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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.

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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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Conflicts of interest: None to report.

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 cm².

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 km². 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 m². 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 cm² 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 cm² 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,

and C; human adenovirus A, B, C, D, and E; human bocavirus 1, 2, and 3; human metapneumovirus, *Chlamydomphila pneumoniae*; *L. pneumophila*; *Haemophilus influenzae*; *Bordetella pertussis*; *S. pneumoniae*; *Moraxella catarrhalis*; and *M. pneumoniae*.)

RESULTS

Of the 58 environmental samples, 8 were positive for at least one of the pathogens in the respiratory multiplex array (Table 1). Air samples were negative with the exception of one (1 of 18, 5.5%), which tested positive for influenza B virus. This sample came from air sampling done during the late afternoon (started at 5:10 PM) on October 26, 2013, at hall 8 of the airport. At the time of the sampling, the hall had a temperature and relative humidity of 23°C and 58%, respectively, and was being used to process pilgrims from Turkey and Thailand who were returning home.

Of the 40 surface samples, 7 (17.5%) were positive for pathogens (Table 1). The most common pathogen contaminants of surfaces were adenovirus (3 of 7, 42.8%) and coronavirus OC43/HKU1 (3 of 7, 42.8%). Potentially pathogenic bacteria (eg, *H. influenzae*, *M. catarrhalis*) were also present on environmental surfaces. Chair handles were the most commonly contaminated surfaces. Out of the 11 chair handles sampled, 4 (36.3%) were contaminated with at least one of the pathogens. The handles of 1 chair were cocontaminated with both coronavirus OC43/HKU1 and *H. influenzae*.

DISCUSSION

RTIs are common during the Hajj, and they are frequently transmitted between pilgrims.² These infections are caused by a variety of organisms, including viruses, bacteria, and fungi. The environment plays an important role in the transmission of these pathogens, which can be via the air or contact with contaminated surfaces.¹³⁻¹⁷ Most studies investigating the role of the environment in the transmission of pathogens have been conducted in health care facilities. These have reported that the air and inanimate surfaces play a major role in the transmission of a number of bacterial, viral, and fungal pathogens.^{15,18} However, the health care environment is unique with many of the pathogens circulating in such environments being specific to the hospital setting and largely reflecting the infection status of the patient population.

Our study investigated the microbial contamination of largely an indoor environment in a public setting, a busy airport terminal accommodating the Hajj pilgrims. Although most air samples were negative for the 22 respiratory pathogens screened for, one sample was positive for influenza B virus. Influenza is responsible for considerable morbidity and mortality worldwide each year¹⁹ and is common during the Hajj.² Influenza viruses have been shown to survive for days in the environment and can easily be transmitted via droplet, airborne (droplet nuclei), or contact transmission.^{13,14,17} Influenza virus has been detected in different indoor environments (eg, schools, homes, office buildings).¹⁷ The low contamination prevalence found in our air samples may be the result of a number of factors, including the restricted range of pathogens screened for, duration of sampling (volume of air sampled), large volume of airport terminal halls and ventilation systems, and environmental conditions (eg, temperature, relative humidity). In addition, because the air samplings were performed at a lower collection flow rate than is optimal for bacteria and fungi, we cannot exclude the possibility that they were not effectively collected by the procedure that was used for this study.

Although the air does not represent a true ecosystem or aeroplankton whereby the microorganisms can grow and reproduce, it does contain microbial forms maintained in suspension coming from the soil, water, plants, or animals, including humans.²⁰ Some studies of microbial air quality have revealed that indoor air in public spaces

contains bacteria, fungi, and viruses, some of which can cause respiratory infections in humans. Goyal et al²¹ investigated contamination of the air by respiratory viruses in 2 large public buildings in the United States by analyzing the content of heating, ventilation, and air conditioning filters of the air-handling units in the buildings. Nine of the 64 filters tested were positive for influenza A virus, 2 filters were positive for influenza B virus, and 1 filter was positive for parainfluenza virus 1. Soto et al²⁰ performed air sampling to detect bacteria and fungi at representative areas within a Spanish university. Most bacteria identified were gram-positive cocci belonging to the *Micrococcus*, *Staphylococcus*, and *Streptococcus* species. Other bacteria (eg, *Neisseria*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*) were also identified. *Cladosporium* was the predominant genus isolated among fungi, in addition to other genera identified at lower rates (eg, *Aspergillus*, *Candida*, *Penicillium*). The study also reported that in the indoor air, bacterial contamination exceeds fungal contamination and the former originates from humans occupying the indoor space, whereas fungi probably come from exogenous sources.

In contrast, studies of the outdoor air suggest that fungal organisms are often present.^{20,22} One study of the aerial contamination in urban areas of a Spanish city calculated that about 5×10^3 viable microorganisms may be daily inhaled outdoor by the citizens of that city.²² Airborne fungal counts surpassed those of bacteria, and fungal isolates were mainly species of *Cladosporium*, *Alternaria*, and *Aspergillus*. Several potentially pathogenic bacteria were also detected, including *Staphylococcus*, *Acinetobacter*, and *Streptococcus*. *Legionella* was occasionally found in 2 environmental areas.²²

A number of studies investigated bacterial and fungal air contamination in Saudi cities (eg, Riyadh, Hofuf, Mecca, Taif).²³ In one report,²⁴ air samples were collected from various public areas in the Mecca region during the 1998 Hajj season. A total of 38 species of fungi and 19 bacterial species belonging to 6 genera were isolated. The most prevalent fungi isolated were *Alternaria*, *Cladosporium*, and *Penicillium* species, whereas *Bacillus*, *Micrococcus*, *Shigella*, and *Pseudomonas* species were the predominant bacteria. The number of total bacterial and fungal colonies was generally higher in densely populated areas compared with less populated places. This is relevant for Hajj where large numbers of pilgrims conjugate in close proximity in confined spaces.

In relation to Hajj, few studies investigated microbial contamination of the air in and around the main pilgrimage locations. In one study,²³ airborne microbial contamination was investigated using a gravitational method (passive sampling using settle plates) at the 4 corners of the holy mosque (Al-Haram Mosque) in Mecca, a key location during the Hajj. Bacteria and fungi concentrations ranged between 1 (21×10^3 CFU/m³) and 4 (57×10^2 CFU/m³) at all corners. A total of 502 bacterial isolates belonging to 11 genera were identified. Gram positives, mainly *Staphylococcus* and *Bacillus* species, were the main bacteria. In addition, a total of 112 fungal isolates belonging to 19 genera were also identified. *Aspergillus* and *Fusarium* species were the predominant fungi. There was no testing for viruses in this study.

Microbial contamination of surfaces in both health care and community settings is common, and pathogens can survive for prolonged periods of time on such surfaces and be transmitted.^{13,15,25,26} Contamination can occur via direct contact with the surface or by microorganisms settling on surfaces from the air. In our study the main viruses detected were adenovirus and coronavirus. These viruses are capable of causing respiratory and enteric symptoms, can survive on surfaces for at least a few hours, and their transmission from such surfaces is possible.^{13,14,27} For example, in another study, adenovirus was detected in bars and coffee shops contaminating drinking glasses, paper, china, cotton cloths, latex, glazed tiles, and polystyrene.¹³ Similarly, coronavirus has been detected in hospitals and apartment buildings contaminating various inanimate surfaces (eg, phones, doorknobs,

computer mice, toilet handles, latex gloves, sponges).^{13,28} In the absence of good hand hygiene, there is a possibility that pilgrims could pick up these viruses on their hands from contaminated surfaces and self-inoculate or further transmit these viruses.

We also detected bacterial contamination on surfaces with *H. influenzae* and *M. catarrhalis*. *H. influenzae* is a small gram-negative coccobacillus capable of causing a wide range of localized and invasive infections and has been recovered from environmental surfaces.²⁹ Most invasive *H. influenzae* infections are caused by serotype b strains. Although the introduction of *H. influenzae* type b conjugate vaccines in the late 1980s made a dramatic effect on the incidence of invasive disease among children, cases of vaccine failure and an increased susceptibility to invasive *H. influenzae* type b and nontype b *H. influenzae* disease have been consistently reported among individuals with various congenital and acquired immune deficiencies.³⁰ Similarly, *M. catarrhalis*, another gram-negative bacterium, is an important and common human respiratory tract pathogen that causes 10%–20% of otitis media episodes in children and is the second most common cause of exacerbations of chronic obstructive pulmonary disease in adults.³¹ Pneumonia caused by *M. catarrhalis* occurs infrequently but is well described in older adults, especially in those with underlying cardiopulmonary disease.³² A large proportion of Hajj pilgrims are older adults with underlying health conditions, hence these organisms could cause serious invasive infections in such susceptible hosts.

Our study has some limitations. We sampled a relatively small number of locations at a particular time during the Hajj. We also sampled the air for a specific time period. Sampling at more locations at various times (crowded and quiet times) for longer periods of time using procedures optimized for viruses in parallel with those for bacteria and fungi would improve the likelihood of detection of pathogens and would inform which periods and locations during the Hajj pathogens are most likely to be present in the air or on surfaces. For instance, studies have shown that human activity and densely populated areas have an effect on the density and variety of microorganisms in the environment.^{20,23} Our study aimed to detect a set of 22 viral and bacterial pathogens. This meant that other respiratory pathogens, including fungi and other viruses and bacteria not included in the panel, would have been missed. Additionally, because the detection was based on molecular techniques alone, it is unclear whether the detected pathogens were infectious or alive at the time of sampling because direct culturing was not performed. On the other hand, there is evidence to support that only a small percentage of microorganisms surviving in natural environments can be cultured and quantified using conventional and standard microbiological media.³³ Considering the 22 pathogens screened for in the multiplex assay, individual culture for these same reagents would have been complex and very expensive. Hence, although the use of molecular techniques, similar to the ones we used, may overestimate the presence of viable agents, it is more practical, less expensive, and less complex than a complex, multiple pathogen, direct culture approach. Further investigations, including both molecular and culture techniques, for the detection of a wider range of respiratory and enteric pathogens seem warranted.

Notwithstanding these limitations, our study gave an insight into some of the respiratory pathogens present in the air and on surfaces during Hajj and valuable information that will help optimize further larger-scale studies into the role of the environment in transmission of respiratory pathogens during mass gathering events, including Hajj. Sampling approaches used in this study may prove useful in designing interventions to reduce RTIs transmission during Hajj. Future interventions may include public health policies encouraging the wearing of protective face masks at certain locations and times during Hajj, emphasis on hand hygiene, and improved cleaning and disinfection of frequently touched surfaces at various locations during the pilgrimage.

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