syncytial virus (0.2%), and influenza A virus (0.2%), including pandemic

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Table 1
Respiratory pathogens in air and surface samples collected from the Hajj terminal at Jeddah airport

Sample type	Date of sampling	Sampling location	Time of sampling	Nationality of pilgrims in the area	Pathogen detected
Air*	10/26/13	Inside terminal, hall 8	5:10 PM	Turkey, Thailand	FLU-B
Surface†	11/3/13	Inside terminal, hall 4: passport inspection table	4:00 PM	Bangladesh	OC-43
Surface	11/3/13	Inside terminal, hall 4: chair handles	4:00 PM	Bangladesh	OC-43, HI
Surface	11/3/13	Inside terminal, hall 4: chair handles	4:00 PM	Bangladesh	MC
Surface	11/3/13	Inside terminal, hall 8: bathroom handles	4:00 PM	Turkey	HAV
Surface	11/4/13	Inside terminal, hall 5: chair handles	4:00 PM	Ethiopia	HAV
Surface	11/4/13	Inside terminal, hall 5: chair handles	4:00 PM	Ethiopia	HAV
Surface	11/5/13	Inside terminal, hall 10: check in counter table	4:00 PM	Mix	OC-43

FLU-B, influenza B virus; *HAV*, human adenovirus A, B, C, D, and E; *HI*, *Haemophilus influenzae*; *MC*, *Moxarella catarrhalis*; *OC-43*, human coronavirus OC-43/HKU1.
*Two-hour sampling time equaling 720 L of air sampled per run.
†Areas sampled were 25 cm^2.

influenza A (H1N1) (0.1%).[7] The threat of new respiratory viruses is always present during Hajj. The novel H7N9 avian influenza A virus first reported in 2013 to have infected humans in China and the Middle East respiratory syndrome coronavirus first reported in 2012 from Saudi Arabia are cases in point.[8,9]

The etiology of RTIs in Hajj is not limited to viruses. A number of other organisms (eg, bacteria, fungi) are responsible for such infections during Hajj. Tuberculosis is an important public health problem because large numbers of pilgrims come from high tuberculosis-endemic areas of the world and may have latent or active tuberculosis.[10] One study examined 64 cases of pneumonia admitted to 2 hospitals in Makkah during the 1994 Hajj season. Diagnosis was established in 46 patients (72%), with *Mycobacterium tuberculosis* being the most prevalent organism (20%), followed by gram-negative bacilli (18.8%), *Streptococcus pneumoniae* (10%), *Legionella pneumophila* (6%), and *Mycoplasma pneumoniae* (6%).[11] In another study of 141 pilgrims with clinical suspicion of pneumonia, 76 (53.9%) were confirmed positive by microbiological tests. The most frequent isolates were *Candida albicans* (28.7%), *Pseudomonas aeruginosa* (21.8%), *L. pneumophila* (14.9%), and *Klebsiella pneumoniae* (9.2%).[12]

Respiratory pathogens can be transmitted via the air or through contact with contaminated environmental surfaces.[13-17] Surveillance of these respiratory pathogens in the environment during Hajj can be indicative of the type of pathogens and strains circulating during the pilgrimage with the possibility of potentially causing epidemics. This surveillance could serve as an early warning system and guide for early interventions to prevent or reduce transmission of novel infectious agents.

In this study we sampled the environment (air and highly touched surfaces) at various locations in the King Abdul Aziz International (KAAI) Airport, Pilgrims City, Jeddah, at the time of the pilgrims' departure post-Hajj to determine the type of respiratory pathogens in the environment and inform future larger-scale studies.

METHODS

Study location

The study was conducted at the Hajj terminal of the KAAI Airport upon pilgrims' departure from the Hajj. KAAI Airport is located in Jeddah and occupies an area of 105 km^2. This airport is the gateway to Makkah, through which most of the international pilgrims reach the Sacred Mosque in Makkah. The Hajj terminal of KAAI Airport is specially built to handle pilgrims that take part in the annual Hajj and is designed in the form of tents occupying an area of 465,000 m^2. It is the fourth largest airport terminal in the world after Hong Kong, Bangkok, and Seoul. It can receive about 50,000 pilgrims a day in the Hajj season. It consists of 12 indoor halls, which act as arrival and departure lounges for pilgrims. It also consists of various indoor public areas (eg, restaurants, indoor and outdoor shopping areas).

Air sampling

Active air sampling was performed from between October 24-28, 2013. Biosamplers (SKC Inc, Eighty Four, PA) were used to sample the air at various locations and times in the Hajj terminal as described in Table 1. The biosamplers were positioned at 1.5 m from the floor and run at a sampling flow rate of 6 L/min for 2 hours, hence the sampling of 720 L of air in each run. Whereas biosamplers are typically used at a sampling flow rate of 12.5 L/min for bacteria and fungi, the same procedure does not work well for viruses. Instead, lower sampling rates (6-8 L/min) have resulted in increased collection efficiencies for airborne viruses in field and laboratory tests (J. Lednicky, 2010, and C. Y. Wu, 2009 and 2010, unpublished data). Microorganisms were collected in 20 mL of collection media composed of 0.5 weight/volume sterile Bovine Serum Albumin Fraction V (Invitrogen, Paisley, UK) in a Phosphate Buffered Saline solution (Invitrogen, Paisley, UK). The media were then aseptically transferred into sterile polypropylene 50 mL clinical-grade centrifuge tubes and frozen at −80°C until further processing.

Surface sampling

Surface sampling using swabs was conducted November 3-5, 2013, using a commercial collection and transport system (Remel, Lenexa, KS). Swabs were used to sample 25 cm^2 areas of frequently touched surfaces at various locations in the airport's Hajj terminal (Table 1). None of the surfaces were cleaned in at least the last 24 hours before sampling. Swabs were moistened in sterile water before use. Sterile 5 × 5 cm sampling templates were used to sample flat surfaces. For nonflat surfaces, areas approximating 25 cm^2 were sampled. Swabbing was done using sterile gloves for each sampling. Swabs were held at an approximately 30° angle to the surface being swabbed, and the swabs were moved across the area in 3 directions (horizontal, vertical, cross section). The swabs were then inserted into the provided transport media and frozen at −80°C until further processing.

Nucleic acid extraction and detection of respiratory pathogens

Nucleic acid (DNA and RNA) was extracted from the samples using the MinElute Virus Spin Kit (Qiagen, Manchester, UK) following the manufacturer's instructions and eluted in 50 μL nuclease-free water. A 5 μL aliquot of each of the nucleic acid extractions was run on the Respiratory Multiplex Array (Randox, Crumlin, UK), which is capable of simultaneously detecting 22 bacterial and viral respiratory pathogens. (These are: Influenza A and B; human respiratory syncytial virus A and B; human parainfluenza virus 1, 2, 3, and 4; human coronavirus 229E/NL63 and OC43/HKU1; human rhinovirus A and B; human enterovirus A, B,