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# Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018

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A R T I C L E I N F O

*Keywords:*
Hajj
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*Klebsiella pneumoniae*
*Streptococcus pneumoniae*

A B S T R A C T

*Background:* Respiratory tract infections are common in the context of the Hajj pilgrimage and respiratory pathogens can be transmitted via contact with contaminated surfaces. We sampled surfaces during the Hajj to detect the presence of respiratory bacteria and viruses.
*Methods:* Frequently touched surfaces at Mecca, Mina, Arafat and Medina were sampled. The common respiratory pathogens were tested by qPCR.
*Results:* 70/142 (49.3%) environmental samples collected were positive for at least one respiratory pathogen. Among the positive samples, *Klebsiella pneumoniae* was the bacterium most frequently tested positive (57.1%), followed by *Streptococcus pneumoniae* (12.9%), *Staphylococcus aureus* (10.0%) and *Haemophilus influenzae* (7.1%). 32.9% positive samples tested positive for rhinovirus and 1.4% for coronavirus. Surfaces with the highest rates of positive samples were kitchen tables (100%), water fountain faucet (73.3%) and edge of water coolers lid (84.6%). Samples collected in Mina were the most frequently contaminated with 68.8% being positive for at least one pathogen and 18.8% positive for a combination of multiple pathogens.
*Conclusion:* These preliminary results indicate that respiratory pathogens are common in environmental surfaces from areas frequented by Hajj pilgrims. Further larger-scale studies are needed to better assess the possible role of environmental respiratory pathogens in respiratory infections in Hajj pilgrims.

## 1. Introduction

The Hajj or Muslim pilgrimage to Mecca, Saudi Arabia, is the "Fifth Pillar of Islam". Muslims who are financially and physically able are required to perform Hajj once during their life. Hajj is one of the largest annual religious mass gatherings in the world. This event gathers from two to three million participants from more than 180 countries [1]. It takes place for 6 days beginning on the eighth and ending on the thirteenth day of Dhu al-Hijjah, the last month of the Islamic calendar. Because the Islamic calendar is lunar and the Islamic year is about eleven days shorter than the Gregorian year, the Gregorian date of Hajj changes from year to year.

The Hajj must be performed in three main locations in Mecca, in Mina and Arafat, which are respectively at 5 and 18 km from Mecca. Most pilgrims sleep in hotels in Mecca and in large tent camps in Mina during the Hajj. Due to overcrowding, pilgrims move slowly between sites, by foot or by bus for hours [2,3]. Most pilgrims also travel to Medina in order to visit the tomb of the Prophet Muhammad.

Respiratory tract infections (RTIs) are a leading cause of hospitalization during the Hajj in Saudi hospitals. Acute upper respiratory tract infection is the most common clinical presentation. The overall prevalence of RTIs, when evaluated among cohorts of pilgrims from different origins, varied from 50 to 93% [4].

The bacterial pathogens most frequently isolated from pilgrims with

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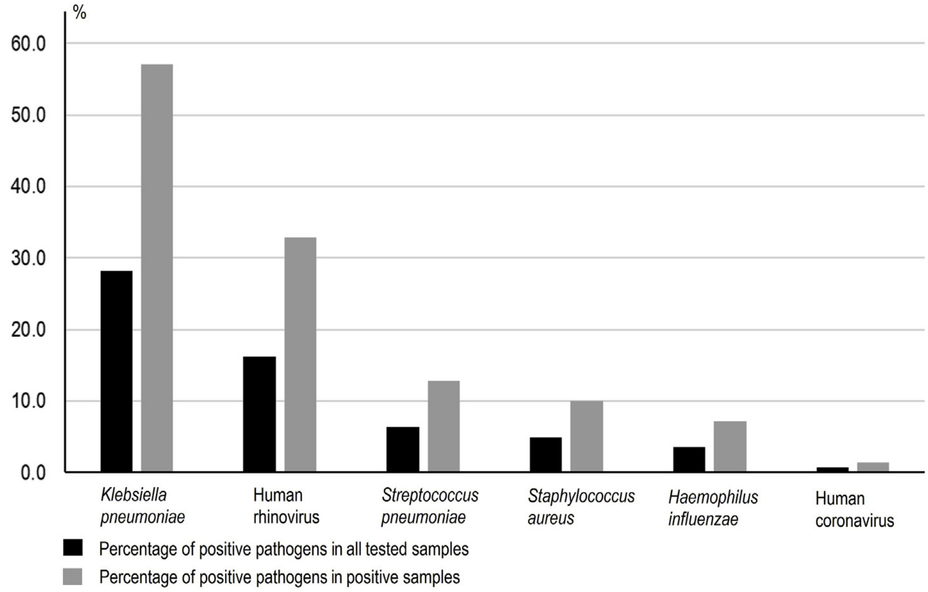


**Fig. 1.** Distribution of pathogens in all tested environmental samples (N = 142) and in positive samples (N = 70).

respiratory diseases are *Haemophilus influenzae, Klebsiella pneumoniae, Streptococcus pneumoniae* and *Staphylococcus aureus* [4]. The most common viral pathogens are human rhinovirus (HRV), non-MERS coronaviruses (HCoVs) and influenza viruses [4–6].

Many pilgrims are elderly people with co-morbidities that could increase the risk of RTIs [3,7]. The high rate of RTIs among Hajj pilgrims are likely due to inter-human transmission, given the overcrowding conditions at the Grand Mosque in Mecca with close contact between pilgrims during rituals (up to 8 persons per $m^2$) and in hotels or other infrastructures in the city, and during the accommodation in tents in Mina with an average of 50–100 people per tent [8–10].

In addition, the environment may play an important role in the transmission of respiratory pathogens through the air or via contact with contaminated surfaces. Many studies conducted in hospitals and/or other health care facilities showed that environmental pathogens are frequently responsible for occupational and nosocomial respiratory infections [11–14].

To assess the potential role of contaminated surfaces in the transmission of respiratory pathogens at the Hajj, we sampled environmental surfaces at various locations along pilgrim route during the Hajj 2016 and 2018 for the molecular detection of common pathogens responsible for Hajj-associated RTIs in pilgrims.

## 2. Materials and methods

### 2.1. Surface sampling

Surface swabbing was conducted from 6 to 19 September 2016 (the Hajj took place from 10 to 15 September) and from 16 to 24 August 2018 (the pilgrimage took place from 19 to 24 August), using a commercial collection and transferred to Sigma-Virocult® medium and stored at −80 °C. Swabs were used to sample 25 $cm^2$ (5 × 5 cm) areas of frequently touched surfaces in facilities used by French pilgrims in Mecca (hotel, commercial center, restaurant), in Mina (tent camp), in a bus from Mina to Mecca, in Arafat (tents and ablution facilities) and in Medina (hotel). Facilities that were investigated were those used by French pilgrims enrolled in prospective cohort surveys that focused on RTIs and respiratory pathogen human carriage.

### 2.2. Identification of respiratory pathogens

DNA and RNA were extracted from the samples using the EZ1 Advanced XL (Qiagen, Hilden, German) with the Virus Mini Kit v2.0 (Qiagen) according to the manufacturer's recommendations. All quantitative real-time PCR were performed using a C1000 Touch™ Thermal Cycle (Bio-Rad, Hercules, CA, USA).

The pathogens tested were based on their frequency in respiratory samples obtained from Hajj pilgrims [4,7,15].

Real-time PCR amplifications were carried out using LightCycler® 480 Probes Master kit (Roche diagnostics, France) according to the manufacturer's recommendations. The *SHD* gene of *H. influenzae*, *phoE* gene of *K. pneumoniae*, *nucA* gene of *S. aureus* and *lytA CDC* gene of *S. pneumoniae* were amplified with internal DNA extraction controls TISS, as previously described [16].

HCoV and human para-influenza virus (HPIV) were detected by one-step duplex quantitative RT-PCR amplifications of HCoV/HPIV-R Gene Kit (REF: 71-045, Biomérieux, Marcy l'Etoile, France), according to the manufacturer's recommendations. One-step simplex real-time quantitative RT-PCR amplifications were performed using Multiplex RNA Virus Master Kit (Roche Diagnostics, France) for influenza A, influenza B, HRV and internal controls MS2 phage [17].

Negative control (PCR mix) and positive control (DNA from bacterial strain or RNA from viral strain) were included in each run. Positive results of bacteria or virus amplification were defined as those with a cycle threshold (CT) value ≤ 35.

## 3. Results

We collected 142 environmental samples (66 and 76 samples in the Hajj 2016 and 2018 respectively, 75 samples at Mecca, 48 at Mina, 8 at Arafat and 11 at Medina) (Supplementary data). A total of 70 samples (49.3%) were positive for at least one respiratory pathogen (Supplementary data). Among the positive samples, *K. pneumoniae* was the most common bacterium that tested positive (57.1%), followed by *S. pneumoniae* (12.9%), *S. aureus* (10.0%) and *H. influenzae* (7.1%). In addition, 32.9% positive samples tested positive for HRV and 1.4% for HCoV. No sample was positive for influenza virus A and B or HPIV (Fig. 1). Overall, the prevalence of positive surfaces was 28.2% for *K. pneumoniae*, 16.2% for HRV and 6.3% for *S. pneumoniae.* Of the 70 positive samples, 12 were positive for more than one pathogen, including 9 positive samples for *K. pneumoniae* and one or two other mostly bacterial pathogens and 3 samples with various associations of bacteria other than *K. pneumoniae* (Supplementary data).

Surfaces with the highest rates of *K. pneumoniae* positive samples

**Table 1**
Distribution of environmental samples positive for *Klebsiella pneumoniae*, Human rhinovirus and *Streptococcus pneumoniae* by surface type (N = 142).

| Surface | N tested | *Klebsiella pneumoniae* n (%) | Human rhinovirus n (%) | *Streptococcus pneumoniae* n (%) |
|---|---|---|---|---|
| Door handles | 22 | 0 (0) | 9 (40.9) | 0 (0) |
| Toilets | 18 | 5 (27.8) | 1 (5.6) | 1 (5.6) |
| Bathroom and ablution equipments | 15 | 9 (60.0) | 2 (13.3) | 0 (0) |
| Handrails | 11 | 2 (18.2) | 2 (18.2) | 0 (0) |
| Ice reserves | 13 | 10 (76.9) | 3 (23.1) | 0 (0) |
| Elevator buttons | 11 | 0 (0) | 1 (9.1) | 0 (0) |
| Air conditioners | 9 | 0 (0) | 1 (11.1) | 0 (0) |
| Restaurant devices | 9 | 2 (22.2) | 1 (11.1) | 0 (0) |
| Edge of water coolers lid | 8 | 5 (62.5) | 0 (0) | 2 (25.0) |
| Faucet of water fountains | 5 | 0 (0) | 1 (20.0) | 3 (60.0) |
| Fridges | 5 | 2 (40.0) | 0 (0) | 0 (0) |
| Tent parts | 5 | 0 (0) | 0 (0) | 2 (40.0) |
| Kitchen tables | 5 | 4 (80.0) | 0 (0) | 1 (20.0) |
| Various[a] | 6 | 1 (16.7) | 2 (33.3) | 0 (0) |
| **Total** | **142** | **40 (57.1)** | **23 (32.9)** | **9 (12.9)** |

[a] Hotel door card, hotel room telephone, vendor machine button, hotel lobby table.

were kitchen tables (80.0%), ice reserves (76.9%), edge of water coolers lid (62.5%) and bathroom and ablution equipments (60.0%). Surfaces with the highest rates of HRV positive samples were door handles (40.9%), while those with the highest rates of *S. pneumoniae* positive samples were tent parts (40.0%) and water fountain faucets (60.0%) (Table 1).

By comparing the distribution of respiratory pathogens by geographical area where samples were collected, Mina positivity rates were significantly higher than in other areas for most pathogens and including multiple contaminations by several pathogens. Of note, all samples positive for *S. pneumoniae* were collected at Mina ($p < 0.0001$) (Table 2).

## 4. Discussion

The microbial contamination of surfaces in both health care and community settings is not uncommon, and pathogens are capable of surviving for prolonged periods on such surfaces and can be transmitted to humans via direct contact with contaminated surfaces [18,19]. Most gram-positive bacteria survive for months on dry surfaces. Many gram-negative species can also survive for months. A few others, such as *Bordetella pertussis*, or *Haemophilus influenzae*, however, persist only for days [20]. However, the human rhinovirus, human coronavirus and influenza virus have all been found to survive in the external environment, for only a matter of hours and occasionally for a day or two [21]. Few studies on the presence of environmental pathogens that may be responsible for human respiratory infections have been conducted in the context of Hajj. Angelakis et al. conducted a study investigating viable bacterial populations in air samples collected around slaughterhouses during the 2012 Hajj season by culture methods. *Bacillus* and *Staphylococcus* spp. were commonly isolated [22]. To our knowledge, only one study aiming at detecting the presence of respiratory pathogens by PCR, was conducted in the context of the Hajj and was based on air and surface samples obtained in Jeddah airport in 2013 [23]. In this survey, pathogens detected on surfaces were adenovirus (n = 3/40, 7.5%), HCoV (n = 3/40, 7.5%), *H. influenzae* (n = 1/40, 2.5%) and *Moraxella catarrhalis* (n = 1/40, 2.5%). No sample was positive for HRV and *S. pneumoniae*. *S. aureus* and *K. pneumoniae* were not tested [23]. Our study showed that respiratory bacteria, notably *K. pneumoniae* and *S. pneumoniae* and HRV were frequently recovered from environmental surface samples in areas frequented by pilgrims. Particularly high levels of contaminated surface were observed in Mina's camp, particularly from the surfaces of the collective kitchen, including tables, drinking sources and the ice supply. Hotel bathroom and ablution area equipments in Mecca were also highly contaminated. Among the French pilgrims surveyed in 2016 [15] and 2018 (unpublished data) who have stayed in the places investigated in the present survey, 16.3% acquired coronaviruses, 16.4% *S. aureus,* 17.3% *K. pneumoniae,* 25.5% *S. pneumoniae,* 26.7% HRV and 30.3% acquired *H. influenzae* during their stay in Saudi Arabia (Fig. 2). High rates of contamination of surface samples by *K. pneumoniae* and HRV suggest a possible source of contamination for pilgrims (Fig. 2). The acquisition of influenza A and influenza B virus was low among pilgrims (2.2% and 0.4% respectively) and no surface sample was positive for these viruses. It is likely that the living conditions in Mina encampment in relative promiscuity and sharing of cooking, food, hygiene and sanitary facilities are responsible for the high rate of environmental contamination by respiratory pathogens from ill pilgrims with possible subsequent transmission to pilgrims through contact with fomites. Although most pilgrims have onset of respiratory symptoms shortly after their arrival in Saudi Arabia (likely resulting from inter-human transmission of respiratory pathogens in various crowed infrastructures in the city), we observed a bimodal pattern of clinical respiratory symptoms with a lower secondary peak occurring in Mina [24]. Our results suggest that the source of contamination for respiratory pathogens might differ in Mecca and Mina, with a possible additional role for contaminated fomites in Mina, besides air-transmission.

*K. pneumoniae* can be transmitted by person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel, or other persons) or by contamination of the

**Table 2**
Distribution of environmental samples positive for *Klebsiella pneumoniae*, Human rhinovirus and *Streptococcus pneumoniae* by geographical area (N = 142).

| Respiratory pathogens | | Mecca | | Arafat | | Mina[a] | | Medina | | Total | | p-value[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n = 75 | % | n = 8 | % | n = 48 | % | n = 11 | % | N = 142 | % | |
| *Streptococcus pneumoniae* | Yes | 0 | 0 | 0 | 0 | 9 | 18.8 | 0 | 0 | 9 | 6.3 | < 0.0001 |
| | No | 75 | 100 | 8 | 100 | 39 | 81.2 | 11 | 100 | 133 | 93.7 | |
| *Klebsiella pneumoniae* | Yes | 14 | 18.7 | 2 | 25.0 | 24 | 50.0 | 0 | 0 | 40 | 28.2 | < 0.0001 |
| | No | 61 | 81.3 | 6 | 75.0 | 24 | 50.0 | 11 | 100 | 102 | 71.8 | |
| Human rhinovirus | Yes | 11 | 14.7 | 1 | 12.5 | 5 | 10.4 | 6 | 54.6 | 23 | 16.2 | 0.01 |
| | No | 64 | 85.3 | 7 | 87.5 | 43 | 89.6 | 5 | 45.4 | 119 | 83.8 | |
| At least one pathogen | Yes | 28 | 37.3 | 3 | 37.5 | 33 | 68.8 | 6 | 54.6 | 70 | 49.3 | 0.005 |
| | No | 47 | 62.7 | 5 | 62.5 | 15 | 31.2 | 5 | 45.4 | 72 | 50.7 | |
| ≥2 pathogens | Yes | 3 | 4.0 | 0 | 0 | 9 | 18.8 | 0 | 0 | 12 | 8.4 | 0.03 |
| | No | 72 | 96.0 | 8 | 100 | 39 | 81.2 | 11 | 100 | 130 | 91.6 | |

[a] 5 samples collected in the bus from Mina to Mecca.
[b] Test Fisher's exact.

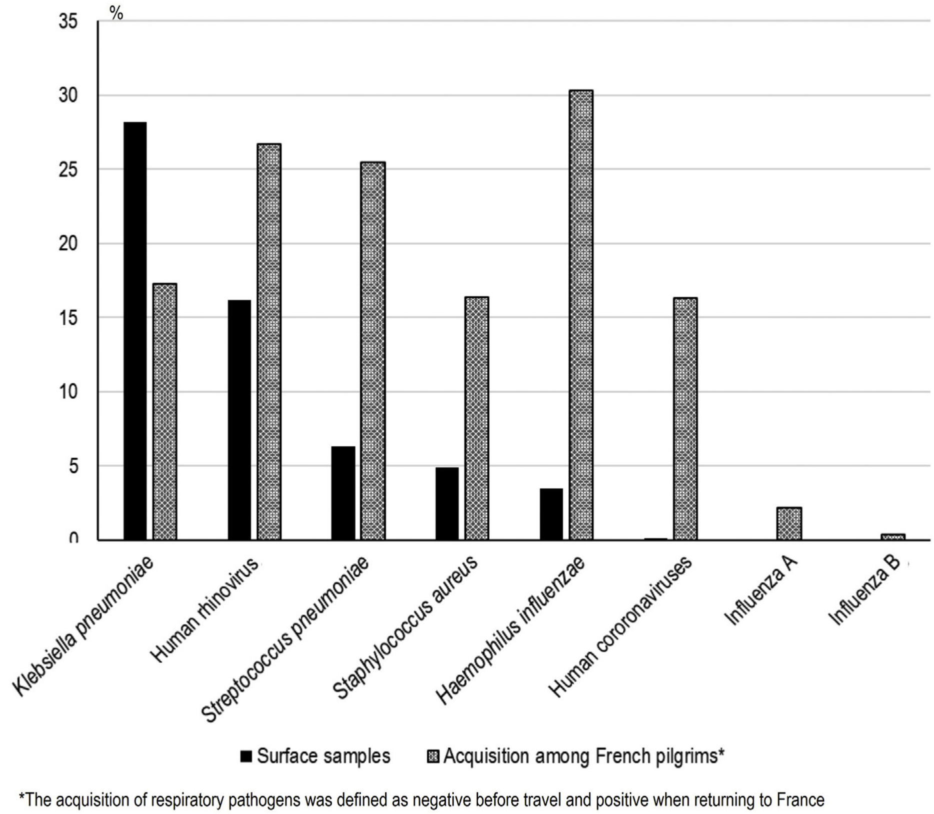


**Fig. 2.** Positivity rates of environmental samples in comparison to acquisition rates as assessed in respiratory samples among French pilgrims surveyed in 2016 [15] and 2018 [unpublished data].

environment. The bacteria do not spread in the air [25]. In a study conducted between June 2009 and June 2010 on 750 environmental surface samples of bathroom, wash basin taps, wash basin, drains, doors and handles in the Outpatient Department at the Al-Azhar University Hospital in Assuit, Egypt, 13.6% samples were positive for *K. pneumoniae*. Wet surfaces of bathrooms, wash basin taps and wash basins were those most frequently found positive, followed by doors and handles, suggesting that environmental surfaces are potential reservoirs for pathogens such as *K. pneumoniae*. [25]. Humans are the main host for *S. pneumoniae*, but direct person-to-person contact is not required for bacteria transmission, thus indicating that environmental reservoir could be involved [26]. In addition, higher rates of pneumococcal transmission are known to occur in overcrowded environments [27,28].

HRV and HCoV can survive on surfaces for at least a few hours. Their transmission by contact with such surfaces has been described [18,29,30]. HCoV has been detected in hospitals and apartment buildings contaminating various inanimate surfaces, such as telephones, computer mice and toilet handles [18,31]. Also, transmission hand-to-hand and by contact with contaminated surface of HRV was identified [32]. Ikonen et al. conducted a recent study on the presence of respiratory viruses on frequently touched surfaces at Helsinki-Vantaa airport, Finland, detected by qPCR. A total of 9/90 (10%) surface samples were positive for at least one respiratory virus with 4/90 (4.4%) HRV, 3/90 (3.3%) HCoV OC43, 3/90 (3.3%) adenovirus and only 1/90 (1.1%) influenza A virus [33]. Another study that focused on surface samples collected from homes and hospital rooms of patients infected with influenza A H1N1, showed that 33/671 samples were positive for this virus [34].

Our study has some limitations. First, we did not collect air samples. Secondly, we tested only 9 respiratory pathogens (based on their higher frequency in French Hajj pilgrims). Additionally, because the detection was based on molecular techniques only, we cannot prove that the detected pathogens were alive and potentially infective. Finally, our sample size is relatively small. Despite these limitations, we demonstrated that DNA and RNA of respiratory pathogens are present at high rates on surfaces in the environment of pilgrims during the Hajj, notably in Mina. Memish et al. hypothesized that, in the absence of good hand hygiene, there is a possibility that pilgrims could pick up environmental pathogens on their hands from contaminated surfaces and self-inoculate or further transmit these pathogens [23]. Respiratory tract infections during Hajj continue to exert a heavy burden on pilgrims [4]. Besides early identification of pathogenic bacterial or viral clusters for faster mitigation of outbreaks and better understanding of disease etiology, we believe that further larger-scale studies are needed to better evaluate the possible role of environmental respiratory pathogens in causing respiratory infections among Hajj pilgrims. In the interim, reinforced hand hygiene and improved cleaning and disinfection of frequently touched surfaces at various locations during the pilgrimage should be recommended with a priority for wet surfaces, kitchen tables and door handles.

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## Declaration of competing interest

Van-Thuan Hoang, Doudou Sow, Khadidja Belhouchat, Thi-Loi Dao, Tran Duc Anh Ly, Florence Fenollar, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, Vincent Pommier de Santi, Philippe Gautret declare that they have no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.101500.

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