Streptococcus pneumoniae acquisition among vaccinated Malaysian hajj pilgrims and its associated factors

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ABSTRACT

Objective: To describe the acquisition of Streptococcus pneumoniae among Malaysian hajj pilgrims and to determine the association with pneumococcal vaccination and respiratory symptoms.

Methods: This was a cross sectional study performed on Malaysian hajj pilgrims in a mass ritual gathering event from October to November 2013 in Makkah. Demographic data and associated factors for the respiratory infection were obtained by interview at Kota Bharu airport on their return. A sputum specimen or throat swab was taken from all subjects for S. pneumonia culture and antimicrobial susceptibility testing.

Results: S. pneumonia was isolated in 1.81% (n=10, 95% CI 0.69, 2.93) out of 549 subjects. Fifty percent of them (n=5) had received pneumococcal vaccination. Difficulty in breathing was significantly reduced in subjects without S. pneumonia isolation (p = 0.042). In multivariable analysis, the uptake of pneumococcal vaccination was found to be statistically significant in reducing S. pneumoniae acquisition (p = 0.018) and those at a younger age were more liable to acquire colonization or infection (p=0.018). Runny nose was significantly associated with the vaccinated group (p=0.040).

Conclusion: The acquisition of S. pneumoniae among Malaysian hajj pilgrims in 2013 was significantly low with the uptake of pneumococcal vaccination.

Keywords: Streptococcus pneumoniae, vaccinated, Malaysian, hajj pilgrims, associated factors

INTRODUCTION

Pneumonia has been identified as the leading cause of hospital admission during hajj [1]. The rituals of hajj pilgrims had exposed a large number of people from all over the world in a certain period of time to various infectious agents. Overcrowding situations was a risk factor for transmission of infections especially for organisms that were transmitted via airborne and droplets. Streptococcus pneumoniae was among the most common organism isolated from respiratory tract among hajj pilgrims with respiratory tract infections [2]. In a recent study, almost 20% of pilgrims had acquired S. pneumoniae nasal colonization post hajj [3]. Generally, adults with certain medical conditions are at highest risk for pneumococcal infection. Those include exceeding age of 65 years old, those with comorbid illnesses such as chronic cardiovascular diseases, chronic pulmonary diseases, chronic liver diseases and diabetes mellitus. The latter was often associated with cardiovascular or renal dysfunction, which increases the risk for severe pneumococcal illness [5].

The data on susceptibility patterns of S. pneumoniae in hajj pilgrims is limited. The resistance rate of S. pneumoniae against many antimicrobial agents was high [6]. In the United States, the penicillin-resistant S. pneumoniae rate had increased from 5.6% in 1991 to 20.4% in 2001 [7]. However, a surveillance study in the United States in the year 2012 showed a lower resistant rate of 2.8% of invasive isolates of S.
pneumoniae [8]. In Asian countries, prevalence rates of penicillin resistance were 0.7% and 57.5% in non-meningeal and meningeal isolates respectively [9]. This study also reported an alarming increase for erythromycin resistant S. pneumoniae in 72.7% followed by 69.7%, and 68.9% for azithromycin and clarithromycin resistance respectively. Erythromycin resistance was more frequently found in children (< 5 years old; 44.8%) than in adults (≥65 years old; 25.1%) (OR, 2.9; 95% CI, 2.2 to 3.8; p<0.0001).

Although pneumococcal vaccination was shown to be effective in preventing pneumococcal infections [10], this vaccine is currently not a mandatory requirement for haj pilgrimage leading to low uptakes of pneumococcal vaccine among haj pilgrims, even in developed countries [3, 11]. However, adults and high risk pilgrims were recommended by the Malaysian government to take a single dose of pneumococcal vaccine prior to travelling for hajj [12]. Two types of vaccines were available in Malaysia, which are Pneumococcal Conjugate Vaccine 13 (PCV 13) or Pneumococcal Polysaccharide Vaccine 23 (PPV 23). PCV 13 is a pediatric vaccine available on the market and offers broader protection against 10 and 13 serotypes. PPV 23 protects against 23 pneumococcal serotypes and is recommended for use, mainly in adults.

Thus, the aim of this study was to describe the prevalence of S. pneumoniae respiratory acquisition among Malaysian hajj pilgrims in 2013, to determine its associated factors and also to make a comparison between vaccination status with regards to pneumococcal acquisition and respiratory symptoms.

METHODS

Study design

This was a cross sectional study performed on returning Malaysian hajj pilgrims commencing from October to November 2013. Data and sample collection were performed on studied subjects at Sultan Ismail Petra Airport (SIPA), Kota Bharu, Kelantan, Malaysia. Sampling was performed using a simple random sampling method.

Sultan Ismail Petra Airport

This airport is one of the busiest domestic airports in Malaysia during the Hajj season with approximately 2000 passengers per Hajj season. It receives passengers from Kuala Lumpur International Airport (KLIA) which comprises of pilgrims and ordinary passengers. The pilgrims had one or two days transit in KLIA from the Kingdom of Saudi Arabia (KSA) before arrival at SIPA.

Data collection

Demographic data, co-morbid illnesses and symptoms of respiratory infection were obtained through interviewer guided questionnaires at the airport on their return.

Culture and antimicrobial susceptibility testing

A sputum specimen and a throat swab were taken from each subject for culture and antimicrobial susceptibility testing (AST). The sputum was placed in a sterile plastic container. Throat swabs were transported in Amies media. Upon receiving them at the Microbiology Laboratory, Universiti Sains Malaysia, both specimens were cultured immediately on blood agar and optochin disc was added to isolate S. pneumoniae. The procedure for culturing and identification of organisms was according to standard microbiology laboratory protocols. AST against erythromycin, oxacillin, vancomycin and trimethoprim-sulfamethoxazole was performed using the disc diffusion method and was interpreted according to the Clinical and Laboratory Standards Institute [13].

Data analysis

Data were analyzed and presented as descriptive statistics. The associated factors such as the demographic data, pneumococcal vaccination and co-morbid illnesses with pneumococcal acquisition were analyzed using multiple logistic regressions. The potential risk factors with p <0.25 in univariate analysis proceeded to multiple logistic regression models for multivariable analysis of numerical and categorical variables like gender, presence of co-morbid illnesses and pneumococcal vaccine uptake.

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee, Universiti Sains
Malaysia (USMKK/PPP/JEPeM (266.3 (3)) prior to conducting the study. Written consent was obtained from the subjects upon enrolment into the study.

RESULTS

Demographic data

A total of 551 subjects agreed to participate in the study. However, some of the data were incomplete due to brief contact during the interview session. The interview sessions were conducted in a short period while pilgrims were waiting for their luggage. Thus, the total subjects for each variable analyzed varied. The mean age of the subjects was 54.07 ±10.36 years (n=522), male were 47.2% (n= 255 out of 540) and 79.3% (n= 388 out of 489, 95% CI) received pneumococcal vaccination.

Associated factors of S. pneumoniae acquisition

S. pneumoniae was isolated in 1.81% (n=10, 95% CI 0.69, 2.93) out of 549 subjects. Eight out of 10 subjects were symptomatic for respiratory infection such as cough (n=8) and runny nose (n=6). Two out of the 10 subjects had influenza like illness (ILI) symptoms (fever, cough and runny nose).

Table 1. Associated factors for acquisition of Streptococcus pneumoniae.

| Variables (n, total analyzed) | Acquisition of Streptococcus pneumoniae | Univariate | Multivariate |
|-------------------------------|----------------------------------------|------------|-------------|
|                               | Yes, n (%) or mean (SD) | No, n (%) or mean (SD) | OR (95% CI) | Wald | p-value | OR (95% CI) | Wald | p-value |
| Age* (549)                    | 48.78 (16.47) | 54.08 (10.22) | 0.95 (0.90-1.01) | 2.38 | 0.123 | 0.91 (0.85-0.99) | 5.56 | 0.018 |
| Gender (M) (540)              | 3 (30.0) | 252 (47.5) | 0.47 (0.12-1.85) | 1.16 | 0.281 | - | - |
| Pneumococcal vaccination (489) | 5 (55.6) | 383 (79.8) | 0.32 (0.08-1.20) | 2.86 | 0.091 | 0.16 (0.04-0.73) | 5.63 | 0.018 |
| Cough (540)                   | 8 (80.0) | 403 (76.0) | 1.26 (0.26-6.01) | 0.08 | 0.771 | - | - |
| Runny nose (540)              | 6 (60.0) | 221 (41.7) | 2.10 (0.59-7.52) | 1.29 | 0.256 | - | - |
| ILI (541)                     | 2 (20.0) | 88 (16.6) | 1.26 (0.26-6.03) | 0.08 | 0.774 | - | - |
| Difficulty in Breathing (541) | 2 (20.0) | 24 (4.5) | 5.28 (1.06-26.23) | 4.14 | 0.042 | 13.50 (2.16-84.24) | 7.76 | 0.005 |
| Myalgia (539)                 | 3 (30.0) | 74 (14.0) | 2.64 (0.67-10.42) | 1.91 | 0.167 | - | - |
| Diabetes (541)                | 0 (0.0) | 77 (14.5) | - | - | 0.997 | - | - |
| COAD (541)                    | 0 (0.0) | 2 (0.4) | - | - | 1.000 | - | - |
| Asthma (541)                  | 0 (0.0) | 17 (3.2) | - | - | 0.999 | - | - |
| Smoking (541)                 | 0 (0.0) | 26 (4.9) | - | - | 0.998 | - | - |
| Chronic renal disease (541)   | 0 (0.0) | 6 (1.1) | - | - | 0.999 | - | - |
| Chronic heart disease (541)   | 1 (10.0) | 16 (3.0) | 3.58 (0.43-29.95) | 1.38 | 0.240 | - | - |

*Years (SD), Simple Logistic Regression, *Backward LR Multiple Logistic Regression model was applied. Age, pneumococcal vaccination, difficulty in breathing, myalgia and chronic heart disease were entered to the model. Multicollinearity and interaction were checked and not found. Hosmer-Lemeshow, (p=0.257), classification table (overall correctly classified percentage=98.3%) and ROC curve (80.0%) were applied to check the model fitness.
Table 2. Comparison of respiratory symptoms between pneumococcal vaccinated and non-vaccinated group.

| Variables (n, total analyzed) | Pneumococcal vaccination | Univariate | Multivariate |
|-------------------------------|--------------------------|------------|-------------|
|                               | Yes, n (%) | No, n (%) | OR (95% CI) | Wald | P-value | OR (95% CI) | Wald | P-value |
| Age (years, SD)* (475)        | 55.61 (10.11) | 55.43 (10.43) | 0.98 (0.96, 1.01) | 2.41 | 0.120 | - | - | - |
| Male gender (498)             | 186 (47.2) | 45 (43.3) | 0.85 (0.55, 1.32) | 0.51 | 0.474 | - | - | - |
| Cough (498)                   | 300 (76.3) | 79 (75.2) | 1.06 (0.64, 1.75) | 0.06 | 0.815 | - | - | - |
| Runny nose (498)              | 153 (38.8) | 52 (50.0) | 0.64 (0.41, 0.98) | 4.20 | 0.040 | 0.64 (0.41, 0.98) | 4.20 | 0.040 |
| ILI‡ (499)                    | 60 (15.2) | 19 (18.1) | 0.81 (0.46, 1.44) | 0.51 | 0.475 | - | - | - |
| Difficulty in Breathing (499) | 19 (4.8) | 6 (5.7) | 0.84 (0.33, 2.15) | 0.14 | 0.710 | 0.92 (0.36, 2.39) | 0.03 | 0.864 |
| Myalgia (497)                 | 54 (13.8) | 12 (11.4) | 1.24 (0.64, 2.41) | 0.40 | 0.530 | - | - | - |

* Mean (SD). ‡ Influenza-like illness which consisted of triad respiratory symptoms (fever, cough and runny nose).
† Backward LR Multiple Logistic Regression model was applied. Multicollinearity and interaction were checked and not found.

Table 3. Comparison of post-hajj Streptococcus pneumoniae acquisition among international hajj pilgrims.

| Study | Country of pilgrims | Year | Reference | Total samples (pre-hajj and post-hajj) | Method used for detection | Pneumococcal acquisition post-hajj, n (%) | Pneumococcal vaccination, n (%) |
|-------|---------------------|------|-----------|---------------------------------------|---------------------------|------------------------------------------|--------------------------------|
| 1     | Malaysia            | 2013 | Our study | 549                                   | Culture                   | 10 (1.81)                                | 388 (79.0)                      |
| 2     | Multi-countries     | 2011 & 2012 | Memish et al. 2015 [2] | 3203                                   | Culture                   | 191 (6.0)                                | 1782 (61.1)*                    |
| 3     | French              | 2012 | Benkouiten et al 2014 [3] | 169                                    | Realtime PCR              | 30 (19.5)                                | 47 (35.9)                       |
| 4     | French              | 2013 | Benkouiten et al 2014 [4] | 255                                    | Realtime PCR              | 29 (36.3)                                | 66 (51.2)                       |

*Influenza or pneumococcal vaccination

Only one subject was asymptomatic possibly in a carrier state. In the univariable analysis, difficulty in breathing was significantly reduced in subjects without S. pneumoniae isolation (p = 0.042) (Table 1). Five out of 10 subjects (50%) with positive isolation of S. pneumoniae had received pneumococcal vaccination. The uptake of pneumococcal vaccination was found to be statistically significant in reducing pneumococcal acquisition (p = 0.018) and those who were younger had more tendencies to acquire colonization or infection (p = 0.018) (Table 1).

**Associated factors of pneumococcal vaccination**

In the univariate analysis between pneumococcal vaccinated and non-vaccinated groups (Table 2), pilgrims who were vaccinated were significantly more likely than those who were unvaccinated to report (or to have) a runny nose (p = 0.040). Other clinical symptoms such as ILI and difficulty in breathing were reduced in the vaccinated group, but they are not statistically significant.

**Antibiotic susceptibility pattern**

Only eight isolates were viable for antimicrobial susceptibility testing. The resistance rate of the isolates to penicillin, erythromycin and trimethoprim-sulfamethoxazole were 25%,
37.5% and 50% respectively. The isolates showed 100% susceptibility to vancomycin.

**DISCUSSION**

Performing Hajj rituals is a risk factor for pneumococcal acquisition [2,14]. There is currently limited microbiological data regarding the prevalence and causes of respiratory infection during Hajj. In our study, we found that the prevalence of *S. pneumoniae* acquisition in Malaysian hajj pilgrims was very low (1.81%) compared to previous studies that reported higher prevalence ranging from 4.8% to 53.8% [2,3,15-18].

The lower pneumococcal acquisition might be caused by several reasons. One of the possible reasons was the higher uptake of pneumococcal vaccination in this study subjects (n=388/79.0%) compared to other previous studies (n=47/35.9%) [3] and (n=66 /51.2%) [4]. Pneumococcal vaccination had been proven to provide protection against *S. pneumoniae* infection [19]. The other reason could be the less sensitive method of isolation. The culture method had lower sensitivity compared to qPCR used in previous study [14].

In another study, PCR showed better sensitivity in detecting *S. pneumoniae* in patients who had been pretreated with antibiotics when culture methods came out as negative for this organism [20]. Thus, using culture methods to detect this organism in our study might have reflected the low prevalence of *S. pneumoniae*.

Furthermore, there is the probability that the majority of Malaysian hajj pilgrims had received antimicrobial agents whilst in KSA. Previous study has revealed that 58.8% of Malaysian hajj pilgrims received antimicrobial agents for upper respiratory tract infection [21]. Prior antimicrobial agent administration could have been one of the contributing factors for low isolation of *S. pneumoniae* in this study.

Among all the studied subjects, 38.2% had isolated *Hemophilus influenzae*. Out of 10 pilgrims who had *S. pneumoniae* isolated, only two had concurrent infection with *H. influenzae*, three had concurrent infections with other organisms and the rest had single isolation of *S. pneumoniae*. A previous study reported that the overgrowth of *H. influenzae* in the nasal cavity in the mouse model might suppress the growth of *S. pneumoniae* [22]. Therefore, the low prevalence of *S. pneumoniae* acquisition in this study could be potentially due to overgrowth of *H. influenzae*.

In this study, those who were younger had more tendencies to acquire colonization or infection (p =0.018). This was thought that younger pilgrims were more active and more exposed to the extremely high density of the crowd during performing hajj rituals. However, gender was not associated with pneumococcal acquisition. In contrast to previous studies, pneumococcal infection could be acquired due to the tendency of smoking in males and subsequently having chronic obstructive airway disease [23]. Males also had a higher prevalence of chronic heart diseases which is also one of the risk factors for pneumococcal acquisition [5].

In this study, the uptake of pneumococcal vaccination was found to be statistically significant in reducing pneumococcal acquisition (p = 0.018). Compared to other similar studies on acquisition of *S. pneumoniae* in the vaccinated group among hajj pilgrims from other countries (Table 3), this study revealed a lower percentage of acquisition of *S. pneumoniae* with almost 80% of the vaccinated population (p = 0.018) (Table 1). We postulated that our hajj pilgrims’ population had achieved herd immunity in spite of our limitations in ascertaining strain serotyped and genotyped that was covered by pneumococcal vaccination. Benkouiten et al 2014 reported the increasing trend of *S. pneumoniae* acquisition in their study in 2013 compared to 2012 in spite of increased pneumococcal vaccination coverage of their hajj pilgrims’ population [3]. We postulated that the herd immunity was not achieved in their hajj pilgrims’ population.

In this study, the resistance rate of *S. pneumoniae* to penicillin, erythromycin and trimethoprim-sulfamethoxazole was 25%, 37.5% and 50% respectively. The results were comparable to a previous study which reported that resistant rates were 22% for penicillin; 35% for erythromycin, clarithromycin, and azithromycin; and 42% for cefaclor [6]. However, our resistance rate was much higher as compared to other previous studies conducted among Malaysian subjects in 1999. They discovered that the resistance rate to penicillin,
erythromycin and trimethoprim-sulfamethoxazole was 7.0%, 1.1% and 9.7% respectively [24]. Diagnosis and treatment of pneumonia in a ritual mass-gathering situation is a medical challenge requiring rapid decision-making and knowledge of its etiology and susceptibility pattern. Therefore, the findings in this study would help clinicians to choose appropriate antimicrobial therapy for Malaysian hajj pilgrims with respiratory symptoms and would also discourage the indiscriminate use of antimicrobial agents and thus prevent further development of bacterial resistance.

The limitations of this study include incomplete data due to brief contact time during the interview sessions. Two of the isolates were not viable to proceed with antimicrobial susceptibility testing because of repeated subculturing to get pure culture.

In conclusion, the acquisition of *S. pneumoniae* among Malaysian Hajj pilgrims in 2013 was significantly low with the uptake of pneumococcal vaccination. Further large-scale studies with culture-based and PCR-based methods were required for the detection of specific serotypes and genotypes, which is imperative to assess the impact of vaccination and the resistance mechanism. Thus, a better understanding of the epidemiology of *S. pneumoniae* during mass gatherings should be obtained.

**Funding:** This study was funded by Ministry of Higher Education Malaysia through Long-term Research Grant Scheme (203.PTS.6728003).

**Acknowledgments:** We gratefully acknowledge Malaysia Airports Sdn Bhd, Sultan Ismail Petra Airport, Kota Bharu, Kelantan and Lembaga Tabung Haji Malaysia for giving permission and providing flights arrival schedules for subjects interview and samples collection at the airport. We appreciate the contributions of three students in Master of Microbiology program; Dr Zety Norfidiyati Ayub, Dr Suhana Hashim and Dr Wan Norliyana Wan Mahmud, who took part in samples and data collection and data transfer into SPSS. We also thank Mrs Rosmaniza Abdullah for her laboratory technical contribution.

**Conflict of interest:** The authors declare that they have no competing interests.

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