Neisseria meningitidis Nasopharyngeal Carriage during the Hajj: a cohort study evaluating the need for ciprofloxacin prophylaxis

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Abstract:

Background: The annual Muslim pilgrimage has the potential of increase risk for acquisition of *Neisseria meningitidis.* Here, we evaluate the Hajj impact on the prevalence of *N. meningitidis* carriage in a paired and non-paired cohort of pilgrims. Secondary objectives were to calculate the compliance with recommended vaccination.

Methods: This a prospective paired (arriving and departing), non-paired arriving and non-paired departing cohort study with the collection of nasopharyngeal samples at the start and the end of the Hajj.

Results: The study included unpaired arriving pilgrims at King Abdul Aziz International Airport (N=1055), unpaired departing cohort (N=373), and a paired cohort (N=628) who were tested on arrival and departure. Meningococcal vaccination was received by all pilgrims, 98.2% received quadrivalent polysaccharide vaccine (ACWY), and 1.8% received meningococcal quadrivalent conjugate vaccine (MCV4). Only 1.61% and 23.03% received pneumococcal and influenza vaccines, respectively. Of the 1055 arriving unpaired pilgrim, 36 (3.4%) tested positive for nasopharyngeal carriage of *N. meningitidis,* and 24 (66.7%) of these were serogroup B, the remainder were non-groupable. *Haemophilus influenza* was detected among 45 (4.3%), and 11 (1%) carriers were positive for both *N. meningitidis* and *H. influenzae.* Out of 373 in the unpaired departing cohort, 6 (1.61%) tested positive for *N. meningitidis*, and 34 (9.1%) were positive for *H. influenzae*. Of the 628 paired cohort pilgrims, 36 (5.7%) pilgrims were positive for *N. meningitidis* at arrival and 16 (2.5%) pilgrims were positive after the hajj.

Conclusion: This the largest study of the epidemiology of *N. meningitidis* among pilgrims. The study showed a significant difference in the carriage between pilgrims from high endemicity and other pilgrims with a predominance of serogroup B. The continued use of ciprofloxacin as prophylactic antibiotics should be reconsidered as well as the consideration to add serogroup B as a required vaccination.

Introduction

Meningococcal disease is a major cause of morbidity and mortality worldwide [1]. Meningococcal disease occurs at a rate of 0.5 -10 cases per 100.000 and may reach a rate of 1,000 in epidemic countries [2]. *Neisseria meningitidis is* classified into 12 serogroups, Only 6 serogroup (A, B, C, W, Y, X) are responsible for most invasive meningococcal disease [3]. Meningococcal disease is the most common presentation of invasive meningococcal infection and causes a substantial burden and death among all age groups [4]. Mass gatherings are associated with outbreaks of respiratory infection diseases including meningococcal disease [5–9]. In 1987, the first international meningococcal disease outbreak following the Hajj occurred and was caused by *N. meningitidis* serogroup A [10–12], and serogroup W135 [13]. The prevalence of asymptomatic *N. meningitidis* carriage ~~increases~~ increased more than 80% and contributed to outbreaks during Hajj in 1987 and 2000-2001 [9,10]. Due to these outbreaks, the Ministry of Health in Saudi Arabia adopted the mandatory vaccination for all ~~hajj~~ Hajj pilgrims, annual vaccination campaigns for all the residents living in the area near the pilgrimage site and mandatory oral ciprofloxacin prophylaxis for all the pilgrims coming from sub-Saharan African meningitis belt countries [1,10,14–16].

Mass gatherings continue to draw larger crowds from around the Globe [13]. These events offer a great potential for a health legacy through intense periods of unprecedented focus and funding for improvement in health systems. However, these events also pose several significant public health challenges within the host country and abroad [17]. ~~Meningococcal disease has been associated with the Hajj.~~ Of factors that increase acquisition of *N. meningitidis* is the Hajj pilgrimage due to crowding and the gathering of

millions in a small place [8,9]. Taking effective preventive measures by the Saudi Ministry of Health, in recent years, curtailed the development of meningococcal outbreaks since the W outbreak in 2000 [7,9,10]. Of those preventive measures, mandatory vaccination of domestic and international pilgrims is required using quadrivalent meningococcal vaccine ~~and~~ as well as the administration of a single dose of ciprofloxacin as chemoprophylaxis against *N. meningitidis* carriage to pilgrims from sub-Saharan African meningitis belt [14]. These practices are based on the evidence of distribution pattern of *N. meningitidis* in the world. Outbreaks in sub-Saharan Africa were caused by serogroup C, W and X meningococcal but most of epidemics were due to serogroup A [18]. Meningococcal serogroup W caused an outbreak in Saudi Arabia among pilgrims during Hajj season 2000 and spread to contacts after returning back to home countries [6,19]. Epidemics related to serogroup B meningococcal disease were reported in Norway and Cuba since 1976 [20]. Late in 1980 group B meningococcal disease spreading from Cuba to São Paulo in Brazil and in 2000 in New Zealand [21,22]. Prophylactic ciprofloxacin can decrease the carriage rate from 8.1% to 0% before and after the Hajj [23]. The recent epidemiologic changes of *N. meningitidis* infection in Africa and other parts of the world; the improvement in vaccination practices among pilgrims; and recent development of conjugate vaccine permit the policy Maker in Ministry of Health, KSA, to re-evaluate aspects of *N. meningitidis* epidemiology and to re-assess the prevention measures that applied during hajj.

In this study, we evaluate the Hajj impact on the prevalence of *N. meningitidis* carriage in pilgrims. Secondary objectives were to calculate the compliance with recommended

vaccination and to re-evaluate the recommendations of a chemoprophylaxis applied during Hajj.

Methods:

Study Area: King Abdul Aziz International Airport (KAAIA) in Jeddah occupies an area of 105 km² and is located 70 kilometers west of the holy city of Makkah, where the pilgrims perform the religious rituals. There are three passenger terminals: the North terminal handles all foreign air carriers, the South terminal handles local flights, and the Hajj Terminal handles pilgrims traveling to Makkah. The KAAIA Hajj terminal is the gateway to Makkah and is designed in the form of tents occupying an area of 465,000 m2. KAAIA can receive about 80,000 pilgrims in 36 hours during the ~~hajj~~ Hajj season. Twelve teams from preventive medicine consisted of physicians, nurses and health inspectors and were distributed in each arrival hall to assess pilgrims, check the vaccination cards, administer chemoprophylaxis single dose of ciprofloxacin tablets) for pilgrims arriving from the sub-Saharan meningitis belt countries and perform other preventive measures, in accordance with international health regulation for pilgrimage posted yearly by Ministry of health , Saudi Arabia [14,15,24].

Mina~~:~~ is a small city located inside a valley in the province of Makkah, about 8 km to the east of the Holy city of Makkah. It covers an area of approximately 20 km². There are more than 100,000 air-conditioned tents in Mina providing temporary accommodation for three million pilgrims. The tents are constructed of fibber glass coated with Teflon in order to ensure high resistance to fire. In these tents Hajj pilgrims stay overnight for five days as part of the Hajj season.

Ethical Approval:

The study was approved by the institutional review board (IRB) of King Fahd Medical City, Riyadh, Saudi Arabia.

Study Population:

The study included unpaired cohort of arriving pilgrims at KAAIA (N=1055), unpaired departing cohort (N=373), and a paired cohort (N=628) who were tested on arrival and departure. Arriving pilgrims were recruited at KAAIA on October 2-October 7, 2014, and departing pilgrims were sampled after performing the ~~hajj~~ Hajj in Mina tents (October 16-October 24, 2014). Nationality of pilgrims was chosen based on the level of meningococcal meningitis endemicity of respective countries. The annual incidence per 100,000 population of >10 cases, 2–10 cases, and < 2 cases represent high, moderate, and low endemicity, respectively [25,26]. To obtain adequate sample size, only countries with more than 5,000 pilgrims were included in the study. We included pilgrims who were 18 years of age and older and verbal consent was obtained. If a pilgrim refused to participate then next pilgrims was asked to take part in the study.

Laboratory testing:

Nasopharyngeal swabs were collected in accordance with WHO guideline, from pilgrims upon arrival and departure. Samples were collected in a charcoal swab with transport media, and were transported in cold boxes at temperature 2-8^{o} C, and were sent to KAIA laboratory within 2 hours of collection at arrival time and to Hira'a General hospital in Makkah laboratory at departure time. Identification of Neisseria species was determined

by biochemical testing, polymerase chain reaction (PCR) and genotyping in Special Infectious Agent Units at King Fahd Medical Research Center of King Abdulaziz University [27,28].

Cultures:

Samples were cultured immediately using direct plating to non-selective and selective media, enriched chocolate agar and 5 % sheep blood agar plates. Samples were directly inoculated on labeled fresh culture plates and streaked for isolation with a sterile disposable loop. All plates were incubated at 36°**C** in humidified 5-10% CO_2 incubator. Plates were examined for growth and typical colonies after 18-24 hours of incubation and again after 48 hours and were examined for colonies with consistent Neisseria morphology. All suspected Neisseria species were subculture for purity on both chocolate and sheep blood plates.

Sample transportation and storage: Frozen samples transported to Special infectious Agents Unit King Fahd Medical Research Center –Jeddah and stored at -80°C for DNA extraction, QIAamp DNA kit(Qiagen), were used following the manufacturer's protocol specifically developed for extraction of DNA from Pharyngeal swab and the extracted DNA was stored at -80°C for PCR analysis.

Polymerase chain reaction (PCR)

In this study we have used *N. meningitidis* species-specific assays *ctrA* considering the probes and guidelines of CDC. The capsule transport gene, *ctrA*, is highly conserved among isolates responsible for invasive meningococcal infections [27,29]. It is, however, not found in all carriage isolates as capsular null (cnl) meningococci are found in carriage

though only very rarely cause disease in immunocompromised patients and thus are not the focus of this current study [30].

In paralleled to the above RT-PCR, a dual-labelled Multiplex Real-Time PCR based FTD bacterial meningitis kit (Luxembourg) were used for detection of *N. meningitidi*s, and *Haemophilus influenzae* from extracted DNA of each sample following the manufacturer's protocol. The test is fully validated with fast-track master mix (Fast-track Diagnostics) and AgPath ID™ One-Step RT-PCR kit (life technologies™). The test contained one positive control and one negative control in each run. The 7500 fast real time PCR instrument (Applied Bio System) was used for this project. Each sample was tested individually in a single tube for the *N. meningitidi*s, and *H. influenzae*.

We performed genogrouping of the positive samples of *N. meningitidis* for the detection of A, B, C, W and Y. In this study, we have used real time Singleplex PCR strategy to identify the six *N. meningitidis* genogroups from the *N. meningitidis* positive samples following genogroup-specific primers and the protocol described by Wang et al [28].

Results:

The study included unpaired cohort of arriving pilgrims at KAAIA (N=1055), unpaired departing cohort (N=373), and a paired cohort (N=628) who were~~,~~ tested on arrival and departure.

Unpaired Arriving Pilgrims:

Of 1055 unpaired arriving pilgrims, 23.03% (n=243) were 18-40 years, 641 (60.76%) were 41–65 years, 149 (14.12%) were >65 years and a mean age of 50 years. Male constituted 63% (n=665) of this cohort. Of those pilgrims, 25.69% (n=271) were from

high endemic countries (the sub-Saharan meningitis belt), 53.2% (n=562) from medium endemic countries, and 21% (n=222) were from low endemic countries (Table 1). In relation to meningococcal vaccination, 98.2% received quadrivalent polysaccharide vaccine (ACWY), and 1.8% received meningococcal quadrivalent conjugate vaccination (MCV4) vaccination. From the unpaired arriving pilgrims, 36 (3.4%) tested positive for *N. meningitides.* The positivity rate was 8.9% among individuals from high endemic region, and **2.3**% from medium endemic region (Tables 1) (P 0.0001). Out of the 36 positive samples, 28 (85.71%) were male, 8 (14.29%) were female. Of the 36 *N. meningitidis* isolates, 24 (66.7%) were serogroup B, and the remaining were non-groupable. In addition, 45 (4.3%) were positive for *H. influenzae*, and 11 (1%) were positive for both *N. meningitidis* and *H. influenzae* (Table 1).

Unpaired departing cohort

Of 373 unpaired departing cohort, 140 (37.5%) were from the high endemicity (meningitis belt) countries, 164 (43.9%) from medium endemic countries, and 69 (18.49%) from low endemic countries (Table 2). Male constituted 60% of this cohort with a mean age of 48 years. All received meningococcal vaccines, 3% received pneumococcal conjugate vaccine and 22% received influenza vaccine. Of the 373 pilgrims, 6 (1.6%) tested positive for *N. meningitides* (two were *N. meningitidis* serogroup B and four were non-groupable). Of the 373 pilgrims, 34 (9.1%) were positive for *H. influenzae*.

Paired cohort results:

A total of 628 paired cohort pilgrims were tested on arrival and on departure. Out of those, 124 (19.75%) were 18 - 40 years, 384 (61.15%) were 41 - 65years, 112 (17.83%)

were > 65 years of age. There were 63.~~69~~7% male and 36.3~~1~~% female. Of those pilgrims, 136 (21.6%) were from high endemic countries, 365 (58.1%) from medium endemic countries, and 127 (20.2%) were from low endemic countries (Table 2). All received meningococcal vaccines: 98.1% received polysaccharide meningococcal vaccine (ACWY), 1.9% received MCV4 vaccine. In addition, 2.2% received pneumococcal conjugate vaccine and 25.6% received influenza vaccine.

Out of the 16 (2.5%) pilgrims in the paired group who tested positive for *N. meningitides* on arrival, only one (0.15%) remained positive after the hajj. On the other hand, eight (1.3%) tested positive on departure and out of those, only 1 (~~1.3~~0.15%) was positive on arrival (P = 0.0003). Thus, the acquisition rate (negative before and positive after) was 7 (1.1%).

Of the paired cohort, 17 (2.7%) tested positive for *H. influenzae* at arrival and only 5 (0.8%) remained positive at the end of the Hajj (P =0.013). Of those who tested negative for *H. influenza* at arrival, 37 (5.9%) tested positive at the end of the hajj.

Comparison of *N. meningitides* Carriage among Arriving Pilgrims:

In the unpaired arriving pilgrims, *N. meningitidis* was detected in 7.1% and 1.9% of those from high endemic countries and other pilgrims respectively (P = 0.001). Among the paired cohort, the rate of *N. meningitidis* among arriving pilgrims was 3.6% in pilgrims from high endemicity and 2.2% from other pilgrims (P =0.035), Figure 1. Of all arriving pilgrims, the carriage rate was 6.3% among pilgrims from high endemic areas compared to 2% in those from other countries (P = 0.0001) (Figure 1).

Discussion: Colonization by *N. meningitidis* can be a major potential source of infection [31,32]. Acquisition of *N. meningitidis* among pilgrims is of concern for the potential spread of this organism into the pilgrims' home countries globally [24,33]. The rate of *N. meningitidis* among arriving pilgrims was low even among pilgrims coming from endemic countries (6.3%). The risk of adverse events of chemoprophylaxis may outweigh the benefits. Evaluation of returning pilgrims to Kuwait did not reveal any colonization among 177 participants [34]. In a study from the United States, the carriage rate of serogroup W was 0.8% among pilgrims and 0.9% among non-pilgrims and thus no prophylactic antibiotic was recommended for returning Pilgrims [35,36]. In a paired cohort group, the prevalence of *N. meningitidis* was 2.5% on arrival and 1.3% on departure, indicating no increase in the acquisition rate of carriage of meningococci [35,36]. The use of oral ciprofloxacin was evaluated in returning Iranian pilgrims, the carriage rates of *N. meningitidis* was 5.2% before and 4.6% after pilgrimage (P = 0.65) in those who did not receive ciprofloxacin compared to the carriage rate of 8.1% and zero before and after pilgrimage in those who had ciprofloxacin on return [23]. In accordance with the Saudi Ministry of Health, mandatory oral ciprofloxacin prophylaxis is given to all pilgrims coming from sub-Saharan African meningitis belt countries [1,10,14–16].

In the paired cohort in this study, 2.5% pilgrims tested positive for *N. meningitidis* on arrival and only 0.15% of them remained positive after the hajj. Data on the possibility of increased carriage among returning pilgrims are variable. Few studies showed no increase in the carriage rate [23,37,38]. In a cohort study, the acquisition rate of *N. Meningitides* was 0.3% among paired cohort and 0.6% among non-paired cohort [39]. In another cohort of French Hajj pilgrims, none of them had *N. Meningitides* on arrival or departure

[40]. A recent review indicated that carriage rates were higher in Hajj pilgrims compared to Umrah pilgrims and that most studies showed the carriage rates to be comparable to the rates in non-epidemic settings [41]. The use of antibiotics during the Hajj may reduce the carriage rate [23]. Another important finding of the study is the fact that *N. meningitidis* serogroup B was isolated among pilgrims. *N. meningitidis* serogroup B is reported in many parts of the world including Europe, Australia, and north America [26,42]. *N. meningitidis* serogroup B represents 50% of all meningococcal cases and caused multiple outbreaks [43,44]. The quadrivalent meningococcal vaccine became a mandatory requirement for all pilgrims in 2001 [9,14]. A serosurvey of pilgrims showed that the majority of pilgrims were vaccinated and protected against meningococcal serogroups A, C, W, and Y [45]. There is a concern of the continued change in the epidemiology of invasive meningococcal disease and the fear of the development of outbreaks related to serogroups B or X. The availability of serogroup B *N. meningitidis* vaccine is an added advantage to be used in cases of outbreaks [46]. The recommendation to use this vaccine for all pilgrims would require further studies of the epidemiology of *N. meningitidis* in pilgrims.

In conclusion, this the largest study of the epidemiology of *N. meningitidis* among pilgrims. The study showed a low rate of carriage and a predominance of serogroup B. The use of ciprofloxacin as prophylactic antibiotics should be reconsidered as well as the consideration to add serogroup B as a required vaccination especially for pilgrims coming from endemic areas.

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Table 1: Characteristics and Carriage Rates of *N. meningitidis* and *H. influenzae* of Unpaired Arriving Pilgrims

	Number of Nasopharyngeal swabs	**Influenza vaccination n (%)**	**Pneumococcal vaccination n (%)**	**Carriage *Neisseria meningitidis* n (%)**	**Serogroup B, n (%)**	**Non-groupable, n (%)**	**Carriage *H. influenzae* on arrival, n (%)**	**Carriage *N. meningitidis* and *H. influenzae* on arrival, n (%)**
High Risk Countries								
Nigeria	85	0 (0)	0 (0)	14(16.47)	5(5.8)	9(10.5)	2 (2.3)	2(2.3)
Ethiopia	93	0 (0)	0 (0)	6 (6.5)	4(4.3)	2(2.1)	12 (13.1)	1(1.0)
Tanzania	95	0 (0)	0 (0)	1 (1.0)	1(1.0)	0(0)	13 (3.1)	1 (1.0)
Subtotal	273 (25.9)	0 (0)	0 (0)	21 (8.78)	10 (3.6)	11 (4.0)	17 (6.2)	4(1.4)
Medium Risk Countries								
India	100	4 (4.0)	0 (0)	0 (0)	0(0)	0 (0)	3(3)	0
Pakistan	98	93 (94.89)	0 (0)	1 (1.0)	1(1.0)	0 (0)	0(0)	0
Bangladesh	79	60 (75.94)	0 (0)	2 (2.5)	2(2.5)	0 (0)	2(2.5)	1 (1.2)
Egypt	98	3 (3.0)	0 (0)	1 (1.02)	1(1.0)	0 (0)	4(4.0)	0
Somalia	98	0 (0)	0 (0)	7 (7.29)	6(6.25)	1(1.4)	4(4.1)	3 (3.1)
Subtotal	467 (44.2)	160 (34.3)	0 (0)	11 (2.3)	10 (2.1)	1 (0.1)	13 (2.8)	4 (0.8)
Low Risk Countries								
Indonesia	98	56 (57.14)	0 (0)	2 (2.0)	2(2.0)	0(0)	7(7.1)	1 (1.0)
Malaysia	95	15 (15.78)	15 (15.7)	0 (0)	0(0)	0(0)	0(0)	0
Albania	30	0 (0)	0 (0)	0 (0)	0(0)	0(0)	1(3.5)	0
USA	92	5 (5.49)	2 (2.1)	2 (2.1)	2(2.1)	0 (0)	7(7.6)	2 (2.1)
Subtotal	315 (29.9)	76 (24.1)	17 (5.4)	4 (1.3)	4 (1.3)	0(0)	15 (4.7)	3 (0.9)
Grand Total	1055	236 (22.36)	17 (1.6)	36 (3.4)	24 (2.27)	12 (1.1)	45(4.2)	11(1.0)

Table 2: Characteristics and Carriage Rates of *N. meningitidis* and *H. influenzae* of Paired Pilgrims

	Number of Nasopharyngeal swabs	Previous influenza vaccination n (%)	Previous pneumococcal vaccination, n (%)	Carriage *Neisseria meningitidis* at arrival, n (%)	Carriage *Neisseria meningitidis* at departure, n (%)	Genogroup B *Neisseria meningitidis* at arrival, n (%)	Genogroup B *Neisseria meningitidis* at departure, n (%)
High Risk Countries							
Ethiopia	64	0 (0)	0 (0)	4(6.2)	0(0)	3(4.6)	0(0)
Tanzania	72	0 (0)	0 (0)	1(1.3)	2(2.7)	1(1.3)	2(2.7)
Subtotal	136 (21.6)	0 (0)	0 (0)	5(3.6)	2(1.4)	4(2.9)	2(1.4)
Medium Risk Countries							
India	73	1(1.3)	0 (0)	0(0)	0(0)	0(0)	0(0)
Pakistan	89	84(94.3)	0 (0)	1(1.1)	0(0)	1(1.1)	0(0)
Bangladesh	27	18(66.6)	0 (0)	1(3.7)	1(3.7)	1(3.7)	1(3.7)
Egypt	86	3 (3.4)	0 (0)	1(1.1)	1(1.1)	1(1.1)	1(1.1)
Somalia	50	0 (0)	0 (0)	6(12.0)	1(0)	5(10.0)	1(2.0)
Subtotal	325 (51.7)	106 (32.6)	1 (0)	9 (2.7)	3 (0.9)	8 (2.4)	3 (1.0)
Low Risk Countries							
Indonesia	59	34(57.6)	0 (0)	1(1.6)	0(0)	1(1.6)	0(0)
Malaysia	68	12(17.6)	12(17.6)	0(0)	1(1.4)	0(0)	1(1.4)
USA	40	2 (3.4)	2 (5.0)	1(2.5)	1(2.5)	1(2.5)	2(5.0)
Subtotal	167 (26.6)	48 (28.7)	14 (8.34)	2 (1.2)	2 (1.2)	2 (1.2)	3 (1.7)
Grand Total	628 (100)	154 (24.5)	14(2.2)	16(2.5)	8(1.2)	14(2.2)	8 (1.2)

Figure 1: Percentage of Arriving Pilgrims with *N. meningitides* based on the Study Group and Endemicity