

Acquisition of Meningococcal Serogroup W-135 Carriage in Turkish Hajj Pilgrims Who Had Received the Quadrivalent Meningococcal Polysaccharide Vaccine

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Invasive meningococcal disease is a recognized public health problem worldwide, with a dynamic and changeable epidemiology. In Turkey, the second most common pathogenic meningococcal serogroup (after serogroup B) is W-135, including an epidemic in 2005, which has been strongly associated with Hajj pilgrims and their close contacts. In two studies conducted in 2010, we assessed meningococcal carriage in intending Turkish pilgrims to the Hajj when they attended to receive a plain polysaccharide vaccine against serogroups A, C, W-135, and Y and, upon their return, to determine the acquisition of meningococcal carriage by the pilgrims themselves and subsequently their household contacts. Nasopharyngeal swabs were obtained from pilgrims before the Hajj and upon their return. Swabs were then obtained from 39 household contacts of pilgrims who were shown to have acquired carriage during the Hajj. Of the 472 pilgrims before the Hajj, 63 (13%) were positive for meningococcal carriage, of which 52 cases (83%) were serogroup W-135. In the 296 pilgrims tested after the Hajj, 81 (27%) were positive for meningococcal carriage, including 74 (91%) with W-135. In 11 family members of pilgrims who acquired W-135 carriage at the Hajj, 10 (91%) had acquired carriage of serogroup W-135. This study illustrates the acquisition of meningococcal carriage, predominantly of serogroup W-135 by pilgrims attending the Hajj, and the transmission of this carriage to their family members on their return, explaining the source of W-135 meningococcal disease in Turkey.

The annual Hajj to Makkah (Mecca) in the Kingdom of Saudi Arabia (KSA) brings together millions of people from around the world and is a well-recognized site for the transmission of airborne infectious diseases such as influenza and invasive meningococcal disease (IMD). In the first few years of the 21st century, a virulent meningococcal serogroup W-135 clone caused an IMD outbreak at the Hajj; this serogroup has been the most important cause of Hajj-associated IMD worldwide for more than a decade (1, 2). The KSA instituted the use of the best-available epidemiologically appropriate meningococcal vaccines during the 1980s (1). Currently, KSA recommends the use of a quadrivalent conjugate vaccine against serogroups A, C, W-135, and Y for citizens and Hajj pilgrims from countries where these vaccines are available (2). A polysaccharide meningococcal vaccine against serogroups A, C, W-135, and Y is used in some countries and regions (3).

The meningococcus, which causes IMD, is an obligate human pathogen that generally colonizes between 5 and 10% of the population in most developed countries. However, carriage rates can increase under conditions of crowding or hyperendemic disease (4, 5). The use of monovalent meningococcal conjugate vaccines in routine universal vaccination programs has been associated with reductions in carriage and consequent herd effects, such as reductions of IMD in unvaccinated age cohorts (6). Quadrivalent conjugate vaccines are expected to confer similar effects with widespread use. However, confirmatory data are lacking. It has been suggested that polysaccharide vaccines may sufficiently protect individuals from IMD during short-term exposures, such as the Hajj, and also prevent acute acquisition of new carriage (7).

In Turkey, meningococcal serogroup B is the most epidemiologically important cause of IMD, and no vaccine is available to adequately address the variety of circulating pathogenic strains (8). Serogroup W-135 IMD is much rarer and associated primarily with Hajj pilgrims and their close contacts. Surrounding na-

tions also report endemic IMD caused by serogroups A, W-135, and C. The current recommendation in Turkey is the use of a plain polysaccharide vaccine against serogroups A, C, W-135, and Y for persons traveling to the Hajj or at high risk for acquiring IMD. Few data are available about the effects of these vaccines on the acquisition of new meningococcal carriage in Hajj pilgrims from Turkey or the transmission of carriage from pilgrims to their close contacts at home.

The present study was conducted to assess meningococcal carriage acquisition during 2010 among Turkish Hajj pilgrims who received polysaccharide vaccine against serogroups A, C, W-135, and Y and the acquisition of meningococcal carriage by household contacts.

MATERIALS AND METHODS

We conducted two studies at Hacettepe University in Ankara: a prospective cohort study in adolescent and adult Hajj pilgrims to compare carriage before and after the Hajj and a second study in close household contacts 2 to 3 months after the Hajj. Both studies followed the principles outlined in the current Declaration of Helsinki, as well as all local and regional ethical requirements for the protection of human research subjects. Ethics committee approval was obtained before study start, and all participants provided written informed consent.

Study participants. Acquisition of asymptomatic nasopharyngeal colonization by meningococci before and after the Hajj was assessed in

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healthy adolescents and adults. Eligible participants were between 15 and 64 years of age, traveling to the KSA to perform the Hajj, and eligible to receive polysaccharide vaccine against meningococcal serogroups A, C, W-135, and Y. One study aim was to support sample size calculations for a proposed study of a meningococcal conjugate vaccine against serogroups A, C, W-135, and Y (Menveo; Novartis Vaccines and Diagnostics, Cambridge, MA). Acquisition of carriage was defined as negative nasopharyngeal swabs before the Hajj and one or more positive swabs after return from the KSA. A second study enrolled consenting household contacts of pilgrims who had acquired nasopharyngeal meningococcal carriage during their stay in the KSA.

Exclusion criteria for both studies included known active illness or chronic disease or drug use that could affect immune status. Persons using local antiseptic solutions taken through the oral or nasal route were excluded to prevent confounding study results.

Study procedures. Participants in the initial study gave written, informed consent and provided nasopharyngeal swabs before and after the Hajj. The study was timed to allow collection of nasopharyngeal samples within 2 weeks before the Hajj to avoid confounding study results. Each participant then received a quadrivalent meningococcal polysaccharide vaccine (Menomune; Sanofi Pasteur, Lyon, France) before traveling to the KSA. Participants were to report to the clinic within 2 weeks after returning from the Hajj, and study personnel attempted to contact those who did not report back. The study was monitored by Novartis personnel.

Participants who were negative for nasopharyngeal carriage before the Hajj but positive after return from the KSA were asked to request that their household contacts participate in a study. Consenting individuals provided nasopharyngeal swabs at the clinic 2 to 3 months later.

Collection and culture of clinical specimens. Charcoal-impregnated, cotton-tipped swabs were used for all throat cultures. One passage in the posterior pharynx, behind the uvula, and one in each tonsil were sampled per participant. Samples were plated directly where possible or transported using Stuart's transport medium.

In the initial study, swabs were plated immediately on receipt on Martin Lewis medium, chocolate agar (GC agar base with 1% hemoglobin and 1% IsoVitaleX), and Columbia agar with sheep blood (8%). The agar plates were incubated at 37°C in a 5% CO₂ atmosphere for 48 h (mobile transport system). Identification of *Neisseria meningitidis* was performed by Gram stain microscopy and biochemical analysis. Gram-negative diplococci showing oxidase positivity, gamma-glutamyltransferase activity, metabolism of glucose and maltose in a cystine-Trypticase agar medium, and a negative ONPG (*o*-nitrophenyl- β -D-galactopyranoside) reaction were identified as *N. meningitidis*.

In the evaluation of household contacts conducted 2 to 3 months after identification of carriage in a pilgrim, samples were cultured on GC agar base (Oxoid, Cambridge, United Kingdom) containing 5% hemoglobin (Oxoid), yeast autolysate, Vitox growth supplement (Oxoid), and VCNT antibiotic supplements (vancomycin, colistin methane sulfonate, nystatin, and trimethoprim; Oxoid).

All suspected meningococcal colonies were characterized by Gram stain and oxidase tests. The Gram-negative diplococci and oxidase-positive meningococci colonies were serogrouped by slide agglutination tests with serogroup-specific antisera (A, B, C, X, Y, Z, W-135, and 29E; Difco/BD, Franklin Lakes, NJ).

Serogrouping by PCR. Single-tube multiplex PCR assay was performed, and serogroup prediction was based on the oligonucleotides in the *siaD* gene for serogroups B, C, W-135, and Y, in orf-2 of a gene cassette required for serogroup A, and in the *ctrA* gene for serogroups 29E, X, and Z (9, 10).

Sample size. The study was designed to be descriptive and to estimate a baseline risk of acquiring *N. meningitidis* carriage during the Hajj. A planned sample size of 360 was calculated assuming a baseline carriage rate of up to 10% and an upper limit of carriage of 35%, allowing for 20% attrition.

TABLE 1 Serotype results for Hajj pilgrims

| Group | No. (%) of subjects ^a | | |
|-------------------------------------|----------------------------------|--|--|
| | Before travel to the Hajj | After return from the Hajj | |
| | | Subjects among Hajj pilgrims who had cultures after return | Negative before the Hajj and positive after the Hajj |
| Provided swabs | 472 (100) | 296 (100) | NA |
| Positive for meningococcal carriage | 63/472 (13) | 81/296 (27) | 39 (100) |
| Serogroup W-135 | 52/63 (83)* | 74/81 (91)* | 39 (100) |
| Serogroup B | 9/63 (14) | 5/81 (6) | 0 (0) |
| Serogroup A | 1/63 (2) | 1/81 (1) | 0 (0) |
| Serogroup Y | 1/63 (2) | 1/81 (1) | 0 (0) |

^a *, Percentages do not add up to 100 because of rounding. NA, not applicable.

Dendrograms for the household contact study. Dendrograms were created using molecular analyst/multi-analyst programs (Bio-Rad, Hercules, CA) by using the unweighted pair group method utilizing arithmetic averages, and a position tolerance of 1.5%. The percent similarity between pulsed-field gel electrophoresis (PFGE) patterns can be used to assess the relationship between the patterns but cannot be used to quantify genetic relatedness. The cophenetic correlation (between similarity on dendrogram and the Dice coefficient-derived degree of similarity) was calculated; a 70% correlation was considered necessary for accuracy in the dendrogram. A clone was defined as isolates with indistinguishable PFGE patterns. A PFGE-based clonal group was defined as isolates with a <3-band difference, a >80% dendrogram similarity, or both.

RESULTS

There were 472 eligible participants gave written, informed consent and provided nasopharyngeal swabs before the Hajj, 63 (13.3%) of whom were positive for asymptomatic meningococcal carriage at baseline, and the majority carried serogroup W-135 and the remainder carried serogroup B (Table 1). Within the first 2 weeks after returning from the Hajj, 296 (62.7%) of the original 472 participants were located and provided additional nasopharyngeal swabs. Among these participants, 81/296 (27.4%) were positive for meningococcal carriage, and 74/296 (25%) carried serogroup W-135.

Of the 39 pilgrims who acquired carriage while in the KSA, all carried serogroup W-135. Of these 39 participants 11 had family members (39 in total) who consented to participate in the study and were tested for meningococcal carriage. Ten of the 39 (25.6%) household contacts tested carried meningococcal serogroup W-135, and the clonal structure of the bacteria was the same as the source case. No other meningococcal carriage was observed in these household contacts (Table 1).

No cases of IMD were detected in study participants or their household contacts.

DISCUSSION

In these studies, 25% of Hajj pilgrims and their close contacts who provided nasopharyngeal swabs had evidence of asymptomatic carriage of meningococcal serogroup W-135. The rate of asymptomatic carriage at the study outset (13.3%) was somewhat higher than would have been expected (10%) (1, 4, 5) and was also inconsistent with the known epidemiology of IMD in Turkey, where

serogroup B is the most prevalent serogroup. A recent carriage study conducted in residence halls in the United Kingdom, where serogroup B is also the most prevalent serogroup, also found that carriage of another serogroup, in this case serogroup Y, was more common than serogroup B carriage (11). It might be of interest to obtain further information about the most commonly carried serogroups in Turkey.

Although the meningococcal carriage rate in household contacts tested in these studies was more than twice as high as would be expected in the general population, the significance of this finding is limited by small sample size. Nevertheless, the results suggest a potential risk for acquiring meningococcal carriage by close contact with asymptomatic persons who return from a high-risk situation. The current data indicate that Hajj pilgrims and their household contacts, similar to university students living in dormitories and new military recruits, have a higher rate of meningococcal carriage than the general population. In some at-risk groups, the reasons for this increased rate of carriage have been hypothesized to include crowding and close contact among people from distinct geographic areas. Insufficient data are available here to determine the cause of this apparent increase in carriage rates.

Of note, meningococcal polysaccharide vaccine did not appear to interfere with the acquisition of new carriage of serogroup W-135 meningococci during the Hajj in these Turkish pilgrims. Although bivalent polysaccharide serogroup A and C vaccines have been found to interrupt new serogroup A carriage among Hajj pilgrims (1), the finding in the present study is consistent with the general understanding of the mechanism of action of polysaccharide vaccines. Although inadequate time may have been allowed for the induction of protective antibodies in some pilgrims, the polysaccharide vaccine was administered consistent with local guidelines, thus reproducing real-world experience. Further, vaccination very shortly before travel is common practice for Hajj pilgrims in many countries. Since the optimal antibody response to polysaccharide meningococcal vaccine arises after 2 weeks, the vaccine should be administered to Hajj pilgrims no later than 2 weeks before their journey.

No evidence suggests that meningococcal polysaccharide vaccine eradicates existing meningococcal carriage, as expected given the immunogenic properties of the vaccine. Conjugate vaccines may confer added protective benefits for Turkish Hajj pilgrims and their household contacts.

The rate of serogroup B meningococcal carriage was low both before and after the Hajj, and all subjects who had serogroup B carriage after the Hajj had tested positive for serogroup B carriage before travel to the KSA. Although these results indicate that serogroup B transmission did not occur in the present study group of Hajj pilgrims, it is unclear whether this finding is generalizable.

Limitations of the present study included the time between vaccination and attendance at the Hajj, which could have prevented the induction of adequate immune antibodies to affect carriage. Despite this caveat, the study followed current practice guidelines and recommendations for travel to the Hajj and therefore represents a real-world experience. Further, no baseline nasopharyngeal swabs were obtained from household contacts before the Hajj. However, comparison of the general data and the literature related to the risk of transmission in household contacts of returning pilgrims suggests the transmission of *N. meningitidis* (12, 13). In addition, nasopharyngeal swabs from household con-

tacts of the serogroup B carriers could have provided valuable information about the dominant epidemiologic cause of IMD in Turkey.

We detected the acquisition of W-135 carriage in Turkish Hajj pilgrims who received a polysaccharide vaccine against serogroups A, C, W-135, and Y, as well as in their household contacts. All acquisition of carriage occurred with serogroup W-135, which was also the only serogroup carried by household contacts after the Hajj. These findings suggest that serogroup W-135-caused IMD in Turkey may be transmitted via the Hajj. Given that serogroup B is the most common cause of IMD in Turkey, additional study is recommended to obtain a fuller understanding of the epidemiologic situation.

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