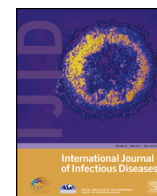


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# Meningococcal serogroup A, C, W, and Y serum bactericidal antibody profiles in Hajj pilgrims



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## SUMMARY

**Background:** The religious seasons of Hajj and Umra in the Kingdom of Saudi Arabia (KSA) have historically been associated with epidemics of meningococcal disease. Due to the effective preventive measures taken in recent years, including vaccination, no meningococcal outbreaks have been reported during Hajj or were Hajj-associated. However, little is known about the immunological profile of pilgrims. The aim of this study was to assess the immunological profile of pilgrims on arrival in KSA against the four meningococcal serogroups, A, C, W, and Y, contained within the quadrivalent vaccine. **Methods:** Following consent, socio-demographic factors and health-related information was collected from pilgrims arriving at King Abdul Aziz International Airport and a blood sample taken. Antibodies were quantified by serum bactericidal antibody assay using baby rabbit complement (rSBA) against the four meningococcal serogroups, A, C, W, and Y.

**Results:** Serum samples were collected from 796 pilgrims; rSBA results were obtained for all four serogroups for 741 of these samples. A total of 48 (6.5%) Hajjis had previously attended Hajj, ranging from 1 to 14 times (median 2 times); 98.2% had received meningococcal quadrivalent vaccine in the last 3 years. Of the 13 who had not, all originated from Bangladesh, with four reporting no previous meningococcal vaccination and nine reporting having received the vaccination more than 3 years ago. For serogroup A, only one pilgrim from Indonesia had an rSBA titre <8. For serogroups C, W, and Y, the percentages of pilgrims with rSBA titres <8 were 9.9%, 17.4%, and 9.4%, respectively. Of note was the high prevalence of non-complement-mediated lysis in pilgrims originating from Nigeria (28/47; 59.6%) and Afghanistan (21/47; 44.7%), but not the other countries. This may be a reflection of the type and pattern of antibiotic usage among these communities.

**Conclusion:** The vast majority of pilgrims are vaccinated and protected against meningococcal serogroups A, C, W, and Y.

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## 1. Introduction

Mass gatherings continue to draw ever larger crowds from all corners of the globe and these pose several significant public health challenges to the health and security authorities both within the host country and abroad. Thus, the introduction, transmission, and implication of infectious diseases during and after mass gathering events remain serious public health

concerns.<sup>1,2</sup> Epidemics of meningococcal disease have been associated with the religious seasons of Hajj and Umra in the Kingdom of Saudi Arabia (KSA), historically caused by serogroup A, but in the 2000s by serogroup W.<sup>3,4</sup> Due to the effective preventive measures taken in recent years, no meningococcal outbreaks have been reported during Hajj or have been Hajj-associated.

The main preventive measures taken by the Saudi Health authorities include mandatory vaccination of pilgrims (domestic and international) with meningococcal quadrivalent vaccine, no more than 3 years and no less than 10 days before arrival in KSA, and the administration of a single dose of ciprofloxacin as chemoprophylaxis against meningococcal carriage for pilgrims

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from Sub-Saharan Africa.<sup>5</sup> Prophylactic ciprofloxacin can decrease nasopharyngeal carriage rates, as evidenced in a study of Iranian pilgrims administered 500 mg of ciprofloxacin 24 h before returning home. Carriage rates were 8.5% before departure from Hajj, decreasing to zero on return, post ciprofloxacin.<sup>6</sup>

The aim of this study was to assess the immunological profile of pilgrims on arrival in KSA against the four meningococcal serogroups, A, C, W, and Y, contained within the quadrivalent vaccine.

## 2. Methods

### 2.1. Study area

King Abdul Aziz International (KAAI) Airport is located in Jeddah and occupies an area of 105 km<sup>2</sup>. This airport is the gateway to Makkah, through which most of the international pilgrims reach the Sacred Mosque in Makkah. The Hajj terminal of KAAI Airport is designed in the form of tents occupying an area of 465 000 m<sup>2</sup>. It is the fourth largest airport terminal in the world after Hong Kong, Bangkok, and Seoul. It can receive about 50 000 pilgrims a day in the Hajj season. Each of its 12 halls acts as an arrival lounge for one flight of pilgrims coming to perform the Hajj, and the same hall acts as a departure lounge for departing pilgrims. A team from preventive medicine is posted in each hall, around the clock, during the arrival of pilgrims. This team reviews the vaccination cards, administers polio drops and/or ciprofloxacin tablets, and performs other special preventive activities, in accordance with the national policy.

### 2.2. Ethics

Approval for this study was gained from the Institutional Review Board of King Fahad Medical City.

### 2.3. Study population

The study population included Hajjis who were arriving through KAAI Airport in Jeddah to perform the Hajj in the Islamic year 1433H (23 September to 20 October 2012). International

Hajjis were stratified into three groups based on the presumed meningococcal disease endemicity level of the country from which they had arrived (Table 1).

Each identified pilgrim from a target country was requested to participate in the study. In the case of refusal, the next pilgrim was asked for consent. Inclusion criteria were Hajji, willing to participate, and age over 18 years; only Hajjis from countries that send more than 5000 pilgrims were included in the study. Hajjis who refused to participate and those who had already received chemoprophylaxis were excluded.

In the absence of a clear estimate of valid vaccination and immunity status against *Neisseria meningitidis*, a prevalence of 50% was used to obtain the largest sample size for a given level of percent point precision. A sample size of 861 was required to estimate the immunity status to within 3% of this value at a confidence level of 95%. To obtain blood samples to determine immunity status, a systematic random sampling technique was used.

After obtaining verbal informed consent, a questionnaire was given to the Hajji to be completed by a Ministry of Health physician. This questionnaire was designed to collect information including socio-demographic factors (age, gender, and the country from which they had arrived), health-related information (history of chronic diseases, receiving immunization against influenza and meningococcal disease), information on the validity of the meningococcal vaccination certificate, and ciprofloxacin tablet administration in their own country and/or in KSA. All questionnaires were prepared in English, and were then translated into multiple languages including Hausa, French, Urdu, Hindi, Bengali, Indonesian, Russian, Malay, Pashto, and Turkish.

### 2.4. Serology

Blood samples (clotted) were collected from pilgrims (before the administration of ciprofloxacin), in accordance with World Health Organization (WHO) guidelines, and transported to the airport primary health care in cold boxes at a temperature between +2 and +8 °C. These were then dispatched to Public Health England, Manchester, UK for testing with the serum bactericidal antibody (SBA) assay for serogroups A, C, W, and Y. Serogroup-specific

**Table 1**  
Characteristics of Hajjis from whom sufficient serum sample volumes were obtained

Country	Number of sufficient serum samples collected from Hajjis	First Hajj (%)	Previous influenza vaccination (%)	Previous pneumococcal vaccination (%)	Previous meningococcal vaccination in the last 3 years	Serum bactericidal antibody control for non-complement-mediated lysis failed for one or more strain
<b>High</b>						
Nigeria	47	46 (97.8)	0 (0)	0 (0)	47 (100)	28 (59.6)
Sudan	20	15 (75.0)	0 (0)	0 (0)	20 (100)	1 (5.0)
Ethiopia	8	8 (100)	0 (0)	0 (0)	8 (100)	0 (0)
Niger	63	54 (85.7)	6 (1.6)	0 (0)	63 (100)	3 (4.8)
Subtotal	138	123 (89.1)	6 (4.3)	0 (0)	138 (100)	32 (23.2)
<b>Medium</b>						
India	99	94 (94.9)	0 (0)	0 (0)	99 (100)	3 (3.0)
Pakistan	105	93 (88.6)	105 (100)	2 (1.9)	105 (100)	7 (6.7)
Bangladesh	75	69 (92.0)	48 (69.6)	0 (0)	62 (82.7)	5 (6.7)
Afghanistan	47	46 (97.9)	46 (97.9)	0 (0)	47 (100)	21 (44.7)
Kazakhstan	6	5 (83.3)	0 (0)	0 (0)	6 (100)	0 (0)
Tajikistan	13	13 (100)	0 (0)	0 (0)	13 (100)	1 (7.7)
Subtotal	345	320 (92.8)	199 (57.7)	2 (0.6)	332 (96.2)	37 (10.7)
<b>Low</b>						
Indonesia	138	137 (99.3)	137 (99.3)	0 (0)	138 (100)	1 (0.7)
Turkey	89	89 (100)	0 (0)	0 (0)	89 (100)	1 (1.1)
Malaysia	14	12 (85.7)	8 (57.1)	8 (57.1)	14 (100)	0 (0)
UK	8	6 (75.0)	0 (0)	0 (0)	8 (100)	0 (0)
France	9	6 (66.7)	0 (0)	0 (0)	9 (100)	0 (0)
Subtotal	258	250 (96.9)	145 (56.2)	8 (3.1)	258 (100)	2 (0.8)
<b>Total</b>	<b>741</b>	<b>693 (93.5)</b>	<b>350 (47.2)</b>	<b>10 (1.3)</b>	<b>728 (98.2)</b>	<b>71 (9.6)</b>

antibody responses were determined by SBA assay using baby rabbit complement (rSBA; Pel-Freez Inc., Rodgerston, AZ, USA) as an exogenous complement source, as described elsewhere.<sup>7</sup> The following strains were used (name, strain, and standardized strain characterization in parenthesis): serogroup A, F8238 (A:4:P1.20.9); serogroup C, C11 (C:16:P1.7-1.1); serogroup W, M,01.240070 (W:NT:P1.18-1.3); and serogroup Y, M,03.241125 (also known as S1975) (Y:2a:P1.5.2). rSBA titres were expressed as the reciprocal of the final serum dilution yielding  $\geq 50\%$  killing at 60 min. For computational purposes, rSBA titres of  $<4$  were assigned a value of 2. The rSBA contains a control for non-complement-mediated lysis. If the control failed then the test was repeated at an appropriate starting dilution, with beta-lactamase. The beta-lactamase is utilized to remove any antibiotics specifically from the penicillinase family. If the control still failed then the serum sample was excluded from the analysis of the rSBA geometric mean titres (GMTs) and percentages over certain thresholds.

### 3. Results

A total of 796 out of the 861 pilgrims had serum samples collected. rSBA results were obtained for all four serogroups for 741 pilgrims; 55 serum samples were insufficient in volume for one or more serogroups. Data from these 55 serum samples were omitted from the analyses.

For pilgrims who had their age recorded, this ranged from 18 to 92 years (median 52 years); the age of 16 pilgrims was not recorded. A total of 64.4% were male, 32.5% female, with gender not recorded for 3.1%. Details of the country of origin, prior attendance at Hajj, previous influenza and pneumococcal vaccines, and prior meningococcal vaccine are given in Table 1. A total of 48 (6.5%) Hajjis had previously attended Hajj, ranging from 1 to 14 times (median 2 times). Overall 47.2% of Hajjis reported prior influenza vaccination, although this varied considerably by country. Only 1.3% of pilgrims had previously received pneumococcal vaccination. A total of 98.2% of pilgrims had received meningococcal quadrivalent vaccine in the last 3 years. Of the 13 who had not, all originated from Bangladesh, with four reporting no previous meningococcal vaccination and nine reporting vaccination more than 3 years ago.

For the four pilgrims who reported no previous meningococcal vaccination, the number with rSBA titres  $\geq 8$  for serogroups A, C, W, and Y were 4 (100%), 4 (100%), 3 (75.5%), and 3 (75.0%), respectively, whilst for  $\geq 128$  the corresponding numbers were 4

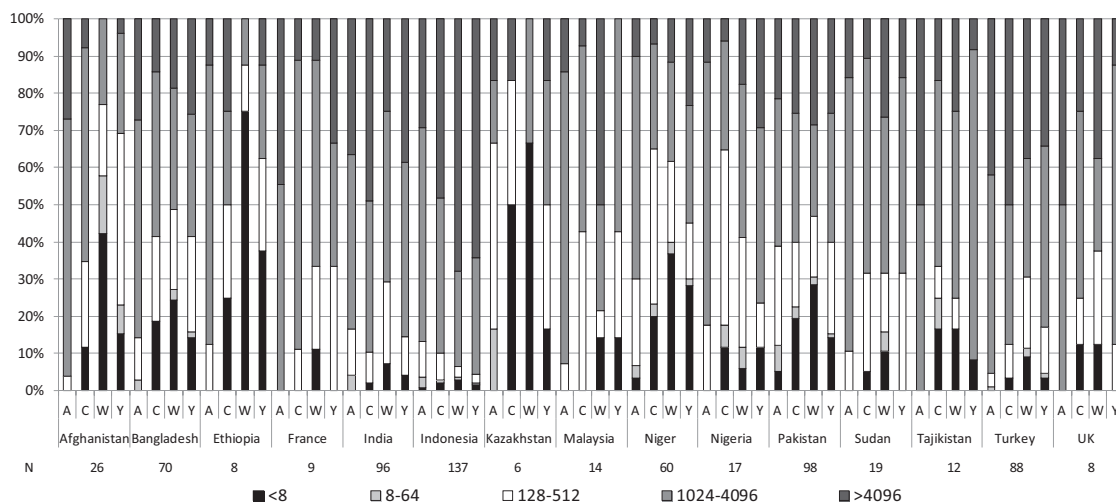
(100%), 4 (100%), 1 (25.0%), and 3 (75.0%), respectively. With regards to the nine pilgrims who reported meningococcal vaccination more than 3 years ago, one showed non-complement-mediated lysis; of the remaining eight, the number with rSBA titres  $\geq 8$  for serogroups A, C, W, and Y were 8 (100%), 3 (37.5%), 5 (62.5%), and 2 (25.0%), respectively, whilst for  $\geq 128$  the corresponding numbers were 7 (87.5%), 3 (37.5%), 5 (62.5%), and 2 (25.0%), respectively.

The number of sera for which the control failed due to non-complement-mediated lysis is given in Table 1. These sera were all excluded from the subsequent analyses. Hajjis from Afghanistan and Nigeria had high percentages of control fails, at 44.7% and 59.6%, respectively.

The percentages of rSBA titres at  $<8$ , 8–64, 128–512, 1024–4096, and  $>4096$  by country of origin of the pilgrims are given in Figure 1. For serogroup A, only one pilgrim from Indonesia had an rSBA titre  $<8$ . For serogroup C, the percentage of pilgrims with rSBA titres  $<8$  was 0.4% (3/741), ranging from 0% in pilgrims from France (0/9) and Malaysia (0/14) to 50% (3/6) in those from Kazakhstan. For serogroup W, 17.4% of all pilgrims had rSBA titres  $<8$ , ranging from 2.9% (4/137) to 75% (6/8) in pilgrims from Indonesia and Ethiopia, respectively. For serogroup Y, 9.4% of all pilgrims had rSBA titres  $<8$ , ranging from 0% in those from France (0/9), Sudan (0/19), and the UK (0/8) to 37.5% (3/8) in those from Ethiopia.

The serogroup A, C, W, and Y rSBA GMTs are given in Figure 2. For serogroup A, the rSBA GMTs (95% confidence interval (CI)) ranged from 444.7 (95% CI 98.7–2003.5) to 4933.4 (95% CI 2710.0–8980.8) for pilgrims from Kazakhstan and France, respectively. For serogroup C, rSBA GMTs ranged from 8.0 (95% CI 0.4–176.6) to 3254.4 (95% CI 2307.8–4589.5) for pilgrims from Kazakhstan and India, respectively. The lowest serogroup W rSBA GMT was seen in pilgrims from Ethiopia at 3.4 (95% CI 0.4–26.9), whilst the highest was seen in those from Indonesia at 5727.7 (95% CI 3948.9–8307.9). For serogroup Y, rSBA GMTs ranged from 20.1 (95% CI 1.6–245.6) to 5162.6 (95% CI 3966.6–6719.1) in pilgrims from Ethiopia and Indonesia, respectively.

Figure 3 depicts the serogroup A, C, W, and Y rSBA GMTs by age group: 18–27 years ( $n = 16$ ), 28–37 years ( $n = 86$ ), 38–47 years ( $n = 154$ ), 48–57 years ( $n = 169$ ), 58–67 years ( $n = 164$ ),  $>68$  years ( $n = 64$ ), and age unknown ( $n = 15$ ) (data not analysed). No trends in rSBA GMT were observed for serogroups A, C, and Y. For serogroup W, the youngest age group, 18–27 years, had the highest



**Figure 1.** Percentages of serogroup A, C, W, and Y serum bactericidal antibody titres at different thresholds by country of pilgrim origin. The putative protective rSBA threshold for serogroup C is a titre of  $\geq 8$ .

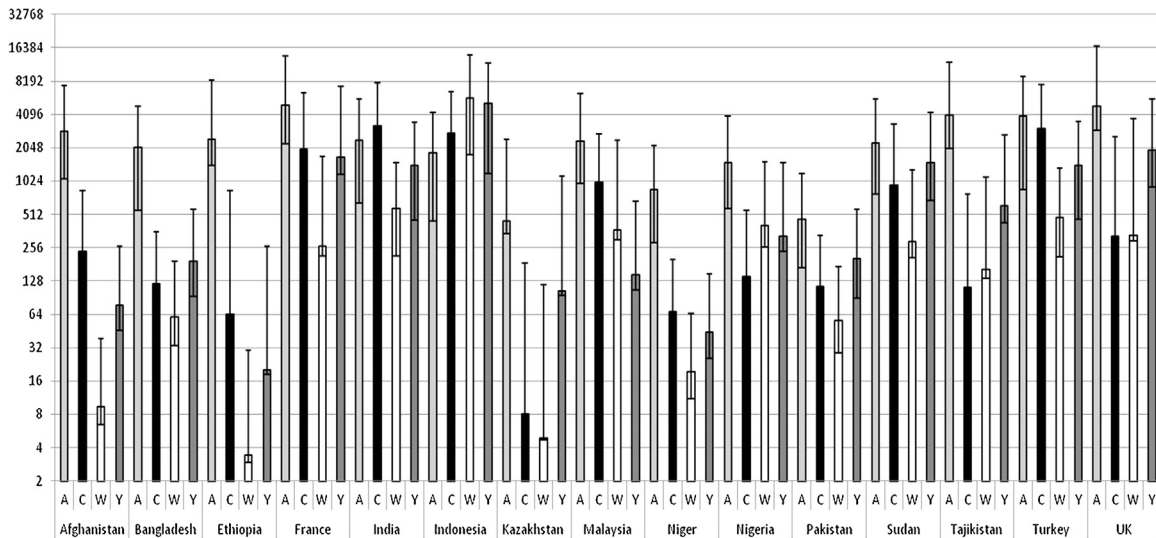


Figure 2. Serogroup A, C, W, and Y geometric mean titres and 95% confidence intervals by country of pilgrim origin.

rSBA GMT of 1468.2 (95% CI 451.2–4777.1), whilst those aged >68 years had the lowest rSBA GMT of 53.2 (95% CI 23.1–122.5).

4. Discussion

Vaccination with meningococcal quadrivalent ACWY vaccine is required for all pilgrims coming to KSA regardless of their country of origin. Pilgrims should show evidence of vaccination no more than 3 years and no less than 10 days before arrival in KSA. In this study, 98.2% of pilgrims were vaccinated with ACWY vaccine; of interest, all of those who were not vaccinated originated from Bangladesh. In a study of Hajj pilgrims in 2013, it was reported that 93% of pilgrims had been vaccinated with ACWY vaccine.<sup>8</sup> In the latter report, 4.4% had received pneumococcal vaccination and 22% had received influenza vaccination as compared to 1.3% and 47.2%, respectively, in the present study.

Provision of a history of meningococcal vaccination does not indicate definitive protection, and the absence of vaccination does not infer that an individual may not be protected, as natural immunity also protects individuals.<sup>9</sup> However, by using the correlate of protection – the rSBA assay – insight can be gained

into the percentage of persons protected.<sup>10</sup> Of note, of the four pilgrims for whom serology was available who had not been vaccinated with ACWY, only one was unprotected against serogroup Y and one against serogroup W. Of those who reported vaccination, only one pilgrim was unprotected against serogroup A, but 9.9%, 17.4%, and 9.4% were unprotected against serogroups C, W, and Y, respectively.

Putative protection for serogroup A was excellent, with only one pilgrim having an rSBA titre <8; however, for serogroups C, W, and Y the percentage of pilgrims protected varied by country. For serogroup C, only 50% of pilgrims from Kazakhstan had rSBA titres ≥8, although it should be noted that only six pilgrims from this country were enrolled in the study. For serogroups Y and W, the highest percentage of pilgrims with rSBA titres <8 was found in those attending from Ethiopia, 37.5% and 75.0%, respectively, although only a total of eight pilgrims were enrolled from that country.

The SBA assay is a bioassay utilizing live meningococci, thus if a serum sample contains antibiotics, these meningococci will be lysed even in the absence of complement. Hence all SBA assays contain a control that measures non-complement-mediated

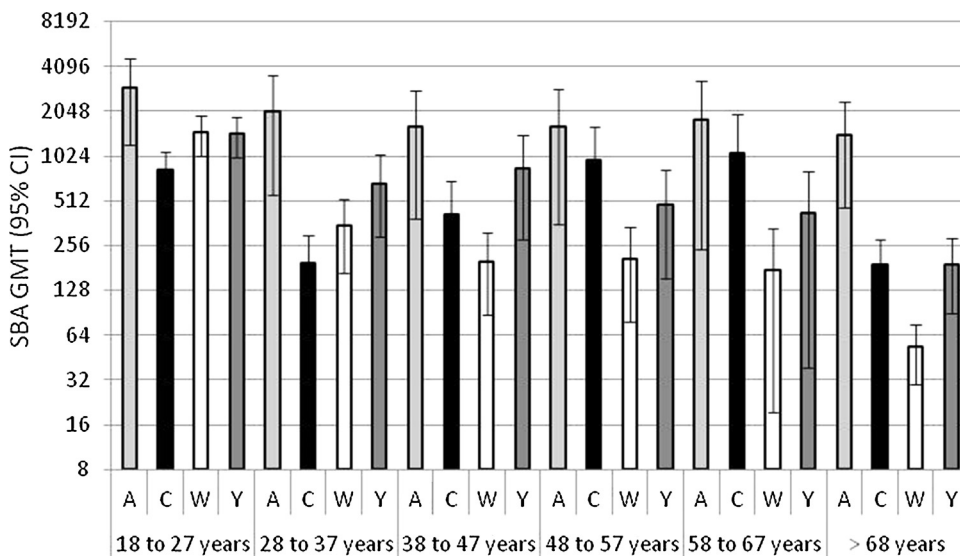


Figure 3. Serogroup A, C, W, and Y geometric mean titres and 95% confidence intervals by age of pilgrim.

lysis.<sup>11</sup> To counteract serum samples containing penicillin, beta-lactamase is added, which shows specificity for penicillins by hydrolyzing the beta-lactam ring. However, for other classes of antibiotic, no easy solution is available and SBA titres cannot be gained. Of note in this study was the high prevalence of non-complement-mediated lysis in pilgrims originating from Nigeria (59.6%) and Afghanistan (44.7%), but not the other countries. This may well be due to the use of antibiotics and related to the antimicrobial stewardship and availability in those countries.

One drawback of this study is that data on the type of meningococcal vaccine – polysaccharide versus conjugate – were not collected. Although both types of vaccine are immunogenic in this age group, conjugates elicit high avidity antibodies and tend to higher SBA levels to all serogroups than the polysaccharide vaccines.<sup>12,13</sup> Given that the cost of conjugate vaccines is higher than that of the polysaccharide vaccines and the polysaccharide vaccines are easily accessible, it may be that the use of polysaccharide vaccines was higher in the less developed countries, particularly those of Sub-Saharan Africa.

Notwithstanding this limitation, with regards to meningococcal vaccination and protection against meningococcal disease, we found that the vast majority of pilgrims are vaccinated and protected.

#### Acknowledgement

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*Conflict of interest:* We declare the following potential conflicts of interest: R.B., H.F., and X.B. have performed contract research on behalf of Public Health England (funded by Pfizer, Novartis Vaccines, Baxter Bioscience, GlaxoSmithKline, Sanofi Pasteur, Alexion Pharmaceuticals Inc., and Merck).

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