Surveillance of Australian Hajj pilgrims for carriage of potentially pathogenic bacteria: Data from two pilot studies

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Institutional review board statement: Ethics approval was granted by the Hunter New England Human Research Ethics Committee (HREC), Australia (Ref: HREC/13/HNE/265). To verify the vaccination records of pilgrims, data were cross-checked with another ongoing trial by our team with a separate ethics approval from the Hunter New England HREC (Ref:13/05/15/3.05).

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Data sharing statement: There are no additional data available.

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INTRODUCTION

Hajj is one of the world’s largest annual mass gatherings, attracting approximately 2-3 million people each year from around the globe. During Hajj there is a high risk of communicable diseases, primarily due to overcrowding, shared accommodation and mingling of local and international pilgrims[1,2]. The implementation of public health measures to prevent the transmission and limit the impact of mass gatherings on pharyngeal carriage of pathogens among Hajj pilgrims is critical to reduce the risk of disease transmission when they return home.

METHODS

In 2014, surveillance was conducted in two phases among Australian Hajj pilgrims: The first phase during Hajj in Mina, and the second phase soon after returning home to Australia. Nasopharyngeal or oropharyngeal swabs were taken from participants then tested, firstly by nucleic acid testing, and also by standard culture.

RESULTS

Of 183 participants recruited in the first phase, 26 (14.2%) tested positive for S. pneumoniae; 4 had received pneumococcal conjugate vaccine (PCV13). Only one tested positive for N. meningitidis (W). Of 93 23rd phase samples cultured, 17 (18.3%) grew S. aureus, all methicillin sensitive, 2 (2.2%) grew N. meningitidis (on subculture; one serotype B, one negative), and 1 (1%), from an unvaccinated pilgrim, grew S. pneumoniae.

CONCLUSION

Relatively high carriage of S. pneumoniae and little meningococcal carriage was found. This indicates the importance of a larger study for improved infection surveillance and possible vaccine evaluation.

Key words: Carriage; Conjugate vaccine; Staphylococcus aureus; Neisseria meningitidis; Streptococcus pneumoniae; Hajj

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Core tip: We conducted this pilot study to understand the impact of mass gatherings on pharyngeal carriage of potentially pathogenic bacteria and to assess the burden of pathogenic microorganisms resistant to antimicrobial agents among travellers returning to Australia following an overseas travel. This study demonstrates that a larger study is feasible and important to inform public health measures to prevent the transmission and limit emergence of antimicrobial resistant pathogens at mass gathering events such as the annual Hajj pilgrimage.

these about 1.5% were methicillin resistant *S. aureus* (MRSA)\(^{22}\). In another study in four Makkah hospitals spanning twelve months from 2004-2005 that included the Hajj season, MRSA accounted for 199 of 512 (39%) *S. aureus* clinical isolates\(^{23}\).

Inappropriate antimicrobial use during Hajj could result in the emergence of drug-resistant organisms, and antibiotic resistant respiratory organisms have been frequently isolated from Hajj pilgrims\(^{24-26}\). The potential for worldwide outbreak of infectious diseases caused by resistant microorganisms such as ciprofloxacin-resistant *N. meningitidis*, penicillin-resistant *S. pneumoniae*, MRSA and extended-spectrum beta-lactamase (ESBL) producing Gram negative bacteria is increasingly recognised\(^{27-30}\). Recently, there was a worrying report of the acquisition of extended-spectrum cephalosporin and colistin-resistant *Salmonella enterica* in a returned French Hajj pilgrim\(^{31}\).

The pharyngeal carriage of bacterial pathogens among Australian pilgrims has not been evaluated. Therefore, we performed two pilot studies, during and after Hajj, to estimate the pharyngeal carriage rate of *N. meningitidis*, *S. pneumoniae* and *S. aureus* among Australian Hajj pilgrims who attended Hajj in 2014, assessed antimicrobial susceptibility patterns and investigated the possible impact of preventive measures such as pre-travel vaccination and facemasks use.

**MATERIALS AND METHODS**

Enhanced surveillance was conducted in two phases among Australian pilgrims: The first phase involved recruiting pilgrims during their tent stay in Mina, Makkah, KSA in the peak period of the Hajj 2014, and the second phase involved recruiting pilgrims after their return from Hajj to Australia (Figure 1).

**First phase (during Hajj)**

During Hajj 2-6 October 2014 Mina, Makkah

193 flocked swabs were collected (*n* = 183) (10 participants swabbed twice)

Post-Hajj Sydney, Australia

All tested by nucleic acid testing

**Second phase (post-hajj)**

Within 2 mo after Hajj Sydney, Australia

30 (out of 183) were followed up after Hajj (*n* = 30)

63 additional Hajj pilgrims from same groups were recruited (*n* = 63)

Two swabs were taken from each participant

All tested by standard culture for growth of pneumococcal, staphylococcal and meningococcal organisms

**Figure 1** Schematic diagram showing recruitment of pilgrims.
using charcoal and non-charcoal Copan Amies agar gel swabs and transported to the laboratory on ice within four hours of collection.

**Phenotypic identification of *N. meningitidis*, *S. pneumoniae* and *S. aureus***

Swabs collected during the second phase were directly plated onto mannitol aztreonam methicillin salt, chocolate and nalidixic acid (Oxoid, Basingstoke, England) and modified New York City (Becton Dickinson, Sparks, MD, United States) agar plates. Bacterial colonies growing following 24-48 h of incubation in 5% CO₂ at 37 °C were identified using the Bruker Microflex LT (Bruker Daltonics Inc., Billerica, MA, United States) matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometer. Antimicrobial susceptibility testing was performed using E-test (AB BIODISK, Solna, Sweden) or the BD Phoenix (Becton Dickinson) automated microbiology system. Serotyping of *N. meningitidis* was performed on all isolates using agglutination serum (Remel Europe Ltd., Dartford, England).

**Nucleic acid test for *N. meningitidis* and *S. pneumoniae***

Swabs collected during the first phase were vortexed in 3 mL of UTM. Nucleic acid (NA) was extracted from 250 µL of UTM sample using the Qiagen EZ1 Virus Mini kit on the Qiagen EZ1 Advanced XL instrument. NA was eluted in a final volume of 60 µL and stored at -80 °C prior to nucleic acid testing (NAT).

**NAT of *S. pneumoniae***

*S. pneumoniae* was detected using a modified version of an assay previously described[33], targeting a 101 base-pair segment of the autolysin-encoding (*lybA*) gene [forward primer, 5’-AGCAGATCTAGAGATGAGC-3’; reverse primer, 5’-TTTTCGGTTGGTTATTGTGC-3’; probe, 5’-6-carboxy-fluorescein (FAM)-TTTTCGGTTTCAGGATGACGG-3’].

Baseline fluorescence was determined using a fluorescence reader (FluorTracker™, Stratagene, La Jolla, CA, United States) before amplification in the Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany). The reaction mix was amplified at 95 °C × 15 min (96 °C × 10 s; 63 °C × 1 min) for 45 cycles and 72 °C × 2 min for one cycle. End point fluorescence was then determined using FluorTracker™, positive samples were defined as a minimum of 2 × increase in fluorescence. These results were confirmed by agarose gel electrophoresis on a 2% gel run at 200 volts for 40 min and stained with SYBR-Safe.

**NAT of *N. meningitidis***

*N. meningitidis* was detected using a previously described assay[34], that uses a single amplification real-time PCR targeting a 110 base-pair segment of the meningococcal capsular transfer gene, *ctrA* [forward primer, 5’-GCTGCGGTAGGTGGTTCAA-3’; reverse primer, 5’-TTTTCGGTTTCAGGATGACGG-3’; probe, 5’-6-carboxy-fluorescein (FAM)-CATTGGCAGCCTGCACTAT-BHQ-1-3’]. The reaction mix was amplified in a Roche LightCycler® 480 (Roche Diagnostics GmbH, Mannheim, Germany) at 95 °C × 5 min (95 °C × 15 s, 60 °C × 1 min) × 45; 40 °C × 30 s with detection on the 640 nmol/L channel during elongation at 60 °C.

**NAT of *N. meningitidis* serogroup***

Samples where *N. meningitidis* was detected were further evaluated using a previously described molecular serotyping method[35]. Samples were tested using five single-plex conventional assays targeting different regions of the *orf-2* and *siaD* genes which are specific for serotypes A, B, C, W and Y. The primer sequences are listed in Table 1. The reaction mixes were amplified at 95 °C × 15 min (95 °C × 30 s; 50 °C/55 °C × 1 min; 72 °C × 30 s) for 40 cycles and 72 °C × 5 min for one cycle. The resultant products were visualised by agarose gel electrophoresis on a 2% gel run at 200 volts for 40 min and stained with SYBR-Safe.

**Ethical approval***

Ethics approval was granted by the Hunter New England Human Research Ethics Committee (HREC), Australia (Ref: HREC/13/HNE/265). To verify the vaccination records of pilgrims, data were cross-checked with another ongoing trial by our team with a separate ethics approval from the Hunter New England HREC (Ref13/05/15/3.05).

### Table 1 The primer sequences for *Neisseria meningitidis*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Gene target</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>orf-2</em></td>
<td>F: CGCAATAGGTGTATATATTCTTCC; R: CGAATAAGTTTCCATATGCTTCC</td>
<td>400</td>
</tr>
<tr>
<td>B</td>
<td><em>siaD</em></td>
<td>F: GGAATCTTACGTTTGCACCA; R: GCAATGCTGAGGATTAAGCATTAA</td>
<td>450</td>
</tr>
<tr>
<td>C</td>
<td><em>siaD</em></td>
<td>F: TCATTAGAGTTTGCGAATAGAAGGT; R: CCTACGAGTTTGCACCAATTGAC</td>
<td>250</td>
</tr>
<tr>
<td>W135</td>
<td><em>siaD</em></td>
<td>F: CACAACCATTTTCATTATATAGTTACTGT</td>
<td>120</td>
</tr>
<tr>
<td>Y</td>
<td><em>siaD</em></td>
<td>F: CTCAAAACCGAAAGGCTTGTTGTTA; R: CTGAAACCGTTTTTTGATAATTGCTAA</td>
<td>120</td>
</tr>
</tbody>
</table>

*Orf2*: Open reading frame; *siaD*: Polysialyltransferase gene.
A total of 246 pilgrims were recruited to this study: 183 in the first phase during Hajj and 93 in the second phase after Hajj; 30 appeared in both groups (Figure 1). The median age for pilgrims was 40 years (range 12-67), 126 (51.2%) were women (Tables 2 and 3).

**First phase (during Hajj)**

One hundred and ninety three samples were collected from 183 study participants. Ten participants provided two swabs, the first collected on their first day in Mina and the other on their last day in Mina (NAT) or on their last day of Hajj. Of the oropharyngeal samples collected from 93 pilgrims, 30 appeared in both groups (Figure 1). Of the 93 participants in this group, 72 reported receiving quadrivalent meningococcal conjugate vaccine before travelling to Hajj: 48 (51.6%) polysaccharide vaccine and 41 (44.1%) conjugate vaccine. Four (4.3%) did not recall their vaccination history but did not disclose whether a facemask, the pneumococcal carriage rate was similar in those who used a facemask compared to those who did not [14.1% (13/92) vs 14.5% (11/76), \( P = 0.95 \)]. There was no statistically significant difference in pneumococcal carriage rates based on age < 50 years (16.7% vs 11.5%, \( P = 0.3 \)) or gender (female vs male = 17.1% vs 9.7%, \( P = 0.2 \)). The only pilgrim with positive \( N. meningitidis \) PCR reported not using a facemask during Hajj.

**Second phase (post-Hajj)**

Of the oropharyngeal samples collected from 93 pilgrims, \( S. aureus \) was isolated in 17 (18.3%), and all were methicillin susceptible (Table 3). \( N. meningitidis \) was isolated in two (2.2%) samples; on subculture, one was serotype B and sensitive to benzylpenicillin and cefotaxime, the other was negative on subculture. Both pilgrims reported receiving the quadrivalent meningococcal polysaccharide vaccine. In this group 89 (95.7%) reported receiving meningococcal quadrivalent vaccine before travelling to Hajj: 48 (51.6%) polysaccharide vaccine and 41 (44.1%) conjugate vaccine. Four (4.3%) did not recall their vaccination history but having attended Hajj before, were likely to have been vaccinated previously.

\( S. pneumoniae \) was isolated from one pilgrim and it could not be serotyped and sensitivity was not done; this pilgrim had not been vaccinated against pneumococcus. Of the 93 participants in this group, 38 (40.9%) reported receiving pneumococcal vaccine, PCV13 in all. Thirty-two (34.4%) reported using a facemask, 59 (63.4%) reported not using a facemask during Hajj and the other 2 (2.2%) did not disclose whether they used a facemask or not. Of 32 pilgrims who used a facemask, \( S. aureus \) was isolated from 8 (25%), and of 59 pilgrims who did not use a facemask \( S. aureus \) was isolated from 9 (15%) \( (P = 0.27) \). Both pilgrims from whom meningococci were isolated reported using a facemask, the pilgrim from whom pneumococcus was recovered did not disclose whether a facemask was used or not. There was no statistically significant difference in staphylococcal carriage rates based on age < 50 years (17.5% vs 23%, \( P = 0.6 \)) or gender (male vs female = 20% vs 13%, \( P = 0.4 \)).

### Table 2  Demographics of participants (during and post-Hajj, \( n = 246 \))

<table>
<thead>
<tr>
<th>Attributes</th>
<th>During Hajj ( n (%) )</th>
<th>Post-Hajj ( n (%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>111 (60.7)</td>
<td>30 (32.3)</td>
</tr>
<tr>
<td>Male</td>
<td>72 (39.3)</td>
<td>63 (67.7)</td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>19-34</td>
<td>38 (20.8)</td>
<td>17 (18.3)</td>
</tr>
<tr>
<td>35-49</td>
<td>58 (31.7)</td>
<td>52 (55.9)</td>
</tr>
<tr>
<td>50-64</td>
<td>26 (15.3)</td>
<td>8 (8.6)</td>
</tr>
<tr>
<td>≥ 65</td>
<td>1 (0.6)</td>
<td>0</td>
</tr>
<tr>
<td>Did not disclose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningococcal vaccine uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>0</td>
<td>4 (4.3)</td>
</tr>
<tr>
<td>Meningococcal polysaccharide vaccine</td>
<td>144 (78.7)</td>
<td>48 (51.6)</td>
</tr>
<tr>
<td>Meningococcal Conjugate vaccine</td>
<td>39 (21.3)</td>
<td>41 (44.1)</td>
</tr>
<tr>
<td>Pneumococcal vaccine uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>145 (79.2)</td>
<td>55 (59.1)</td>
</tr>
<tr>
<td>Pneumococcal conjugate vaccine (PCV13)</td>
<td>38 (20.8)</td>
<td>38 (40.9)</td>
</tr>
<tr>
<td>Facemasks used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used facemasks</td>
<td>76 (41.5)</td>
<td>32 (34.4)</td>
</tr>
<tr>
<td>Did not use facemasks</td>
<td>92 (50.3)</td>
<td>59 (63.4)</td>
</tr>
<tr>
<td>Did not disclose</td>
<td>15 (8.2)</td>
<td>2 (2.2)</td>
</tr>
</tbody>
</table>

### Table 3  Carriage rate of \( S. pneumoniae, N. meningitidis \) and \( S. aureus \)

<table>
<thead>
<tr>
<th></th>
<th>During Hajj</th>
<th>Post-Hajj</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First day of Mina (NAT)</td>
<td>Last day of Mina (NAT)</td>
<td>Standard culture</td>
</tr>
<tr>
<td>( S. pneumoniae )</td>
<td>1/10</td>
<td>26/183</td>
<td>1/93</td>
</tr>
<tr>
<td>( N. meningitidis )</td>
<td>--</td>
<td>1/183</td>
<td>2/93</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>--</td>
<td>--</td>
<td>17/93</td>
</tr>
</tbody>
</table>

\( ^1 \) \( P \) value is for the difference in carriage detection rates for the last day of Mina vs post-Hajj by standard culture. NAT: Nucleic acid testing; \( N. meningitidis \); \( S. pneumoniae \); \( S. aureus \); \( S. pneumoniae \) polysaccharide vaccine; \( S. pneumoniae \) conjugate vaccine.

PCV13: Pneumococcal conjugate vaccine 13-valent.
participants had taken antibiotics (either amoxicillin, amoxicillin/clavulanic acid and/or roxithromycin) while at Hajj; however, none had taken antibiotics within 2 wk prior to swab collection. *S. aureus* was isolated from two of those who reported using antibiotics during Hajj and *S. pneumoniae* from another one.

**DISCUSSION**

We found a 14.2% pneumococcal carriage rate in pilgrims during the Hajj 2014, which is moderately high. About 2 in 5 received conjugate pneumococcal vaccine before travel. Carriage was similar irrespective of whether pneumococcal vaccine had been given, reflecting the likelihood that many pilgrims were already colonised before being vaccinated and that vaccination is more potent in preventing acquisition than in extinguishing carriage.

Prevalence of pneumococcal carriage was almost double the rate reported among French pilgrims during the early phase of the Hajj 2012 (7.3%), but lower than the rate (19.5%) found a few days before the pilgrims’ departure from KSA[36]. In a study of 3203 pilgrims (1590 at the beginning, and 1613 at the end of Hajj), Memish et al[37] demonstrated that, although the overall carriage rate of pneumococci among African and Asian pilgrims in the early weeks of the Hajj 2011 and 2012 was 4.4%, the prevalence of PCV13 vaccine-serotypes was only 1.1%. In the same cross-sectional investigation, the overall carriage rate was 7.5% during the later phase of Hajj and 3.6% belonged to PCV13 vaccine-serotypes[37]. Subsequently the investigators conducted a prospective cohort study during the Hajj 2013 demonstrating that 1.8% pilgrims before and 7.1% (*P < 0.01*) pilgrims immediately after the conclusion of Hajj carried pneumococci; 35.5% serotypes are covered by PCV-13[38]. However, the carriage rates reported in all studies including ours, was much lower than the high carriage rate of 62% found by Benkouiten et al[39] among French pilgrims during the Hajj 2013. The pneumococcal carriage rate in the post-Hajj phase was very low (1.1%). We are unaware of any other pneumococcal carriage study in pilgrims after their return to their home countries for comparison. High PCV13 uptake (39%) in the post-Hajj cohort may have reduced the carriage rate or it could be an effect of antibiotic use (17.2% reported receiving antibiotics while at Hajj). Also, there was a time difference of up to two months between collection of Hajj and post-Hajj samples, enough time for most pilgrims to have lost carriage of Hajj-associated pneumococci. The diagnostic tests used differed between our study phases (PCR was used for first phase, and standard culture for the second phase) which may explain the low detection rate in the post-Hajj phase.

The uptake of PCV13 in the first cohort of our study, 21%, and in the second cohort (post-Hajj), 40.9%, was higher than any other report. This reflects pilgrims’ participation in a vaccine trial involving PCV13. However, we did not find significant difference in pneumococcal carriage rate between vaccinated and unvaccinated pilgrims. Although not significant, it was lower in the vaccinated group (Table 4), possibly because of the small sample size or because a large proportion of the serotypes were not covered by PCV13. Although serotype characterisation was not performed in our pilot study, other studies suggest that between a quarter and half of the serotypes at Hajj are not covered by PCV13. None of the pilgrims in our cohorts reported having received pneumococcal polysaccharide vaccine, because only a few (3.3%) suffered from chronic diseases for which pneumococcal vaccination is recommended, and only one was aged over 65 years. In another study, overall pneumococcal polysaccharide vaccine uptake among Australian pilgrims ranged between 14% and 29%[40]. International studies have shown that the overall uptake of pneumococcal vaccine in Hajj pilgrims ranged between 2.5% and 36%[41-43].

The low meningococcal carriage rate of 0.6% during Hajj is not surprising because of more universal vaccination, nearly half with quadrivalent conjugate vaccine. During Hajj 2012 and 2013, Benkouiten et al[39] failed to detect *N. meningitidis* in nasal and/or throat swabs collected from French pilgrims. However, a study conducted in Mina during Hajj 2003 among 344 pilgrims from 29 different nations identified a carriage rate of 3.2%[44], following the 2000-2001 W epidemic.

The post-Hajj meningococcal carriage rate of 1.1% is less than in other studies. After the worldwide meningococcal W outbreak following 2000 Hajj, the carriage among Singaporean pilgrims two weeks after the Hajj 2001 was 15% for serogroup W with 55% persisting as carriers for 5-6 mo[45]. During the following year, El Bashir et al[46] demonstrated a carriage rate of 6.3% among United Kingdom pilgrims for all serogroups 2-6 wk after the pilgrims’ return from Hajj. Twenty one percent of the pilgrims reported receiving antibiotics for respiratory illnesses during Hajj[8]. This high rate of antibiotic use compares with 17.2% reported by participants in our study. In 2010, Ceyhan et al[47] reported that 27% of returned Turkish Hajj pilgrims were positive for meningococcal carriage, mostly W-135. Airport-based surveillance studies conducted in 2001 in Thailand[48] and the United States[49] demonstrated a meningococcal carriage rate of 0% and 2.6%, respectively. This is similar to the 1.4% carriage rate in a more contemporary study in Iran in 2012[50]. In the

<table>
<thead>
<tr>
<th>Table 4 Pneumococcal carriage rates according to the uptake of 13-valent pneumococcal conjugate vaccine in first phase of study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCR positive for pneumococci n (%)</strong></td>
</tr>
<tr>
<td>PCV13</td>
</tr>
<tr>
<td>No PCV13</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

PCV13: Pneumococcal conjugate vaccine 13-valent.
latter two studies respectively, 15% and 58.5% pilgrims reported using antibiotics during Hajj[48-49]. Other studies conducted in Iran and Kuwait demonstrated that a single dose of ciprofloxacin before travel essentially eradicated meningococcal carriage[50,51]. The low carriage rate several weeks after Hajj in our study could possibly be indicative of the effect of a fairly high uptake (44.1%) of conjugate meningococcal vaccine. By contrast, the reported uptake of conjugate meningococcal vaccine among international pilgrims at Hajj 2013 was only 0.2%[32]. Worldwide, few pilgrims receive the conjugate vaccine because of its costs. In a surveillance study conducted in 2009 involving 1400 Hajj pilgrims of 14 nationalities, Ashgar et al[56] found the carriage rate of meningococci among arriving Hajj pilgrims to be 5.9%, increased by the end of the pilgrimage to 11.1% (P = 0.03)[56]. They also reported circulation of meningococcal strains resistant to azithromycin, ceftriaxone, ciprofloxacin, levofloxacin, meropenem and rifampicin[28].

Due to the public health significance, monitoring of antimicrobial susceptibility of clinical specimens for meningococci and pneumococci is important[52], particularly since pilgrims from high-risk countries in the African meningitis belt are routinely given ciprofloxacin prophylaxis on arrival for pilgrimage into KSA. Transnational dissemination of multi-drug resistant organisms has been reported[28]. This is relevant in the context of pneumococcal disease since about 20% of the pneumococcal isolates at Hajj are penicillin resistant[29]. Circulation of drug resistant pneumococci has been of concern in other mass gatherings, such as the reporting of pathogenic multi-drug resistant strains of S. pneumoniae circulating in Spain at the time of Barcelona Olympic in 1992 (however, the Olympic Organising Committee did not recommend pneumococcal vaccine for visitors)[54,55]. Today, antibiotic resistance is widespread and, considering the high incidence of pneumonia, the high carriage rate of pneumococci and circulation of multi-drug-resistant pneumococci, vaccination is recommended for all high-risk pilgrims and the conjugate vaccine is preferred[20,27,53].

The effect of facemasks use on pharyngeal bacterial carriage at Hajj has not been established yet, although a large trial is underway to examine the effectiveness of facemasks against viral infections[32]. In other settings such as among healthcare workers, the effectiveness of facemasks against pharyngeal bacterial colonisation, including S. pneumoniae was evaluated but no significant effect was observed[56]. Even though we did not find any significant effect of facemasks use on the pharyngeal/nasopharyngeal carriage rate of S. pneumoniae or S. aureus, interestingly a prospective study conducted in the Netherlands among pig farmers demonstrated that the use of facemasks was significantly associated with lower MRSA nasal carriage[57]. Perhaps a larger facemask study could demonstrate its true effect on pharyngeal colonisation of bacteria. We found an 18% carriage rate of S. aureus in the second (post-Hajj) phase of the study, but did not detect MRSA. This compares with a nasal carriage rate of 25% among arriving international pilgrims and 20.9% among departing pilgrims during the Hajj 2009[58] and similar to the nasal carriage rate of methicillin-susceptible S. aureus (28%) elsewhere in Australia[59,60].

To our knowledge, this is the first Australian carriage surveillance study for potentially pathogenic bacteria such as pneumococci, meningococci and S. aureus among Hajj pilgrims. We were able to validate pneumococcal and meningococcal conjugate vaccine uptake from a parallel trial. In the Hajj 2009, roughly one in five to seven S. aureus isolates were MRSA[58]. S. aureus has been cultured from sputum samples (between 3.8% to 7.7% isolates) among Hajj pilgrims with respiratory infections but MRSA was not cultured[15,59]. However, MRSA was isolated in samples collected from various body sites in about 1.5% pilgrims during the Hajj 2004[22].

Limitations of our study include a relatively small sample size and an inconsistent sampling site (i.e., mostly nasopharyngeal in the first phase and oropharyngeal in the second phase). Different diagnostic methods were employed in the two different phases of the study with NAT in first phase of the study and phenotypic methods in the second phase which did not allow us to compare two datasets directly, and because of the differences in study designs it was not possible to make valid comparison with the reports of other investigators, so we limited the discussion to only narrative synthesis. In addition, only a few strains of carriage organisms were studied, especially we did not assess for other potentially vaccine preventable pathogens such as H. influenza, and pneumococcal isolates were not serotyped. The discordance in the number of participants between first and second phase was due to unavailability of some participants for post-Hajj sampling within 2 mo after Hajj, because often pilgrims make side trips to other countries after Hajj and do not return to Australia directly. To address these limitations, a larger study is currently underway.

In conclusion, this study found a moderately high carriage rate of S. pneumoniae amongst pilgrims during the Hajj 2014 in the background of a conjugate pneumococcal vaccine trial, but a low meningococcal carriage rate. This pilot study demonstrates that a larger study is feasible and important to inform public health measures to prevent the transmission and limit the impact of significant infectious diseases at mass gathering events such as the annual Hajj pilgrimage. Further information on the serotype of circulating pneumococcal isolates will optimise the use of pneumococcal vaccination in pilgrims.

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