

# *Neisseria meningitidis* Serogroup W-135 Carriage among US Travelers to the 2001 Hajj

Peter M. Dull,<sup>1,2</sup> Jalaa' Abdelwahab,<sup>4</sup> Claudio T. Sacchi,<sup>2</sup> Margaret Becker,<sup>3</sup> Corie A. Noble,<sup>2</sup> Gwen A. Barnett,<sup>2</sup> Robyn M. Kaiser,<sup>2</sup> Leonard W. Mayer,<sup>2</sup> Anne M. Whitney,<sup>2</sup> Susanna Schmink,<sup>2</sup> Gloria W. Ajello,<sup>2</sup> Jennifer Dolan-Livengood,<sup>5</sup> David S. Stephens,<sup>2,5</sup> Marty S. Cetron,<sup>3</sup> Tanja Popovic,<sup>2</sup> and Nancy E. Rosenstein<sup>2</sup>

<sup>1</sup>Epidemic Intelligence Service, Epidemiology Program Office, <sup>2</sup>Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, and <sup>3</sup>Division of Global Migration and Quarantine, National Center for Infectious Diseases, and <sup>4</sup>Public Health Prevention Service, Epidemiology Program Office, Centers for Disease Control and Prevention, and <sup>5</sup>Emory University School of Medicine, Atlanta, Georgia

In 2000, a large international outbreak of meningococcal disease caused by *Neisseria meningitidis* serogroup W-135 was identified among pilgrims returning from the Hajj in Saudi Arabia. To assess ongoing risk, we evaluated *N. meningitidis* carriage among US travelers to the 2001 Hajj. Of 25 *N. meningitidis* isolates obtained, 15 (60%) were nongroupable and 8 (32%) were serogroup W-135 when tested by standard slide-agglutination techniques. Two additional nongroupable isolates were characterized as serogroup W-135 when tested by polymerase chain reaction. Nine of 10 serogroup W-135 isolates were indistinguishable from the Hajj-2000 clone. None of the departing, but 9 (1.3%) of the returning, pilgrims carried serogroup W-135 ( $P = .01$ ); all carriers reported previous vaccination. Carriage of *N. meningitidis* serogroup W-135 increased significantly in pilgrims returning from the Hajj. Although the risk of disease to pilgrims appears to be low, the risk of spread to others of this pathogenic strain remains a concern.

*Neisseria meningitidis* is a major cause of meningitis worldwide; even with prompt antibiotic therapy, the case fatality rate is 10%–15%. Among survivors, 12%–19% have long-term neurologic sequelae, including hearing loss, loss of limbs, and cognitive deficit [1]. Meningococci are spread from person to person through direct contact with oropharyngeal secretions, and asymptomatic meningococcal carriers are the primary source of *N. meningitidis* transmission. The baseline carriage rate in the United States is estimated to be 5%–10%, and carriage rates are higher among adolescents and young adults [2, 3]. Fewer than 1% of individuals who acquire carriage go on to develop meningococcal disease. The balance between carriage of the organism and the development of disease after acquisition is affected by a

combination of host and environmental factors and *N. meningitidis* characteristics.

In 1987, a large epidemic of serogroup A meningococcal disease occurred among pilgrims to the Hajj, a large annual gathering of Muslims in Medina and Mecca, Saudi Arabia. Cases in secondary contacts of returning pilgrims were also reported in many countries [4]. At that time, a study by Centers for Disease Control and Prevention (CDC) researchers of passengers returning from Saudi Arabia to JFK International Airport in New York City showed that pilgrims were significantly more likely to be carriers of serogroup A meningococcus than were nonpilgrims [5]. Primarily on the basis of these data, the CDC began recommending vaccination against meningococcal disease for all travelers to the Hajj. The following year, Saudi Arabia began a policy of requiring that all Hajj visitors produce a certificate of vaccination against meningococcal disease before entry. Most countries administered a bivalent vaccine that contained polysaccharides of serogroups A and C; in the United States, the licensed meningococcal vaccine contains polysaccharides of serogroups A, C, Y, and W-135. Importantly, although the vaccine is pro-

Received 28 January 2004; accepted 30 June 2004; electronically published 29 November 2004.

Reprints or correspondence: Dr. Nancy Rosenstein, Epidemiology Section, Meningitis and Special Pathogens Branch, C-09, Div. of Bacterial and Mycotic Diseases, NCID, CDC, 1600 Clifton Rd., Atlanta, GA 30333 (nar5@cdc.gov).

The Journal of Infectious Diseases 2005;191:33–9

© 2005 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2005/19101-0006\$15.00

tective ( $\geq 85\%$ ) for older children and adults, it does not reliably prevent pharyngeal carriage [6].

In March 2000, along with an outbreak of serogroup A meningococcal disease, the first reported large outbreak of serogroup W-135 meningococcal disease occurred in Saudi Arabia in association with the Hajj pilgrimage. Subsequently, >400 laboratory-confirmed cases of meningococcal disease caused by *N. meningitidis* serogroup W-135 were identified worldwide, including 5 cases in the United States [7]. Among the 5 Hajj-associated cases of serogroup W-135 infection in the United States in 2000, 2 were in vaccinated pilgrims and 2 were in close contacts of vaccinated pilgrims.

Because serogroup W-135 is an infrequent causative agent of meningococcal disease [8], limited information was available on its epidemiology; therefore, there was limited guidance as to the appropriate public health response. In February and early March of 2001, to further investigate the transmission of *N. meningitidis* among Hajj pilgrims, to assist in making recommendations to prevent additional cases, and to better understand the molecular epidemiology of meningococcal carriage, we studied oropharyngeal carriage among travelers going to and returning from the Hajj.

## SUBJECTS, MATERIALS, AND METHODS

Approximately 9000 US pilgrims attended the Hajj in Saudi Arabia in 2001. The last arrival date permitted for international flights to Jeddah was 27 February, and the official dates of the Hajj were 3–7 March. The study population included cohorts of Hajj pilgrims departing between 17 and 25 February 2001 on flights leaving from JFK International Airport in New York City to Jeddah, Saudi Arabia. Flights transporting large groups of pilgrims were identified through contact with travel agents, tour-group operators, and airlines. CDC workers approached potential study participants, and documents were provided in either English or Arabic, according to individual preference. After giving informed consent, all adult ( $\geq 18$  years old) pilgrims were asked to answer a brief set of questions and to provide a swab sample from the oropharynx for culture. The outbound passenger questionnaire included identifiers (name and birth date) and return flight information so that, on their return, the repeat-culture results could be compared. Informed consent was obtained from all participants, and the human-experimentation guidelines of the US Department of Health and Human Services and/or those of the authors' institution(s) were followed.

Approximately 3 weeks later (9–15 March), all passengers (pilgrims and nonpilgrims) disembarking from flights inbound to JFK International Airport directly from Jeddah, Saudi Arabia, were approached for study enrollment. After giving informed consent, all adult ( $\geq 18$  years old) passengers were asked to answer a brief set of questions and to provide a swab sample

from the oropharynx for culture. On the inbound passenger questionnaire, the following information was collected: demographics, seat number, recent travel, recent antibiotic use, meningococcal vaccination history, and potential risk factors for meningococcal carriage. Contact information was also solicited, so that passengers could be informed of positive culture results.

Each study participant on inbound flights was given a packet that contained an information sheet and a 500-mg tablet of ciprofloxacin. If there were no contraindications, participants were advised that they might take the antibiotic immediately or wait for their culture results. All inbound passengers whose oropharyngeal cultures yielded *N. meningitidis* were contacted by telephone and advised of the results. All passengers were advised that they could find their results on a CDC Web site, listed with a unique identifier identical to one printed on each information sheet. Antimicrobial chemoprophylaxis was not offered to outbound travelers, because of the low risk of disease in this vaccinated population.

## Definitions

Vaccinated persons were defined as those who reported meningococcal vaccination  $\leq 5$  years before their arrival date in Saudi Arabia. Because it can take 7–10 days to mount an effective immune response and protection lasts up to 5 years, “protected” persons were defined as those who reported meningococcal vaccination  $>10$  days and  $\leq 5$  years before their arrival date in Saudi Arabia. Those inbound passengers answering yes to the question, “Are you returning after performing the Hajj?” were defined as pilgrims; those answering no to the same question were defined as nonpilgrims.

A carrier was defined as a person with any *N. meningitidis* isolated from an oropharyngeal swab as identified by standard microbiological procedures [9]. An isolate that is nongroupable by standard slide agglutination (SASG) may possess capsule-specific genes that are amplified by serogroup-specific polymerase chain reaction (PCR). Therefore, for purposes of the analysis, an *N. meningitidis* serogroup W-135 carrier was defined as a person with *N. meningitidis* isolated from an oropharyngeal swab and identified as serogroup W-135 by SASG and/or serogroup-specific PCR.

## Laboratory Methods

**Collection of clinical specimens.** Swabs were collected from the high posterior oropharyngeal wall by trained health professionals, and samples were plated directly onto a selective medium, BBL-modified Thayer-Martin medium (MTM II; Becton Dickinson Microbiology Systems). Plates were maintained in CO<sub>2</sub>-enriched, sealed containers. Within 2–4 h, the containers were transported to an incubator at the New York City Public Health Department Laboratory, where they were incubated overnight at 35°C in 5% CO<sub>2</sub>.

**Identification of *N. meningitidis* serogroups.** Plates were examined for the presence of colonies showing the typical morphology of *N. meningitidis*. Suspect colonies were screened for oxidase reactivity, and positive samples were gram stained. If gram-negative diplococci were present, a sample of the growth was transferred to a BBL sheep-blood agar plate (TSA II 5% SB; Becton Dickinson Microbiology Systems) and, after overnight incubation, serogrouped by SASG [9]. Cultures were subsequently transported to Atlanta and confirmed at the CDC by use of SASG and molecular techniques.

All presumptive *Neisseria* isolates were confirmed as *N. meningitidis* by a real-time PCR assay targeting the *ctrA* gene, which encodes a capsule transport protein [10]. All *ctrA*-positive samples were further tested for possession of a serogroup-specific capsule polymerase gene by use of 5 separate real-time PCR assays for serogroup A, B, C, W-135, or Y [11]. Isolates that did not give positive results in any of the serogroup-specific assays were characterized as nongroupable for the purposes of the study. All subsequent analysis was performed by use of serogrouping as defined by serogroup-specific PCR results.

**Additional genotypic and phenotypic characterization.** Meningococcal strains were characterized by use of pulsed-field gel electrophoresis (PFGE) [12] and sequencing of the 16S rRNA genes [13]. All 16S rRNA gene sequences have been deposited in GenBank. The *N. meningitidis* serogroup W-135 strains were additionally characterized by PorA variable-region (VR) typing, as described elsewhere [14].

The isolates initially identified as nongroupable by SASG but subsequently shown by PCR to possess serogroup W-135 capsule genes were further studied by use of primers internal to each gene coding for capsule biosynthesis (*syn*) and capsule transport (*ctr*) proteins, and the PCR products were sequenced [15]. To further assess phenotypic characteristics, the isolates were tested in a serum bactericidal assay by incubation in 10% normal human serum for 30 min by methods described elsewhere [16].

## Statistical Methods

Univariate analysis was performed in EpiInfo 6.04b (CDC, Atlanta, GA) and SAS 8.1 for Windows (SAS) software, by use of Fisher's exact test with Mantel-Hansel odds ratios to assess the associations of individual variables with carriage of *N. meningitidis* and with carriage of *N. meningitidis* serogroup W-135, as defined by serogroup-specific PCR. A matched analysis was performed of the carriage status of pilgrims enrolled in both the outbound and inbound portions of the study. Because many enrolled outbound pilgrims were not encountered on the inbound enrollment flights, a separate unmatched analysis of carriage status was performed to evaluate outbound, compared with inbound, passengers.

## RESULTS

### Descriptive Epidemiology

**Outbound passengers.** Participants were recruited among passengers departing on 7 consecutive direct flights from JFK International Airport to Jeddah, Saudi Arabia, 17–25 February 2001. A questionnaire and corresponding swab were available for 452 outbound passengers. The median age was 50 years (range, 21–86 years); 63% were men. All outbound study participants carried Hajj visas and were pilgrims. Of the 443 who answered the question, 15% had received an antibiotic during the preceding 2 weeks. Meningococcal vaccination was reported by 98.9% of pilgrims, and 92.5% were protected at their time of anticipated arrival in Saudi Arabia, on the basis of the timing of vaccination.

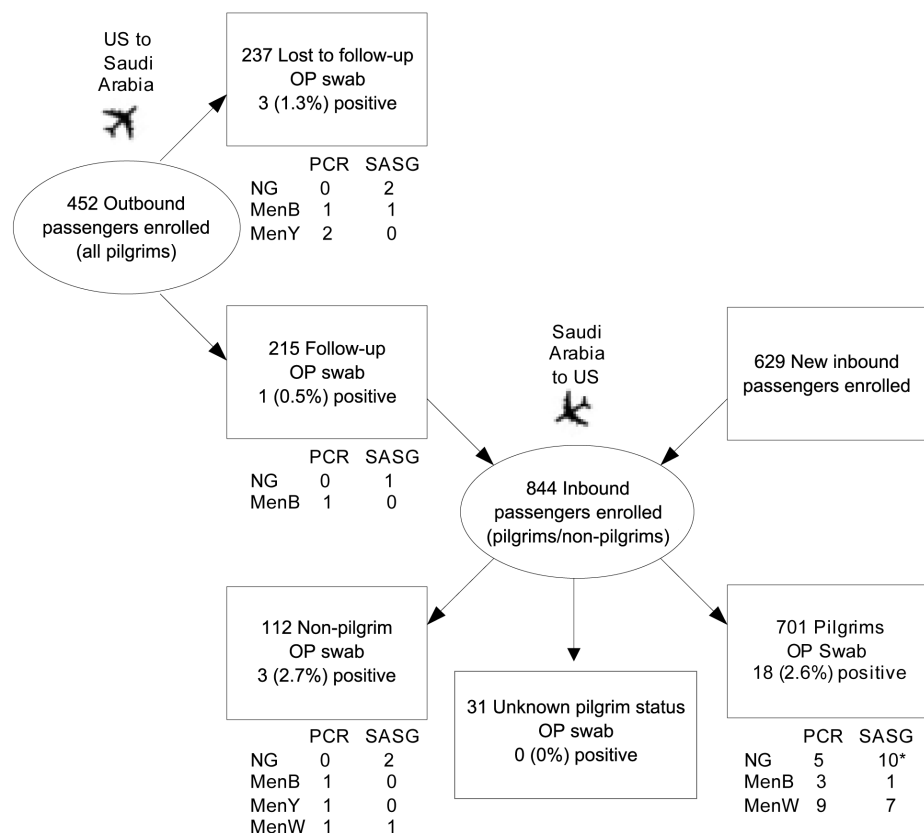
**Inbound passengers.** Participants were recruited among ~1400 inbound passengers on 5 direct flights to JFK International Airport from Jeddah, Saudi Arabia, 9–15 March 2001. A questionnaire and corresponding swab were available for 844 inbound passengers. Of those who answered the questions, 61.5% were men and 86.2% were pilgrims. The median age was higher among pilgrims than nonpilgrims (47.0 vs. 35.0 years;  $P < .0001$ , Wilcoxon test). Antibiotic use during the preceding 2 weeks was reported by 44.8% of subjects. There were 215 pilgrims among the 844 inbound passengers who were also enrolled during the outbound portion of the study.

Vaccination coverage was higher among pilgrims than nonpilgrims (97.5% vs. 71.0%;  $P < .0001$ ). Similarly, on the basis of vaccination history, more pilgrims than nonpilgrims were protected on their arrival in Saudi Arabia (81.9% vs. 60.2%;  $P < .0001$ ).

### Carriage Rates

**Unmatched analysis.** Of the 452 oropharyngeal cultures obtained from outbound passengers (pilgrims only), 4 cultures tested positive for *N. meningitidis* (0.9%); of these, 2 were serogroup Y and 2 were serogroup B according to the results of serogroup-specific PCR (figure 1). No *N. meningitidis* serogroup W-135 isolates were identified. Of the 844 oropharyngeal cultures obtained from inbound passengers (pilgrims and nonpilgrims), 21 cultures tested positive for *N. meningitidis* (2.6%); of these, 10 were serogroup W-135, 5 were nongroupable, 4 were serogroup B, and 1 was serogroup Y (figure 1). One isolate that was not groupable by SASG was not viable after transport. Four isolates of *Neisseria lactamica* were also recovered from inbound passengers.

**Matched analysis.** Of the 215 pilgrims who provided oropharyngeal cultures during both phases of the study, 1 who had *N. meningitidis* serogroup B isolated during the outbound phase was again colonized with *N. meningitidis* serogroup B 22 days later, during the inbound phase. This strain was not further molecularly characterized. Four other pilgrims who had neg-



**Figure 1.** Schematic of the methods and results of the present study. Oropharyngeal cultures were obtained from 452 outbound passengers (all pilgrims) leaving JFK International Airport in New York City en route to Jeddah, Saudi Arabia, for the Hajj. Two hundred fifteen of these pilgrims provided second samples on their return, along with 629 additional enrollees (pilgrims and nonpilgrims). MenB/Y/W, serogroup B/Y/W-135; NG, not groupable; OP, oropharyngeal; PCR, polymerase chain reaction; SASG, serogroup by standard slide agglutination. \*One isolate was not viable for molecular studies.

ative cultures on the outbound phase had *N. meningitidis* (2 nongroupable, 1 serogroup B, and 1 serogroup W-135) isolated during the inbound phase.

**Molecular subtyping.** Of the 14 available isolates (from inbound or outbound passengers) identified as nongroupable by SASG (figure 1), 2 were identified as *N. meningitidis* serogroup W-135 by serogroup-specific PCR (table 1). These 2 isolates and the 8 *N. meningitidis* serogroup W-135 isolates identified by SASG were additionally characterized by PFGE, PorA VR typing, and sequencing of the 16S rRNA genes. Of these 10 isolates, 8 had identical molecular markers that were characteristic of the W(ET)-37 clone, which had previously been identified to be the cause of the *N. meningitidis* serogroup W-135 outbreaks associated with the Hajj in 2000—PFGE type 40, PorA VR type 5,2, and 16S type 31 (table 1) [7]. A single isolate (M7890) differed from these 8 by only 1 bp (16S type 54). Molecular markers of the remaining *N. meningitidis* serogroup W-135 isolate (M7888) were very different from those seen in the other 9 isolates.

**Analysis of isolates with discordant results as determined by SASG and serogroup-specific PCR.** The 2 *N. meningitidis*

serogroup W-135 isolates from inbound passengers identified as nongroupable by SASG were additionally studied by PCR for the presence and characteristics of genes coding for *syn* and *ctr* operons [15]. The first isolate (M7904) yielded the expected PCR products for all *syn* and *ctr* genes studied, including the serogroup Y or W-135 polymerase gene (the primers used did not distinguish between these 2 serogroups). The second isolate (M7888) yielded the expected PCR products for all genes except *synA*, where the product was ~800 bp larger than expected. Nucleotide-sequence analysis revealed an IS1301 insertion [15] in the gene. In a bactericidal assay that used exposure to 10% normal human serum for 30 min, isolate M7904 survived at rates comparable to an encapsulated control strain, whereas isolate M7888 was rapidly and completely killed. Colony immunoblots performed with W-135- and Y-specific monoclonal antibodies to serogroups W-135 and Y (supplied by W. Zollinger, Walter Reed Army Institute of Research, Washington, DC) showed that isolate M7904, but not isolate M7888, expressed the W-135 capsule. Isolate M7888 did not express capsule because of an IS1301 insertion in *synA*.

**Table 1. Phenotypic and molecular characterization of *Neisseria meningitidis* serogroup W-135 strains from inbound passengers.**

Strain	SASG	Serogroup-specific PCR	16S type	PFGE pattern	PorA VR type
W(ET)-37 clone	W135	W135	31	40	5,2
M7890	W135	W135	54 <sup>a</sup>	40	5,2
M7854	W135	W135	31	40	5,2
M7857	W135	W135	31	40	5,2
M7901	W135	W135	31	40	5,2
M7903	W135	W135	31	40	5,2
M7895	W135	W135	31	40	5,2
M7900	W135	W135	31	40	5,2
M7887 <sup>b</sup>	W135	W135	31	40	5,2
M7904 <sup>b</sup>	NG	W135	31	40	5,2
M7888	NG	W135	6	78	18–1,3

**NOTE.** NG, nongroupable; PFGE, pulsed-field gel electrophoresis; PorA VR, PorA variable region; SASG, serogroup by standard slide agglutination.

<sup>a</sup> 16S type 54 differs by 1 bp from 16S type 31.

<sup>b</sup> Husband-wife pair.

### Risk Factors for Carriage

The results of univariate analysis revealed no difference in overall rates of carriage by age or sex (data not shown). Among inbound passengers, vaccination status, protection status, smoking, previous upper respiratory-tract infection, antibiotic use, and the presence of chronic medical conditions were not associated with either overall carriage (table 2) or serogroup W-135 *N. meningitidis* carriage (data not shown). Among inbound pilgrims, there was no association of carriage (overall or serogroup W-135) with crowding at the Hajj (number of persons per hotel room in transit or number of persons per tent during other stages of the Hajj).

Inbound pilgrims were more likely to be carriers of *N. meningitidis* than were outbound pilgrims (2.6% vs. 0.9%;  $P = .03$ ). None of the outbound, but 9 (1.3%) of the inbound, pilgrims were carriers of *N. meningitidis* serogroup W-135 ( $P = .01$ , Fisher's 2-tailed exact test). Of the 9 inbound pilgrims who were carriers of *N. meningitidis* serogroup W-135, an oropharyngeal culture had been obtained from 1 at the time of departure 12 days earlier; no *N. meningitidis* was isolated at that time. Among inbound passengers, the prevalence of carriage of *N. meningitidis* serogroup W-135 was not significantly different between pilgrims (9/701 [1.3%]) and nonpilgrims (1/112 [0.9%]).

### DISCUSSION

In comparison to pilgrims traveling to the Hajj from the United States in 2001, returning pilgrims had an increased overall rate of carriage of *N. meningitidis*, a substantial portion of which was serogroup W-135. The carriage rate of pathogenic *N. meningitidis* was low, but the present findings do suggest that there

was ongoing transmission of *N. meningitidis* serogroup W-135 in Saudi Arabia during the period of the Hajj in 2001. From 9 February to 22 March 2001, Saudi Arabian health officials reported that there were >109 cases of meningococcal meningitis, including 35 deaths; of these, >50% were due to *N. meningitidis* serogroup W-135 [17]. This was consistent with reports from other countries of invasive serogroup W-135 meningococcal disease in 2001 Hajj pilgrims and their close contacts, including the European Union [18], Africa [17], and Mauritius [19]. A high rate of carriage (15% of 171 pilgrims) of a single clone of *N. meningitidis* serogroup W-135 were also identified in pilgrims returning to Singapore, with evidence of spread to household contacts [20]. Finally, a study among Saudi Arabian residents 1 week before the 2001 Hajj found a 4.7% carriage rate of *N. meningitidis*, 40% of which were serogroup W-135 [21], similar to the rate that we found among nonpilgrims (2.7% overall, 33% of which were serogroup W-135) and among returning pilgrims (2.6% overall, 50% of which were serogroup W-135) arriving from Saudi Arabia. It is possible that, even during the brief period of exposure during the Hajj, the intensity of close contact with other pilgrims and with the local population caused pilgrims to acquire a carriage profile similar to the endemic carriage profile. Although the numbers of positive cultures in our study do not permit a meaningful comparison, "routine" visits to an endemic area (for business or tourism) without the accompanying intense crowding present during the Hajj might not permit such high efficiency of transmission. The clonal spread of this strain among US residents has not been apparent—rates of serogroup W-135 meningococcal disease remain extremely low [22]; similarly, no reported outbreaks in other developed nations have occurred.

The overall carriage rate of *N. meningitidis* (<3%) in our study

**Table 2. Risk factors for oropharyngeal carriage of *Neisseria meningitidis* among inbound passengers from Jeddah, Saudi Arabia, 9–15 March 2001.**

Risk factor	<i>N. meningitidis</i> carriage rate (%)		<i>P</i>
	Carrier (n = 22 <sup>a</sup> )	Noncarrier (n = 822 <sup>a</sup> )	
Vaccinated	18 (85.7)	721 (94.4)	.12
Protected <sup>b</sup>	16 (76.2)	580 (79.7)	.78
Smoked cigarettes	2 (9.1)	58 (7.5)	.68
Exposed to cigarette smoke	6 (30.0)	168 (23.8)	.59
Sore throat	12 (54.6)	436 (56.4)	.83
Fever	5 (23.8)	174 (24.0)	1.00
Cough	11 (50.0)	489 (62.1)	.19
Used any antibiotic	7 (33.3)	372 (49.1)	.19
Pilgrim	19 (86.4)	682 (86.2)	1.00
Any medical condition	0 (0)	21 (3.2)	.06

<sup>a</sup> Denominators vary because of missing information.

<sup>b</sup> Vaccination >10 days and <5 years before arrival in Saudi Arabia.

was low relative to previous reports, which were 5%–10% and higher [3]. Standardized sampling was performed, and the rate likely reflects an accurate assessment, given the techniques that we used. Several possibilities may account for the low carriage rate, including the frequent use of antibiotics (~45%) during travel, infrequent cigarette use, older participant age (median age, 45 years), the higher socioeconomic status of US pilgrims, and the clustering of transmission among groups traveling together. The latter 2 factors may explain, in part, the discrepancy between the carriage rates among pilgrims found in our study and those in the Singapore study [20]. Antibiotic use was variable in duration and in type, including the frequent use of  $\beta$ -lactam antibiotics, which are not expected to affect carriage.

Despite low rates of carriage, transmission from and disease in returning pilgrims were still possible. In the United States, during the months after the 2001 Hajj, a case of serogroup W-135 meningococcal disease was identified through passive surveillance in a vaccinated 61-year-old Brooklyn, NY, resident who presented, 8 days after returning from Saudi Arabia, with meningococcemia and survived. An oropharyngeal culture had been obtained from this pilgrim on departure (no growth) but not on return from the Hajj. This isolate was also indistinguishable from the W(ET)-37 clone from the 2000 Hajj by all molecular methods used in the present study (data not shown).

A unique epidemiologic link between 2 carriers emphasizes the limitations of SASG for assessing the extent of spread of an invasive clone and the importance of meningococcal phase variation. An *N. meningitidis* serogroup W-135 isolate (M7887) was recovered from the oropharynx of an elderly male pilgrim. His wife was also an *N. meningitidis* carrier, but her isolate was not groupable by SASG (M7904). When they were further evaluated, the strains were found to be identical by PFGE, serotyping, serosubtyping, and 16S rRNA sequencing. Phenotypic testing was performed by use of the serum bactericidal assay, and the wife's isolate showed survival similar to that of an encapsulated control strain, which suggests the presence of a capsule. This isolate was reevaluated by SASG and was again not groupable, which suggests that a phenotypic change had not occurred during culture passage. Molecular studies found that this isolate contained a wild-type *syn* and *ctr* operon that included the *synG* gene (encoding the serogroup W-135-specific polymerase). Phase variation modifying the qualitative or quantitative characteristics but not eliminating capsule production at a site outside of *synG* was predicted for this isolate. A recent study of nongroupable *N. meningitidis* isolates from a meningococcal carriage study in Georgia characterized several classes of distinct genetic alteration types. The majority of nongroupable strains were genetically related or identical to the groupable strains recovered from the same population [15]. The unique epidemiologic link in the husband-wife pilgrim pair in our study suggests that the isolates were identical except for

capsule expression. High-frequency phase variation in the expression of virulence factors that included the capsule has been recognized in *N. meningitidis* both in vitro and in vivo [15].

The pathophysiology of meningococcal colonization and disease involves steps that include (1) altering capsule production and either enhancing epithelial adherence or up-regulating capsule expression and (2) facilitating invasive disease or transmission from mucosal surfaces [23]. Accumulating evidence of the pathogenic potential of strains that are not groupable by SASG [24, 25] brings into question assumptions that were previously made in SASG-based oropharyngeal carriage studies. Because, historically, nongroupable strains have been routinely discounted in carriage studies, underreporting of the carriage of potentially pathogenic strains is possible. More relevant to the public health community is that, in meningococcal disease-outbreak settings in developed countries, the decision to initiate mass vaccination is determined, in part, by the isolation of invasive disease isolates of an identical serogroup in unrelated persons. Without molecular characterization of such isolates, true carriage and genotype-specific attack rates may be underestimated, and the decision to initiate vaccination may not be made or may be delayed. In an outbreak setting, consideration should be given to evaluating, by molecular techniques, all potentially epidemiologically linked isolates.

Although the overall carriage rate in the present study was low, the high proportion of *N. meningitidis* serogroup W-135 carriage suggests that there are high rates of transmission in Saudi Arabia and that the continued use of an appropriately timed quadrivalent meningococcal polysaccharide vaccine for US Hajj pilgrims is recommended. Continued surveillance for serogroup W-135 meningococcal disease in Saudi Arabia, the United States, and globally will be important in evaluating the continuing risk. A better understanding of the genetic and consequent phenotypic behavior of *N. meningitidis* is essential to the interpretation of the true role and pathogenic potential of nongroupable isolates.

## Acknowledgments

We thank the following persons for their significant contributions to the field work of collecting and tracking samples and questionnaires, as well as data entry: Sandra Arthur, John Bateman, Moira Booth, Evelyn Colon, Veronica Dekozan, Ellen DeMott, Emmanuel Ntekop, Rachel Pechersky, Michael Phillips, Sarah Reagan, Gregory Reitz, Saima Saeed, Sheryl Shapiro, Dan Singer, Don Spatz, Christina Tan, and Reina Turcios.

## References

1. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr* 1981;99:540–5.
2. Cartwright KA, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidemiol Infect* 1987;99:591–601.

3. Broome CV. The carrier state: *Neisseria meningitidis*. J Antimicrob Chemother **1986**; 18(Suppl A):25–34.
4. Novelli VM, Lewis RG, Dawood ST. Epidemic group A meningococcal disease in Haj pilgrims [letter]. Lancet **1987**; 2:863.
5. Moore PS, Harrison LH, Telzak EE, Ajello GW, Broome CV. Group A meningococcal carriage in travelers returning from Saudi Arabia. JAMA **1988**; 260:2686–9.
6. Advisory Committee on Immunization Practices. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **2000**; 49(RR-7):1–10.
7. Mayer LW, Reeves MW, Al-Hamdan N, et al. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electrophoretic type-37 complex. J Infect Dis **2002**; 185:1596–605.
8. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992–1996. J Infect Dis **1999**; 180:1894–901.
9. Popovic T, Ajello G, Facklam R. Manual for the laboratory diagnosis of meningitis caused by *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. Geneva: World Health Organization, **1999**.
10. Frosch M, Weisgerber C, Meyer TF. Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B. Proc Natl Acad Sci USA **1989**; 86:1669–73.
11. Mothershed EA, Sacchi CT, Whitney AM, et al. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. J Clin Microbiol **2004**; 42:320–8.
12. Popovic T, Schmink S, Rosenstein NA, et al. Evaluation of pulsed-field gel electrophoresis in epidemiological investigations of meningococcal disease outbreaks caused by *Neisseria meningitidis* serogroup C. J Clin Microbiol **2001**; 39:75–85.
13. Sacchi CT, Whitney AM, Reeves MW, Mayer LW, Popovic T. Sequence diversity of *Neisseria meningitidis* 16S rRNA genes and use of 16S rRNA gene sequencing as a molecular subtyping tool. J Clin Microbiol **2002**; 40:4520–7.
14. Sacchi CT, Whitney AM, Popovic T, et al. Diversity and prevalence of PorA types in *Neisseria meningitidis* serogroup B in the United States, 1992–1998. J Infect Dis **2000**; 182:1169–76.
15. Dolan-Livengood JM, Miller YK, Martin LE, Urwin R, Stephens DS. Genetic basis for nongroupable *Neisseria meningitidis*. J Infect Dis **2003**; 187:1616–28.
16. Kahler CM, Martin LE, Shih GC, Rahman MM, Carlson RW, Stephens DS. The ( $\alpha$ 2→8)-linked polysialic acid capsule and lipooligosaccharide structure both contribute to the ability of serogroup B *Neisseria meningitidis* to resist the bactericidal activity of normal human serum. Infect Immun **1998**; 66:5939–47.
17. WHO. Meningococcal disease, serogroup W135 (update). Wkly Epidemiol Rec **2001**; 76:213–6.
18. Henserson S, Handford S, Ramsay M. *Neisseria meningitidis* W135: 2a: P1,2,5 arising from successive pilgrimages to Mecca. Eurosurveillance Weekly 2001; 5. Available at: <http://www.eurosurveillance.org/ew/2001/010419.sp>. Accessed 8 November 2004.
19. Issack MI, Ragavoodoo C. Hajj-related *Neisseria meningitidis* serogroup w135 in Mauritius. Emerg Infect Dis **2002**; 8:332–4.
20. Wilder-Smith A, Barkham TM, Earnest A, Paton NI. Acquisition of W135 meningococcal carriage in Hajj pilgrims and transmission to household contacts: prospective study. BMJ **2002**; 325:365–6.
21. Balkhy HH, Memish ZA, Osoba AO. Meningococcal carriage among local inhabitants during the pilgrimage 2000–2001. Int J Antimicrob Agents **2003**; 21:107–11.
22. Centers for Disease Control and Prevention (CDC). Active bacterial core surveillance reports, emerging infections program network, *Neisseria meningitidis*. Available at: <http://www.cdc.gov.ncidod/dbmd/abcs/survreports.htm>. Accessed 8 November 2004.
23. Hammerschmidt S, Hilse R, van Putten JP, Gerardy-Schahn R, Unkmeir A, Frosch M. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. EMBO J **1996**; 15:192–8.
24. Tsang RS, Law D, Squires SG. Two serologically non-groupable *Neisseria meningitidis* strains from clinical specimens identified by molecular method as serogroup B meningococci. Can Commun Dis Rep **2001**; 27:9–12.
25. Vogel U, Morelli G, Zurth K, et al. Necessity of molecular techniques to distinguish between *Neisseria meningitidis* strains isolated from patients with meningococcal disease and from their healthy contacts. J Clin Microbiol **1998**; 36:2465–70.