



HAL
open science

Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018

Van-Thuan Hoang, Doudou Sow, Khadidja Belhouchat, Thi-Loi Dao, Tran Duc Anh Ly, Florence Fenollar, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, et al.

► **To cite this version:**

Van-Thuan Hoang, Doudou Sow, Khadidja Belhouchat, Thi-Loi Dao, Tran Duc Anh Ly, et al.. Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018. *Travel Medicine and Infectious Disease*, 2019, pp.101500. 10.1016/j.tmaid.2019.101500 . hal-02469837

HAL Id: hal-02469837

<https://amu.hal.science/hal-02469837v1>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Environmental investigation of respiratory pathogens during the Hajj 2016 and 2018

Van-Thuan Hoang^{1,2,3}, Doudou Sow^{1,2,4}, Khadidja Belhouchat^{1,2}, Thi-Loi Dao^{1,2,3}, Tran Duc Anh Ly^{1,2}, Florence Fenollar^{1,2}, Saber Yezli⁵, Badriah Alotaibi⁵, Didier Raoult^{2,6}, Philippe Parola^{1,2}, Vincent Pommier de Santi^{1,2,7}, Philippe Gautret^{1,2*}

¹Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

²IHU-Méditerranée Infection, Marseille, France

³Thai Binh University of Medicine and Pharmacy, Thai Binh, Viet Nam

⁴Service de Parasitologie-Mycologie, Faculté de médecine, Université Cheikh Anta Diop, Dakar, Senegal

⁵The Global Centre for Mass Gatherings Medicine, Ministry of Health, Riyadh, Saudi Arabia

⁶Aix Marseille Univ, MEPHI, Marseille, France

⁷French Military Center for Epidemiology and Public Health Marseille, France

*Corresponding author:

Philippe Gautret

VITROME, Institut Hospitalo-Universitaire Méditerranée Infection, 19-21 Boulevard Jean Moulin 13385 Marseille Cedex 05, France. Phone: + 33 (0) 4 13 73 24 01. Fax: + 33 (0) 4 13 73 24 02. E-mail address: philippe.gautret@club-internet.fr

Abstract

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.

Keywords: Hajj; respiratory pathogens; environmental sampling; human rhinovirus; *Klebsiella pneumoniae*; *Streptococcus pneumoniae*

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 kilometers 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 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 (HCoV) 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 m²) and in hotels or other infrastructures in the city, and during the accommodation in tents in Mina with an average of 50 to 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.

Materials and Methods

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 cm² (5x5 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.

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 .

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 (Figure 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 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).

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 (Figure 2). High rates of contamination of surface samples by *K. pneumoniae* and HRV suggest a possible source of contamination for pilgrims (Figure 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 crowded 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 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.

Funding

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program « Investissements d’avenir », reference ANR-10-IAHU-03, the Région Provence-Alpes-Côte d’Azur and European funding FEDER PRIMI.

Conflict of 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.

References

1. Memish ZA, Alimuddin Zumla, Rafat F Alhakeem. Hajj: infectious disease surveillance and control. *Lancet* 2014;383:2073–82.
2. Madani TA, Ghabrah TM, Al-Hedaithy MA, et al. Causes of hospitalization of pilgrims in the Hajj season of the Islamic year 1423 (2003). *Ann Saudi Med* 2006; 26:346–51.
3. Ibrahim O. Al-Orainey. Tuberculosis infection during Hajj pilgrimage. *Saudi Med J* 2013;34:676-80

4. Hoang VT, Gautret P. Infectious diseases and mass gatherings. *Curr Infect Dis Rep.* 2018;20:44. <https://doi.10.1007/s11908-018-0650-9>.
5. Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA. Hajj-associated viral respiratory infections: A systematic review. *Travel Med Infect Dis* 2016;14:92-109. <https://doi.10.1016/j.tmaid.2015.12.008>.
6. Al-Tawfiq JA, Benkouiten S, Memish ZA. A systematic review of emerging respiratory viruses at the Hajj and possible coinfection with *Streptococcus pneumoniae*. *Travel Med Infect Dis* 2018;23:6-13. <https://doi.10.1016/j.tmaid.2018.04.007>.
7. Hoang VT, Meftah M, Anh Ly TD, Drali T, Yezli S, Alotaibi B, et al. Bacterial respiratory carriage in French Hajj pilgrims and the effect of pneumococcal vaccine and other individual preventive measures: A prospective cohort survey. *Travel Med Infect Dis.* 2018. <https://doi.org.10.1016/j.tmaid.2018.10.021>.
8. Khamis NK. Epidemiological pattern of diseases and risk behaviors of pilgrims attending Mina hospitals, Hajj 1427 H (2007 G). *J Egypt Public Health Assoc.* 2008;83:15-33.
9. Abubakar I, Gautret P, Brunette GW, Blumberg L, Johnson D, Pomeroy G, et al. Global perspectives for prevention of infectious diseases associated with mass gatherings. *Lancet Infect Dis* 2012;12:66-74. [https://doi.10.1016/S1473-3099\(11\)70246-8](https://doi.10.1016/S1473-3099(11)70246-8).
10. Al-Tawfiq JA, Zumla A, Memish ZA. Respiratory tract infections during the annual Hajj: potential risks and mitigation strategies. *Curr Opin Pulm Med.* 2013;19:192-7. <https://doi.10.1097/MCP.0b013e32835f1ae8>.
11. Yezli S, Memish ZA. Tuberculosis in Saudi Arabia: prevalence and antimicrobial resistance. *J Chemother* 2012;24:1-5
12. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol* 2011;32:687-99.

13. Fernstrom A, Goldblatt M. Aerobiology and its role in the transmission of infectious diseases. *J Pathog* 2013;2013:493960.
14. La Rosa G, Fratini M, Della Libera S, Iaconelli M, Muscillo M. Viral infections acquired indoors through airborne, droplet or contact transmission. *Ann Ist Super Sanita* 2013;49:124-32.
15. Hoang VT, Sow D, Dogue F, Edouard S, Drali T, Yezli S, et al. Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: A prospective cohort study. *Travel Med Infect Dis.* 2019. <https://doi.org/10.1016/j.tmaid.2019.03.003>
16. Memish ZA, Assiri A, Turkestani A, Yezli S, Al Masri M, Charrel R, et al. Mass gathering and globalization of respiratory pathogens during the 2013 Hajj. *Clin Microbiol Infect.* 2015;21:571.e1-8. <https://doi.org/10.1016/j.cmi.2015.02.008>.
17. Ninove N, Nougairede A, Gazin C, Thirion L, Delogu I, Zandotti C, et al. RNA and DNA bacteriophages as molecular diagnosis controls in clinical virology: a comprehensive study of more than 45,000 routine PCR tests. *PLoS One* 2011;6: e16142.
18. Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* 2007;73:1687-96
19. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis.* 2013;26:338-44. <https://doi.org/10.1097/QCO.0b013e3283630f04>.
20. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis.* 2006;6:130. <https://doi.org/10.1186/1471-2334-6-130>.
21. Walther BA, Ewald PW. Pathogen survival in the external environment and the evolution of virulence. *Biol Rev Camb Philos Soc.* 2004;79:849-69.

22. Angelakis E, Yasir M, Azhar EI, Papadioti A, Bibi F, Aburizaiza AS, et al. MALDI-TOF mass spectrometry and identification of new bacteria species in air samples from Makkah, Saudi Arabia. *BMC Res Notes*. 2014;7:892.
23. Memish ZA, Almasri M, Assirri A, Al-Shangiti AM, Gray GC, Lednicky JA, et al. Environmental sampling for respiratory pathogens in Jeddah airport during the 2013 Hajj season. *Am J Infect Control*. 2014;42:1266-9. <https://doi.10.1016/j.ajic.2014.07.027>.
24. Hoang VT, Sow D, Dogue F, Edouard S, Drali T, Yezli S, et al. Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: A prospective cohort study. *Travel Med Infect Dis*. 2019. <https://doi.org.10.1016/j.tmaid.2019.03.003>
25. MM. Afifi. Detection of Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* and *Escherichia coli* of Environmental Surfaces at Upper Egypt. *Int J Biol Chem*. 2013;7:58-68
26. Zafar MA, Wang Y, Hamaguchi S, Weiser JN. Host-to-Host Transmission of *Streptococcus pneumoniae* is driven by its inflammatory toxin, Pneumolysin. *Cell Host Microbe*. 2017;21:73-83. <https://doi.10.1016/j.chom.2016.12.005>.
27. Givon-Lavi N, Fraser D, Porat N, Dagan R. Spread of *Streptococcus pneumoniae* and antibiotic-resistant *S. pneumoniae* from day-care center attendees to their younger siblings. *J. Infect. Dis* 2002;186:1608-14
28. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect. Dis*. 2004;144–54 [https://doi.10.1016/S1473-3099\(04\)00938-7](https://doi.10.1016/S1473-3099(04)00938-7).
29. Yezli S, Otter JA. Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment. *Food Environ Virol* 2011;3:1-30.

30. van Doremalen N, Bushmaker T, Munster VJ. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. *Euro Surveill* 2013;18.
31. Dowell SF, Simmerman JM, Erdman DD, Wu JS, Chaovavanich A, Javadi M, et al. Severe acute respiratory syndrome coronavirus on hospital surfaces. *Clin Infect Dis* 2004;39:652-7.
32. Winther B, McCue K, Ashe K, Rubino JR, Hendley JO. Environmental contamination with rhinovirus and transfer to fingers of healthy individuals by daily life activity. *J Med Virol*. 2007;79:1606-10.
33. Ikonen N, Savolainen-Kopra C, Enstone JE, Kulmala I, Pasanen P, Salmela A, et al. Deposition of respiratory virus pathogens on frequently touched surfaces at airports. *BMC Infect Dis*. 2018;18:437. <https://doi.org/10.1186/s12879-018-3150-5>.
34. Killingley B, Greaux J, Digard P, Wise H, Garcia F, Varsani H, et al. The environmental deposition of influenza virus from patients infected with influenza A(H1N1)pdm09: Implications for infection prevention and control. *J Infect Public Health*. 2016;9:278-88. <https://doi.org/10.1016/j.jiph.2015.10.009>.

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

Surface	N tested	<i>Klebsiella pneumoniae</i> n (%)	Human rhinovirus n (%)	<i>Streptococcus pneumoniae</i> 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*	6	1 (16.7)	2 (33.3)	0 (0)
Total	142	40 (57.1)	23 (32.9)	9 (12.9)

*Hotel door card, hotel room telephone, vendor machine button, hotel lobby table

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

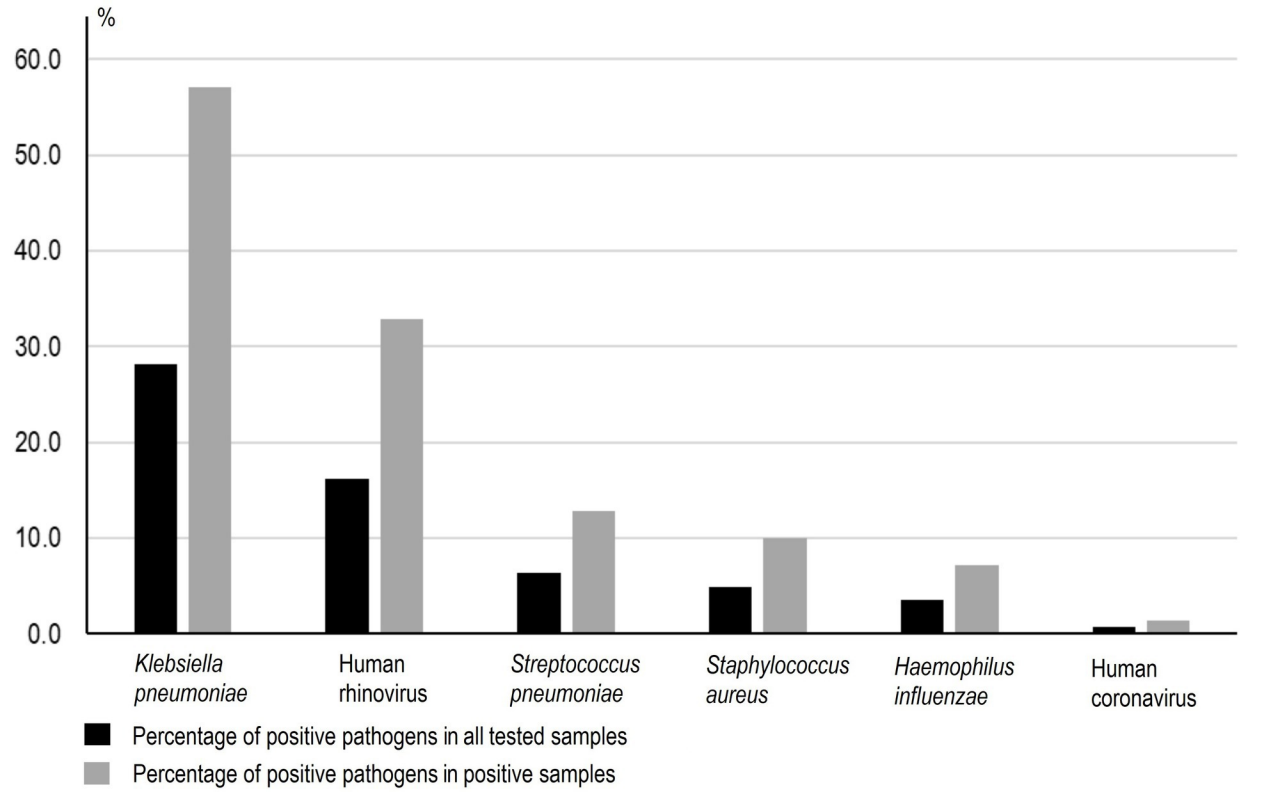
Respiratory pathogens		Mecca		Arafat		Mina*		Medina		Total		p-value**
		n=75	%	n=8	%	n=48	%	n=11	%	N=142	%	
<i>Streptococcus pneumoniae</i>	Yes	0	0	0	0	9	18.8	0	0	9	6.3	<0.0001
	No	75	100	8	100	39	81.2	11	100	133	93.7	
<i>Klebsiella pneumoniae</i>	Yes	14	18.7	2	25.0	24	50.0	0	0	40	28.2	<0.0001
	No	61	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	

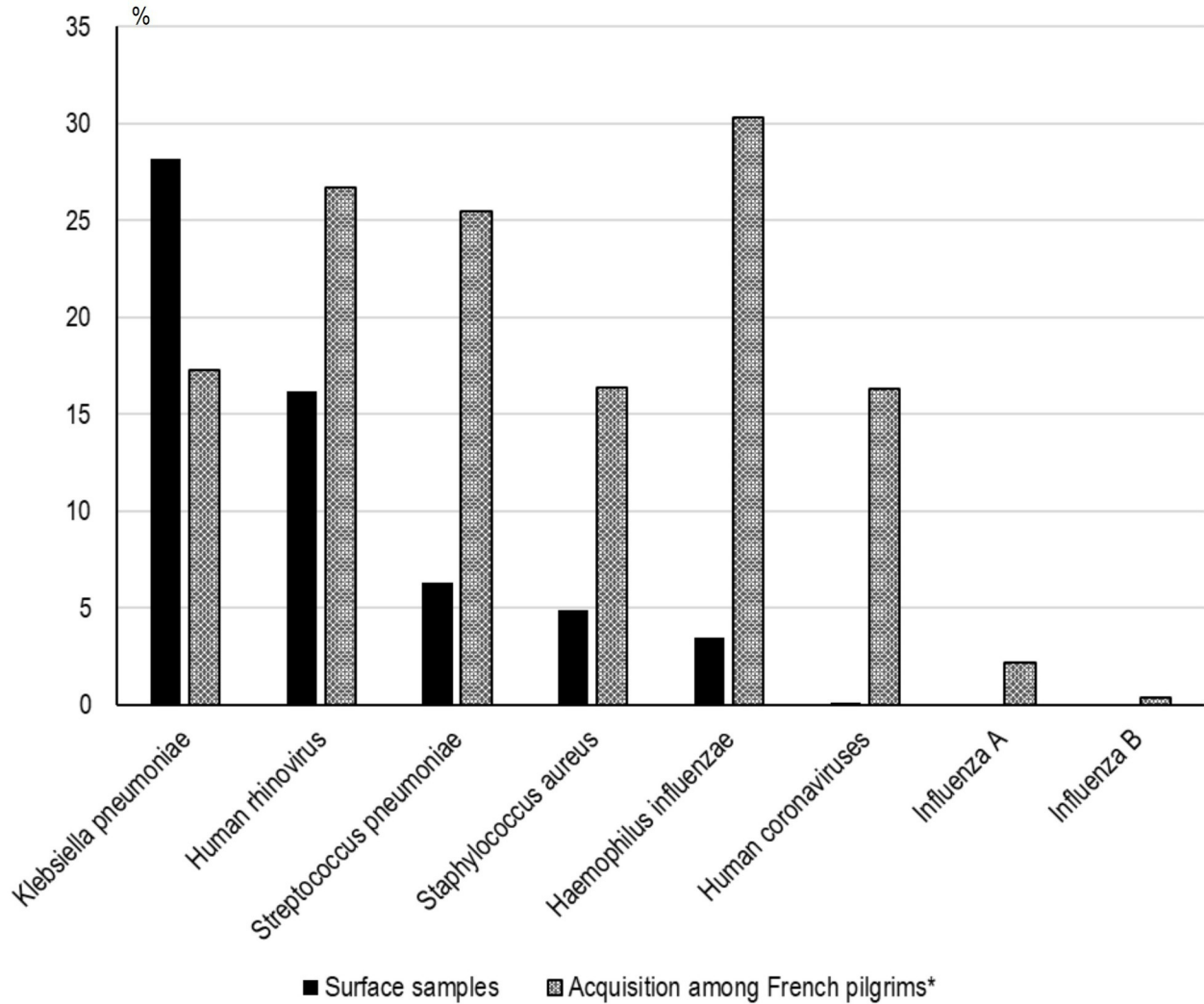
* : 5 samples collected in the bus from Mina to Mecca

** : Test Fisher's exact

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

Figure 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]





*The acquisition of respiratory pathogens was defined as negative before travel and positive when returning to France