Acquisition of *Streptococcus pneumoniae* Carriage in Pilgrims During the 2012 Hajj

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To investigate the nasal carriage of some respiratory bacterial pathogens that are responsible for infections associated with person-to-person transmission, we conducted a cohort survey of pilgrims departing to Mecca for the 2012 Hajj season. In this report, we demonstrate the acquisition of *Streptococcus pneumoniae* nasal carriage in returning Hajj pilgrims.

**Keywords.** bacteria; cohort study; epidemiology; Hajj; pneumonia.

The Hajj is the oldest and largest annual mass gathering in the world. Inevitable overcrowding within a confined area with individuals from different parts of the world in close contact with others leads to a high risk of acquiring and spreading infectious diseases during the pilgrims’ stay [1].

We investigated the nasal carriage of respiratory bacterial pathogens responsible for person-to-person transmission in a cohort survey of pilgrims departing from Marseille, France, to Mecca for the 2012 Hajj season.

**METHODS**

The study design has been published previously [2]. Nasal swabs were systematically collected from each participant in the month before departing for the Kingdom of Saudi Arabia (KSA) and in the 3 days before leaving the KSA. In a number of cases, the physician (K. B.) also collected additional nasal samples at the onset of symptoms during the pilgrimage in the KSA.

Each sample was tested independently for *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Bordetella pertussis*, and *Mycoplasma pneumoniae* using quantitative real-time PCR (qPCR). Total nucleic acids were purified from a 400-µL sample using a BioRobot EZ1 Advanced XL (Qiagen) according to the manufacturer’s instructions. The sequences of all primers and probes used in this study were previously reported [3], and the primers and probes were used at a final concentration of 500 nM or 62.5 nM, respectively. PCR assays were performed using a qPCR MasterMix (Eurogentec, Angers, France). Each run included a positive PCR control consisting of DNA extracts from clinical specimens and a negative PCR control consisting of MasterMix without DNA. Five microliters of DNA or positive/negative control was added to 20 µL of a reaction mixture containing primers (ply and lytA; ctrA and crgA; IS481; or P1 gene), 15 µL of MasterMix, and PCR-specific probes. The reactions were performed using a C1000TM Thermal cycler (CFX96TM Real-Time System, Bio-Rad, Marnes-la-Coquette, France). The following cycling conditions were applied: 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 second and 60°C for 35 seconds, then 45°C for 45 seconds.

Samples were considered positive for *S. pneumoniae* if they were positive for both the ply and lytA genes.

The Pearson χ² test and Fisher exact test were applied to analyze the categorical variables, as appropriate. *P* values ≤.05 were considered significant. Statistical analyses were performed using SPSS, version 17.2.

**RESULTS**

Of the 169 participants enrolled in the study, 167 (98.8%) responded to the pretravel questionnaire. More than half of the respondents (57.5%) reported suffering from at least 1 chronic disease [2]. Among the participants, 61 (36.5%) and 99 (59.3%) had an indication for pneumococcal vaccination according to the French and US guidelines, respectively.

A total of 137 posttravel questionnaires (81.1%) were completed. During their stay, 90.4% of pilgrims suffered from at least 1 respiratory symptom [2], 79.5% sought healthcare from a doctor, and 62.1% were prescribed antibiotics. None of the
pilgrims presented with pneumonia. Overall, 47 participants (35.9%) reported receiving the 23-valent pneumococcal polysaccharide vaccine (PPSV23; Pneumo 23) before traveling to KSA. Of those who had an indication for pneumococcal vaccination according to the French guidelines, 22 (47.8%) reported receiving the PPSV23 vaccination before traveling to KSA.

Of the 169 participants, 165 (97.6%) and 154 (91.1%) underwent, respectively, a pre-Hajj nasal swab before departing for the KSA and a post-Hajj nasal swab in the 3 days before leaving the KSA. Seventy pilgrims (41.4%) underwent an additional nasal swab at the onset of acute respiratory symptoms during their pilgrimage.

None of the participants tested positive for *N. meningitidis*, *B. pertussis*, or *M. pneumoniae* during any point of the study period. *Streptococcus pneumoniae* was detected in 12 participants (7.3%) before departing for the KSA, in 5 of the 70 (7.1%) symptomatic pilgrims who were sampled during their pilgrimage (7.1% vs 7.3%; *P* = .971), and in 30 pilgrims (19.5%) during the 3 days before leaving the KSA (19.5% vs 7.3%; *P* = .001) (Figure 1).

Of the 17 pilgrims positive for *S. pneumoniae*, either before departing for the KSA or during the pilgrimage, 13 (76.5%) tested negative before leaving the KSA; of these, 6 (46.2%) received antibiotics (Figure 1).

Of the 34 pilgrims positive for *S. pneumoniae* while in the KSA, 32 (94.1%) were negative before traveling to the KSA; of these 34, 16 (47.1%) and 22 (64.7%) had an indication for pneumococcal vaccination according to the French and US guidelines, respectively. Among the 47 pilgrims who reported receiving the PPSV23 vaccination before traveling to the KSA, 9 (19.1%) tested positive for *S. pneumoniae* before leaving the KSA.

A lower, but not statistically significant, prevalence of *S. pneumoniae* was detected before leaving the KSA among the pilgrims who received antibiotics compared to other pilgrims (17.0% vs 25.5%, respectively; *P* = .224), and between those who reported influenza-like illness (defined according to the presence of the triad of a cough, sore throat, and subjective fever) compared to those who did not (40.9% vs 59.1%, respectively; *P* = .106). However, pilgrims with influenza-like illness received antibiotics more frequently than other pilgrims (81.5% vs 44.2%, respectively; *P* < .001).

Of the 30 pilgrims positive for *S. pneumoniae* before leaving the KSA, 5 (16.7%) were coinfected with at least 1 virus, but none of the 14 (46.7%) who did not receive antibiotics were coinfected. Of the 125 pilgrims who were negative for *S. pneumoniae* before leaving the KSA, 12 (9.6%) were coinfected with at least 1 virus (16.7% vs 9.6%, *P* = .326), whereas among the 41 (36.0%) who did not receive antibiotics, 3 were coinfected (7.3%) (none vs 7.3%, *P* = .562).

**DISCUSSION**

This is the first prospective longitudinal study in a single cohort of pilgrims using qPCR to investigate carriage of *Streptococcus pneumoniae* before departing for the KSA, during the pilgrimage in the KSA, and just before leaving the KSA. We observed a 2.7-fold increase in the nasal carriage rate of *S. pneumoniae* in the pilgrims returning from the 2012 Hajj pilgrimage, with about 1 of 5 pilgrims testing positive before leaving the KSA. Although such colonization is usually asymptomatic, symptomatic individuals colonized with *S. pneumoniae* can serve as a reservoir for the person-to-person transmission of pneumococcus in France upon their return.

Pneumonia is a leading cause of hospitalization of pilgrims in Saudi hospitals, including intensive care units, during the Hajj period [4, 5]. *Streptococcus pneumoniae* remains one of the most common pathogens isolated from these patients [5]. Pneumococcal infections, including pneumonia, continue to cause substantial morbidity and mortality worldwide [6], especially among the elderly and in those individuals with certain underlying conditions for which pneumococcal vaccination is recommended [7, 8]. Currently, 2 types of pneumococcal vaccines are available: the PPSV23 vaccine and the 13-valent protein-polysaccharide conjugate vaccine (PCV13). It appears as though one-third of the participants in this study had an indication for pneumococcal vaccination according to the French guidelines; however, only half of these pilgrims reported receiving the PPSV23 vaccination before traveling to the KSA. Although PCV13 is available for use in adults aged 50 years and older [9], it has not yet been recommended for healthy adults by the Advisory Committee on Immunization Practices (ACIP). However, the ACIP recommends that adults with immunocompromising conditions who are eligible for pneumococcal vaccination should be vaccinated with both the PCV13 and PPSV23 vaccines [10]. Based on our results, vaccination against pneumococcal diseases should be considered in pilgrims attending the Hajj according to their country’s current recommendations, particularly those pilgrims with medical risk factors for invasive pneumococcal disease. As the conjugate vaccine has proven to be effective in preventing both the symptomatic disease caused by the serotypes in the vaccine [9, 10] and in the asymptomatic colonization of the nasopharynx [11], the vaccination of all target groups may be possibly considered, in the context of the Hajj.

Several limitations to our study need to be considered. First, nasopharyngeal swabs would have been more adequate samples for culture and serotyping of *S. pneumoniae*. Second, collected samples were placed into viral transport medium, which contains antibiotics [12]. Also, a high proportion of pilgrims were prescribed antibiotics during their stay due to lack of knowledge.
of the causative agent of upper respiratory tract infections, and this possibly affected the recovery of pneumococcus. However, this had no impact on the PCR results, as demonstrated above. Third, samples obtained at the beginning of the pilgrimage were stored at room temperature (20°C) for periods up to 30 days before being processed. However, these limitations may have resulted in the degradation of the bacteria and/or genetic material, which may have contributed to underestimation of the frequency of infection.

In conclusion, although our results cannot be extrapolated to all pilgrims due to small sample size, this report demonstrated the acquisition of \textit{S. pneumoniae} nasal carriage in a cohort of pilgrims returning from the 2012 Hajj pilgrimage. Further large-scale studies with culture-based and PCR-based methods

![Figure 1. Summary of the 44 Hajj pilgrims who tested positive for \textit{Streptococcus pneumoniae} during the study period. A total of 146 (88.5%) pre-Hajj samples were collected from participants in the month prior to their departure for the Kingdom of Saudi Arabia (KSA), with a mean storage time of 13 days (range, 5–37 days) at 20°C before they were processed. Nineteen (11.5%) pre-Hajj samples were collected on the day of departure for the KSA (at the Marseille airport) and were stored at 20°C for 30 days after the collection date before they were processed. Additionally, 70 samples were collected from ill pilgrims during the pilgrimage in the KSA, with a mean storage time of 22 days (range, 10–27 days) at 20°C before they were processed. Finally, a total of 154 post-Hajj samples were collected from pilgrims just before leaving the KSA, with a mean storage time of 6 days (range, 5–8 days) at 20°C before they were processed. A, Symptoms during the pilgrims’ stay in the KSA, as reported by the pilgrims just prior to returning to France or after returning to France. B, Antibiotics prescribed during their stay in the KSA. Abbreviations: ILI, influenza-like illness; KSA, Kingdom of Saudi Arabia.](https://cid.oxfordjournals.org/content/cid/58/15/e108)
are needed to confirm the results of this small-scale preliminary study; for detection of specific serotypes, which is important to assess the impact of vaccination; and to better understand the epidemiology of nasopharyngeal carriage of \textit{S. pneumoniae} during mass gathering events.

\textbf{Notes}

\textit{Acknowledgments.} We thank Donia Mouelhi and Quentin Bayard for their technical contributions.

\textit{Financial support.} This work was supported by the Marseille Public Hospitals Authority (AORC 2012).

\textit{Potential conflicts of interest.} All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

\textbf{References}