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ORIGINAL ARTICLE

Observational Study

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

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Abstract

AIM

To estimate the pharyngeal carriage rate of *Neisseria meningitidis* (*N. meningitidis*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*) among Australian Hajj pilgrims.

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 2nd 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

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.

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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 importation of pathogens from arriving pilgrims may result in local transmission of infection within the Kingdom of Saudi Arabia (KSA). Similarly, there may be further dissemination of infectious diseases after pilgrims return home.

Respiratory infections are of particular concern at Hajj; transmission may occur from symptomatic individuals or asymptomatic carriers[3,4]. In susceptible populations, the pharyngeal colonisation of pathogens may contribute to serious bacterial infections, including pneumonia, sepsis and meningitis[5]. Localised meningococcal outbreaks and their further dissemination have been linked to international travel, migration, attendance at Hajj and participation in sporting events[6-11]. Intercontinental spread of serogroup A meningococcal disease in 1987 affected thousands of Hajj pilgrims globally; mandating bivalent meningococcal (A and C) vaccine for all Hajj pilgrims helped with disease control[7]. Investigation of the 1992 meningococcal outbreak in Makkah, KSA showed an extremely high meningococcal carriage rate of 86% among devotees who attended congregational prayers in the Holy Mosque[12]. Following the Hajj-associated outbreaks of *Neisseria meningitidis* (*N. meningitidis*) W in 2000 and 2001, visas for entry into KSA for Hajj and Umrah pilgrims were changed to require the quadrivalent meningococcal vaccine (covering serogroups A, C, W and Y)[13,14].

Currently, respiratory infections are the most common illnesses during Hajj[15,16]. Cough is almost de rigeur, occurring virtually in all Hajj pilgrims[17,18]. Pneumonia is the leading cause of hospital admission during Hajj, with the commonest causative organisms being *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae, Klebsiella pneumoniae* and *Mycobacterium tuberculosis*[19-21].

In a study of samples from nares, axilla, groins and open wounds of Hajj pilgrims, a 20.9% carriage rate of *Staphylococcus aureus* (*S. aureus*) was found. Of

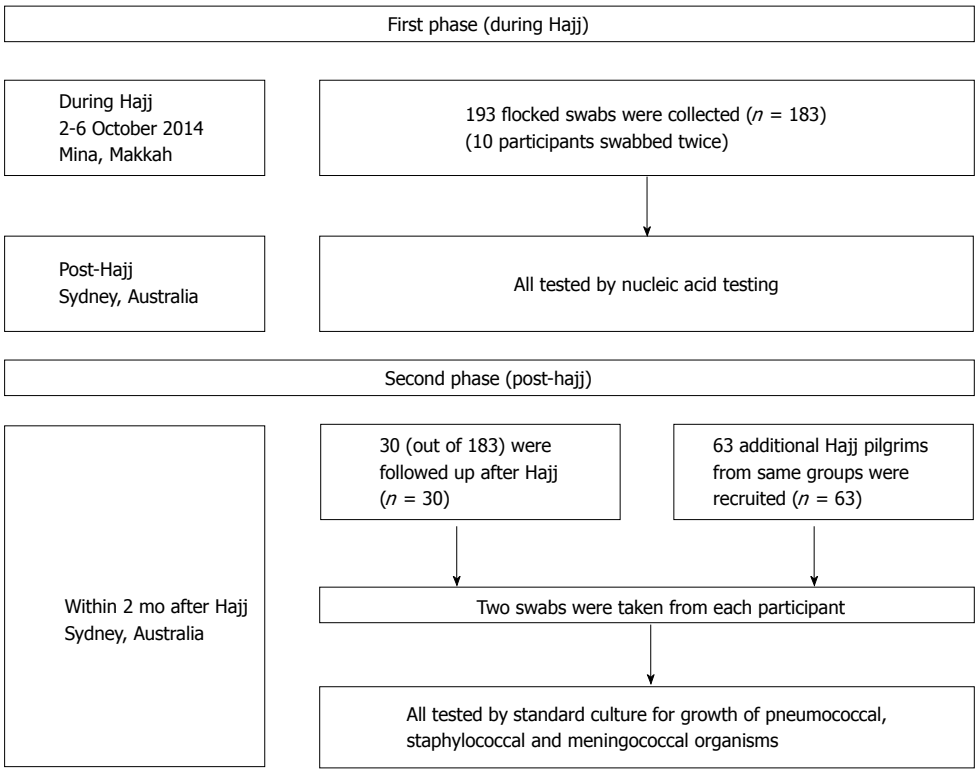

Figure 1 Schematic diagram showing recruitment of pilgrims.

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)

This study was explained to pilgrims in their tents. From those who consented, nasopharyngeal or oropharyngeal swabs were collected while in Mina from all participants on the fifth day of their stay in tents; in a subset of pilgrims who had respiratory symptoms (cough, sore throat and/or rhinorrhoea), swabs were also collected on the first day of recruitment. Following collection, flocked swabs were placed in universal transport medium (UTM) (Vircell). The swabs were transported approximately 5 km on ice to the collaborating laboratory in Makkah where they were stored at -20 ℃ before being shipped under similar conditions to the testing laboratory in Australia. This carriage study was nested within an ongoing randomised controlled trial examining the efficacy of facemasks against respiratory viruses; this methodology is published[32].

Second phase (post-Hajj)

Within 2 mo of their return from Hajj, Australian pilgrims were consented for follow-up swabbing. Oropharyngeal swabs were collected in mosques, community centres, local events (such as "family fun" days) and participants' residences in Greater Sydney, New South Wales. From each pilgrim, 2 oropharyngeal swabs were obtained

Table 1 The primer sequences for *Neisseria meningitidis*

Serotype	Gene target	Primer sequences	Product size (bp)
A	*orf-2*	F: CGCAATAGGTGTATATATTCTTCC; R: CGTAATAGTTTCGTATGCCTTCTT	400
B	*siaD*	F: GGATCATTTCAGTGTTTTCCACCA; R: GCATGCTGGAGGAATAAGCATTAA	450
C	*siaD*	F: TCAAATGAGTTTGCGAATAGAAGGT; R: CAATCACGATTTGCCCAATTGAC	250
W135	*siaD*	F: CAGAAAGTGAGGGATTTCCATA; R: CACAACCATTTTCATTATAGTTACTGT	120
Y	*siaD*	F: CTCAAAGCGAAGGCTTTGGTTA; R: CTGAAGCGTTTTCATTATAATTGCTAA	120

Orf2: Open reading frame 2; *siaD*: Polysialyltransferase gene.

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% CO_2 at 37 ℃ 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 Hajj 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 ℃ 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 (*lytA)* gene [forward primer, 5′-ACGCAATCTAGCAGATGAAGC-3′; reverse primer, 5′-TGTTTGGTTGGTTATTCGTGC-3′; probe, 5′-6-carboxy-fluorescein (FAM)-TTTGCCG AAAACGCTTGATACAGGG-BHQ-1-3′].

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 ℃ × 15 min (96 ℃ × 10 s; 63 ℃ × 1 min) for 45 cycles and 72 ℃ × 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′-TTGTCGCGGATTTGCAACTA-3′; probe, 5′-6-carboxy-fluorescein (FAM)-CATTGCCACGTGTCAGCTGCACAT-BHQ-1-3′]. The reaction mix was amplified in a Roche LightCycler® 480 (Roche Diagnostics GmbH, Mannheim, Germany) at 95 ℃ × 5 min (95 ℃ × 15 s, 60 ℃ × 1 min) × 45; 40 ℃ × 30 s with detection on the 640 nmol/L channel during elongation at 60 ℃.

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 ℃ × 15 min (95 ℃ × 30 s; 50 ℃/55 ℃ × 1 min; 72 ℃ × 30 s) for 40 cycles and 72 ℃ × 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 2 Demographics of participants (during and post-Hajj, *n* = 246)

Attributes	During Hajj *n* (%)	Post-Hajj *n* (%)
Gender		
Female	111 (60.7)	30 (32.3)
Male	72 (39.3)	63 (67.7)
Age in years		
0-18	0	1 (1)
19-34	38 (20.8)	17 (18.3)
35-49	58 (31.7)	52 (55.9)
50-64	28 (15.3)	8 (8.6)
≥ 65	1 (0.6)	0
Did not disclose	58 (31.7)	15 (16.1)
Meningococcal vaccine uptake		
Not vaccinated	0	4 (4.3)
Meningococcal polysaccharide vaccine	144 (78.7)	48 (51.6)
Meningococcal Conjugate vaccine	39 (21.3)	41 (44.1)
Pneumococcal vaccine uptake		
Not vaccinated	145 (79.2)	55 (59.1)
Pneumococcal conjugate vaccine (PCV13)	38 (20.8)	38 (40.9)
Facemasks use		
Used facemasks	76 (41.5)	32 (34.4)
Did not use facemasks	92 (50.3)	59 (63.4)
Did not disclose	15 (8.2)	2 (2.2)

PCV13: Pneumococcal conjugate vaccine 13-valent.

RESULTS

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 when symptomatic and second collected on their 5th day.

Twenty-six (14.2%) participants had *S. pneumoniae* detected by NAT (Table 3). Thirty-eight (20.8%) participants had a confirmed history of receiving 13-valent pneumococcal conjugate vaccine (PCV13) within six months prior to travel. Of the 26 pilgrims who were PCR-positive for *S. pneumoniae,* 4 reported receiving PCV13 and the other 22 reported not receiving the vaccine.

Of the 183 study participants only one (0.6%) tested positive for *N. meningitidis* (serogroup W). Receipt of quadrivalent polysaccharide meningococcal vaccine was reported by 144 (78.7%) participants - 39 (21.3%) reported receiving quadrivalent meningococcal conjugate vaccine. The pilgrim with positive *N. meningitidis* PCR reported receiving the polysaccharide vaccine. Seventy-six (41.5%) participants reported using a facemask, while 92 (50.3%) reported not using a facemask at anytime during Hajj; the other 15 (8.2%) did not disclose

Table 3 Carriage rate of *Streptococcus pneumoniae, Neisseria meningitidis and Staphylococcus aureus*

	During Hajj		Post-Hajj	*P*[1]
	First day of Mina (NAT)	Last day of Mina (NAT)	Standard culture	
S. pneumoniae	1/10	26/183	1/93	< 0.01
N. meningitidis	--	1/183	2/93	0.26
S. aureus	--	--	17/93	

[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*: *Neisseria meningitidis*; *S. pneumoniae*: *Streptococcus pneumoniae*; *S. aureus*: *Staphylococcus aureus*.

if they used 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). Sixteen (17.2%)

Table 4 Pneumococcal carriage rates according to the uptake of 13-valent pneumococcal conjugate vaccine in first phase of study

	PCR positive for pneumococci *n* (%)	PCR negative for pneumococci *n* (%)	Total
PCV13	4 (10.5)	34 (89.5)	38
No PCV13	22 (15.2)	123 (84.8)	145
Total	26 (14.2)	157 (85.8)	183

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 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*[8] 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*[46] 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[47] and the United States[48] demonstrated a meningococci 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[49]. In the

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%[42]. 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*[28] 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)[28]. 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[53]. 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[20,37,53].

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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through a multidisciplinary Approach".

COMMENTS

Background

Pharyngeal acquisition of pathogenic microorganisms during Hajj, one of the world's largest mass gatherings, is a known risk. Before this study, the carriage rate of common bacterial pathogens among Australian pilgrims had not been investigated.

Research frontiers

There is high risk of acquiring a carriage of potentially pathogenic bacteria during Hajj, the author propose investigating this at a larger scale.

Innovations and breakthroughs

This study emphasises that international travel, including mass gatherings, is a significant risk factor for the acquisition of and subsequent colonisation or infection with bacteria.

Applications

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.

Terminology

Carriage: The harbouring or transporting of a microorganism for example in the human body; Hajj: The Muslim pilgrimage to Mecca, which takes place in the last month of the arabic calendar and which all Muslims are expected to make at least once during their lifetime if they can afford to do so. It is one of the Five Pillars of Islam; Pilgrimage: A pilgrimage is a journey of spiritual significance; Pilgrim: A person who journeys to a sacred place for religious reasons.

Peer-review

It is an interesting surveillance study for carriage of pathogenic bacteria, the first one among Hajj pilgrims.

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