# Meningococcal carriage among Hajj pilgrims, risk factors for carriage and records of vaccination: a study of pilgrims to Mecca.

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## Abstract

Background

Hajj brings millions of pilgrims from different countries into a confined place of worship. A number of outbreaks of meningococcal disease have been reported immediately after the Hajj. The Saudi government requires that all pilgrims receive a quadrivalent meningococcal vaccine at least 10 days before the Hajj. We conducted a study to determine the uptake of meningococcal vaccine and antibiotic use. We also investigated risk factors of meningococcal carriage and carriage of *Neisseria meningitidis* pathogenic serogroups A, C, W, and Y.

Methods

A cross-sectional oropharyngeal carriage survey was conducted in 2973 Hajj pilgrims in September 2017. A real-time polymerase chain reaction (rt-PCR) assay was used to identify *N. meningitidis* from the oropharyngeal swabs. A questionnaire investigated potential risk factors for carriage of *N. meningitidis.*

Results

Overall, 2,249 oropharyngeal swabs were obtained. The overall prevalence of carriage of *N. meningitidis* was 4·6% (95% CI: 3.4% - 6%). Carriage of pathogenic serogroups was not associated significantly a with any of the meningococcal risk factors evaluated. A majority of pilgrims (77%) were vaccinated but 22·58 % said they were carrying unofficial vaccination cards

Conclusion

Carriage with serogroups A, C, W, and Y was not significantly associated with any of the risk factors investigated. Almost a quarter of pilgrims were unlikely to have been vaccinated, highlighting a need to strengthen compliance with the current policy of vaccination to prevent meningococcal disease outbreaks during and after the Hajj.

## Introduction

*Neisseria meningitidis* is a gram-negative aerobic bacterium which causes invasive meningococcal disease, a communicable disease spread via respiratory droplets.[1] There are twelve identified serogroups of *N. meningitidis*, which can be distinguished from each other by their polysaccharide capsule. However, there are six serogroups (A, B, C, W135, X ,and Y) that cause invasive disease.[2] Meningococcal carriers who have bacteria in the oropharynx but do not present with any symptoms, are the main source of invasive infections.[1, 3]

Over two million Muslims visit Mecca in Saudi Arabia every year to perform the Hajj, one of the largest mass gatherings in the world,[4] Pilgrims typically stay in the tents during the five days of each Hajj season where they share accommodation with other pilgrims.[5] Overcrowding during the Hajj and an increase in the number of pilgrims inside Hajj tents has, in the past, facilitated the spread of meningococcal disease, and there have been several meningococcal outbreaks during Hajj pilgrimage.[4] As a consequence of these outbreaks, the Saudi authorities developed and upgraded their Hajj vaccination policy to mandatory quadrivalent meningococcal vaccination, which must be administered to all Hajj pilgrims before arriving to Saudi Arabia for the Hajj.[6] A vaccine that protects against all serogroups is currently unavailable.[7] The existing Hajj vaccination policy does not indicate the type of quadrivalent (ACYW135) vaccine that should be administered, i.e., a quadrivalent meningococcal conjugate vaccine (MCV-4) or a quadrivalent meningococcal polysaccharide vaccine (MPSV-4).[8]Meningococcal polysaccharide vaccination can prevent severe meningococcal illnesses but it does not prevent the acquisition of carriage.[1] A quadrivalent conjugate vaccine may prevent acquisition of new carriage but it does not clear existing carriage which may take two months or more to clear naturally. [9] A quadrivalent meningococcal

ACWY glycoconjugate vaccine was shown to have little impact on carriage one month post vaccination.[9] This may have an impact on Hajj vaccination policy, which currently only requires vaccination 10 days prior to travelling to the Hajj.[10]

Self-medicating with antibiotics has long been a custom among pilgrims during the Hajj period to protect themselves against diseases transmitted via the respiratory route.[11] This has most likely played a role in eliminating carriage in previously reported Hajj studies.[12, 13] Conversely, this custom of administration of non-prescribed antibiotics by Hajj pilgrims may contribute to increasing antibiotic resistance.[11]

The aim of this study was to determine the uptake of the meningococcal vaccine and the use of antibiotics by Hajj pilgrims. The study also aimed to investigate the rate of *N. meningitides* carriage among pilgrims and to determine the risk factors associated with the carriage of *N. meningitides* serogroups (A, C, W and Y).

## Methods

### Study design and setting

A cross-sectional study was conducted in Jeddah, Saudi Arabia at the Hajj terminal of King Abdulaziz International Airport (KAIA) after the 2017 Hajj. Most of the international pilgrims pass through the Hajj terminal when visiting the Sacred Mosque in Mecca.[14]

### Sampling methods

Two stage cluster sampling was used in the Hajj terminal at KAIA to select participants for the study. Departing flights from the Hajj terminal were selected as clusters as the first stage using simple random sampling from the daily Hajj flight schedules. Subsequently, at the second stage, systematic random sampling of each flight cluster

was undertaken using seat numbering. Departing pilgrims were recruited in the airport lounge and only those who provided informed consent were included in the study.

## Data collection

All pilgrims selected for inclusion in the study were provided with written information regarding the aims of the study. Upon their agreement to participate, they were asked to sign a consent form. An electronic data capture tool, 'Open Data Kit' (ODK) was used to collect questionnaire data.[15] Assistance was provided to pilgrims with clarifying questions and on how to use electric tablets.

## Questionnaire design, piloting and translation

Twenty electronic tablets (Asus Zenpad 8 Z580C) supported by designed data questionnaire forms for 15 languages including Arabic, Albanian, Bengali, Bosnian, Chinese, English, French, Hindi, Indonesian, Kurdish, Malay, Pashto, Russian, Turkish and Urdu were used to collect data from pilgrims. All translated questionnaire forms were piloted prior to conducting the study.

## Samples collection and storage

A Dacron / polyester tip swab was gently rolled over the tonsils and posterior pharynx and inserted into a vial containing transport medium containing skimmed milk, tryptone, glucose and glycerine (STGG). All swabs were kept at 4°C in an ice box for one hour followed by storage in a portable freezer at -20 °C. They were then kept securely at the airport for two weeks prior to being transported to King Abdullah University of Science and Technology (KAUST) where they were stored at -80 °C and then shipped on a dry ice to London School of Hygiene & Tropical Medicine for laboratory investigations. No cold chain breakdown was recorded during shipment

## Laboratory analysis

An aliquot of 300 µl STGG medium was extracted from each vial and purified using QIAamp cador Pathogen Mini Kit (Cat No. /ID: 54106), following the manufacturer's protocol. Extracted DNA was eluted in 100 µl of elution buffer and stored at -20°C. Quantitative PCR was then used to detect *N. meningitidis ctrA*, *sodC*, and *porA* target genes separately using primers and probes as described previously.[16, 17] It is recommended to use a duplex real time -PCR approach that targets both *porA* and *ctrA* genes as these two are effective in detecting most *N. meningitidis* invasive strains. Nongroupable (NG) strains can also be identified by using *sodC* assay, which is superior to *ctrA* assay in detecting NG strains of *N. meningitidis.*[18]

All positive samples were tested for capsular biosynthesis genes for *N. meningitidis* serogroups A, B, C, W, X ,and Y using primers and probes as described previously.[17] All PCR reactions were performed with 5µl of extracted DNA, 10µl of qPCRBIO Probe mix Hi-ROX (PCR Biosystems PB20·22) in a reaction volume of 20µl using the 7500 ABI platform (Applied Biosystem, USA). Samples were tested in duplicates and considered as positive when the sample had a cycle threshold (Ct) value below 40.[19] All samples were tested in parallel with positive controls for each serogroup (kindly supplied by Dr Odile Harrison, University of Oxford).

## Statistical analysis

A descriptive analysis of all survey variables was performed using Stata commands for survey data (SVY) to account for the multistage sampling design. All analyses were weighted for probability of selection. Flight numbers and number of pilgrims in each flight were used to calculate the weight used in the analysis to ensure that the probability of selection for each pilgrim sampled from flight was the same as the overall

probability of selection for all pilgrims. A logistic regression model was developed to examine the associations between risk factors for meningococcal carriage (age, sex, education, type of meningococcal vaccine, timing of meningococcal vaccination, smoking status, marital status, country classification by income, length of stay in Saudi Arabia and number of pilgrims inside the tent where the participant slept) and the binary outcome variable "meningococcal carriage of serogroup ACWY''. Vaccination time was calculated from the date of receiving vaccination until the Hajj dates and was categorised as 0: ≤ 60 days, 1: ≥ 61 days. It is suggested that two months are needed to naturally clear any existing carriage of *N. meningitidis* and therefore was used as a cut-off point.[10] The Wald test was used to assess evidence for any associations between the outcome "meningococcal carriage of serogroup ACWY" and each of the potential variables mentioned above. Due to lack of observations in some categories of variables, variables such as age, education, and country classification by income were re-categorised to fit the regression model. Age was categorised as 0: ≤ 34 years, 1: 35 to 44 years, 2: 45 to 54 years, 3: 55 to 64 years, and 4: ≥ 65 years. Education was classified as 0: low (Illiterate pilgrims or those who could only read and write), 1: middle (pilgrims with qualification of two years college, high school, or less than high school), and 2: high (pilgrims with doctoral, master's, or bachelor's degree). The type of meningococcal vaccine was categorised as 0: bivalent A and C or quadrivalent polysaccharide, 1: quadrivalent conjugate, and 2: unknown type. Country classification by income, as defined by the World Bank report for 2017-2018,[20] was also categorised as 0: low and low middle-income countries, 1: upper middle-income countries and 2: high-income countries. Variables with a $p > 0.1$ were considered statistically insignificant. All analyses were conducted using the STATA 16 software. [21]

## Results

### Demographic and other baseline characteristics

Of the initial 2,973 participants, 2,249 (75·56%) completed the electronic questionnaire and agreed to be swabbed. Table 1 summarises pilgrims' demographic data. Participants came from China, Europe, East Africa, the Middle East, North Africa, North America, Post-soviet states, South Asia, Southeast Asia, West Africa, and South Africa. The total length of stay of pilgrims inside Saudi Arabia during the Hajj journey (n=2973) averaged 33.8 (SD: 12.92) days in the 2017 Hajj season.

### Meningococcal vaccination status and antibiotic use

The survey showed that 22·6 % of the study participants stated that they had not been vaccinated against meningococcal disease; 12·5 % of those were not vaccinated and not carrying any vaccination certificates and 11% self-reported that they were unvaccinated and carrying an unofficial purchased vaccination certificate. Very few pilgrims (0·5 %) had received the bivalent polysaccharide (A and C) meningococcal vaccine. A majority of pilgrims (71.2 %) had received the mandatory meningococcal quadrivalent vaccine. Most of the pilgrims (74·4) had received their vaccine from hospitals, (13·7%) from private clinics, (3·9%) from pharmacies, (3·4%) from mosques and (4·5%) from other places. Figure 1 illustrates the vaccination status of pilgrims by country classification by income.

Approximately one-third of all vaccinated participants (35·7%) received their vaccines at least two months before the Hajj. More than half of the participants (55·8%) (49·4 – 62) said that they took antibiotics during and after the Hajj (Table 2).

## Meningococcal carriage rate

A total (37%) of the 2,249 swabs were positive for *ctrA*, *SodC*, and *porA* genes. Target *ctrA* and *porA* genes characteristic of *N. meningitidis* were found among 103 of the total 2,249 samples tested, giving an overall carriage prevalence of 4·6%. (95% CI: 3.4% - 6%); 34 (1·13%) of these samples was positive for serogroups responsible for disease - serogroup A (n=2), B (n=10), C (n=10), W (n=3), X (n=6), and Y (n=3). Both *N. meningitidis* B and C were identified in seven pilgrims, A and B in one, and C and X in one.

## Logistic regression analysis

The logistic analysis was restricted to the 1,736 pilgrims who received the meningococcal vaccine and provided an oropharyngeal swab. None of the variables studied was found to be associated with the outcome of carriage at 10% significant level. Meningococcal carriage of serogroup A, C, W, and Y *N*. *meningitidis* was higher among those who received a vaccine more than 60 days before the Hajj compared to those who received it 60 days or less before the Hajj but the difference between groups was not statistically significant (adjusted OR 1·6; (0·55–4·8, *p*-value 0·36) (Table 3) No other statistically risk factors for *N. meningitidis* carriage were found.

## Discussion

According to the Hajj and Umrah vaccination policy, all pilgrims should receive the mandatory meningococcal quadrivalent (ACYW) vaccine at least 10 days before the Hajj.[8, 22] Pilgrims are also required to submit a certificate to proof that they have received the vaccine.[22] Surprisingly, a quarter of the pilgrims (22·6 %) in this study self-reported not having been vaccinated against meningococcal disease and of those

10·1 % were carrying unofficial vaccination certificates. The finding of a high percentage of pilgrims being unvaccinated was unexpected and indicates the need to determine whether pilgrims have been vaccinated prior to arrival for Hajj pilgrimage to prevent any future meningococcal outbreaks. An immediate strengthening of the visa/vaccination regulations at Hajj is necessary .The development of a Harmonised Hajj Health Information System (HHIS), a synchronised information and data sharing platform among all Hajj stakeholders, would be beneficial.[23] The HHIS would capture pre-Hajj data, for instance pilgrims' demographic information, pre-existing health conditions and vaccination status.[23] To ensure that all pilgrims are receiving mandatory vaccines for the Hajj, vaccines could be given in specific vaccination centres authorised by the Saudi embassies in each country where pilgrims originate from and the vaccination status linked electronically to the HHIS.

Our study identified there were more carriers among those vaccinated more than two months prior to travelling to the Hajj than among those vaccinated less than two months before arrival, although the difference between groups was not statistically significant. Little information was found in the literature on the question of the association between time of meningococcal vaccination and carriage of *N. meningitidis.* Read et al. conducted the only published study that has investigated this association with regards to meningococcal conjugate vaccine and showed that the natural elimination of existing carriage may take two months or more and that the conjugate vaccine can only prevent acquisition of new carriage.[9]

There are a number of possible reasons for our findings. Firstly, the number of carriers was relatively small with only 18 being positive for a meningococcus of pathogenic serogroups A,C,W and Y, therefore a larger study might have found a significant effect. Secondly, over 50% of the pilgrims had received a polysaccharide vaccine and

polysaccharide vaccines have little or no impact on carriage [24]; only 16% were known to have received the quadrivalent conjugate vaccine which has been shown to prevent new acquisition of carriage. The Saudi Hajj vaccination policy does not indicate the type of quadrivalent vaccine required and leaves it to pilgrims to decide.[8]

As many pilgrims come from developing countries, the cost of the conjugate vaccine could influence uptake for many.[8] Pilgrims from developing countries make life-long savings to be able to travel for the Hajj[25] and for many the option of receiving a cheaper polysaccharide vaccine is one that is more appropriate for their financial status.. Finally, although the analysis took into account reported use of antibiotics, it is possible that unreported use of antibiotics, which was likely widespread, might have confounded the difference between the groups. The high use of antibiotics reported in our study is consistent with other research which found that over 60% of pilgrims who travelled to Saudi Arabia carried antibiotics from their homeland with them and that 39·2% acquired non-prescribed antibiotics in Saudi Arabia.[26] Other studies have reported misuse and overuse of antibiotics among pilgrims [27] which, if continued, will make Hajj pilgrimage at risk of spreading antibiotic resistance.[28]

The overall prevalence of meningococcal carriage in our study was low (4·6%.), and that of serogroups A, B, C, W, X, and Y that cause the meningococcal disease was very low (1·13%) with many meningococci being non-groupable. This finding is in agreement with the findings of Memish et al. who also found a low prevalence of carriage of *N*. *meningitidis* in pilgrims attending the 2014 Hajj.[29] A surprising finding was the high proportion of carriers carrying meningococci of more than one serogroup. Carrying more than one serogroup of *N.meningitidis* in the throat is rare but can occasionally happen.[30] Contrary to expectations, we did not find any significant

association between *N*. *meningitidis (*serogroupable and non-serogroupable) carriage and any of the risk factors found in other studies (age, sex, education level, smoking status, marital status, type of meningococcal vaccination, timing of vaccination ,country classification by income, and number of pilgrims inside the tents).[31]. Detection of serogroup X among the pilgrims is of concern as *N*. *meningitidis* serogroup X has the potential to cause epidemics, as experienced recently in the African meningitis belt.[32] Currently, there is no licensed vaccine against serogroup X *N. meningitidis*[33] although a pentavalent conjugate vaccine containing a serogroup X conjugate is being developed by the Serum Institute of India and is undergoing clinical trials.[34]

This study had a number of limitations. Only pilgrims that had completed the Hajj were included and there was a lack information on events before the Hajj, for example those who attended a Hajj camp before the pilgrimage. Our findings are limited by the use of a cross-sectional design, given that conducting a large-scale longitudinal study is challenging and costly in circumstances similar to the Hajj. In addition, information on antibiotic use was self-reported and some pilgrims may have confused use of antibiotics with other non-antibiotic medications. The percentage of those unvaccinated is limited to those who have reported not being vaccinated and the number could be larger than recognised. Despite these limitations, this study was one of the largest studies undertaken on meningococcal carriage in pilgrims after completing the Hajj. Results have raised many questions regarding the need for further investigation including the issue of pilgrims traveling with unofficially purchased vaccination cards. Our study determined the prevalence of unvaccinated pilgrims, although it did not reveal the reasons why people had not been vaccinated or purchased unofficial vaccination cards. Pilgrims from different countries may possibly

have different reasons. A future qualitative phase of the study may provide information to explore the issue of unofficial vaccination cards more comprehensively.

As one of the primary reasons for the authorities insisting on meningococcal vaccination prior to the Hajj is to prevent spread of meningococci among pilgrims during the Hajj, as well as to protect them against invasive meningococcal disease, more studies are needed to ascertain the importance of using a conjugate rather than a polysaccharide vaccine and on the optimum vaccination time prior to traveling to the Hajj.

## Contributions

AK.Alasmari wrote the first draft of the article with further contributions from all co-authors. A.K.Alasmari designed the study with support from R.Behrens, and D. Heymann. A.K.Alasmari was responsible for data collection with logistic support from A. Assiri. A.K.Alasmari did laboratory analysis of the samples with advisory support from J.H. Houghton. A.K.Alasmari did statistical analysis, with support from P. Edwards. All authors reviewed and approved the final version of the article.

## Ethical approval

Ethical approvals were obtained from the ethics committees of the London School of Hygiene and Tropical Medicine (approval #11260) and King Abdullah University of Science and Technology prior to conducting the research (16IBEC21).

## Conflicts of interest

We have no conflict of interest to declare.

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Figure 1: Vaccination status of pilgrim by country classification by income.

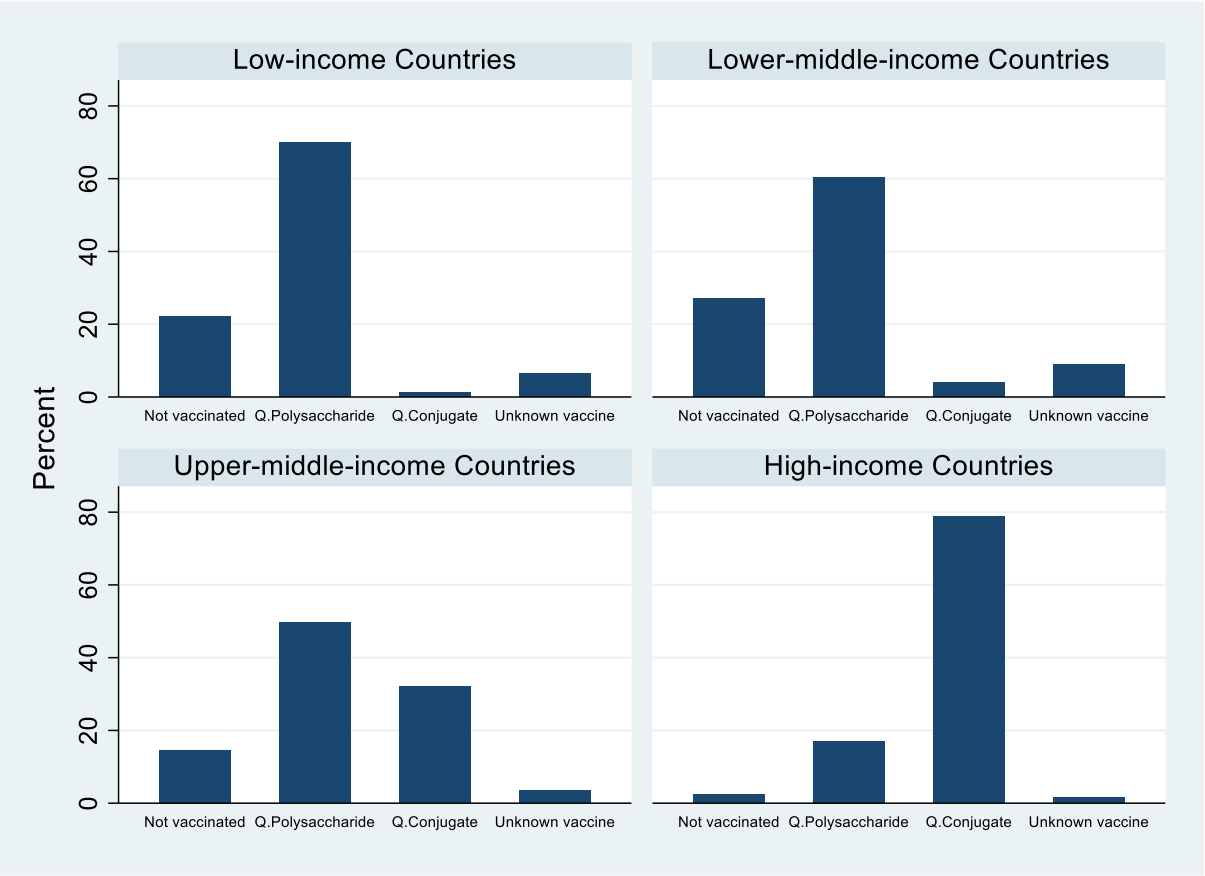

Table 1: Demographic characteristics of pilgrims

| **Characteristics** | **Total (%)** |
|---|---|
| **Sex** | |
| Female | 32 |
| Male | 68 |
| **Age in years** | |
| 11 - 17 | 0·5 |
| 18 - 24 | 2·9 |
| 25 – 34 | 12·6 |
| 35 – 44 | 26·7 |
| 45 – 54 | 26·3 |
| 55 – 64 | 22·5 |
| 65 or above | 8·5 |
| **Education level** | |
| Illiterate | 5 |
| Can read and write | 11 |
| Less than high school | 9 |
| High school | 19 |
| Two years college | 9 |
| Bachelor degree | 27·5 |
| Master's degree | 15 |
| Doctoral degree | 4·5 |
| **Country classification by income** | |
| Low | 6 |
| Low-middle | 57 |
| Upper-middle | 28 |
| High | 9 |
| **Marital status** | |
| Married | 87 |
| Unmarried | 13 |

Table 2: Pilgrim's social and vaccination profile

| **Variable** | **Total percentage %** |
|---|---|
| **Vaccination status** | |
| Unvaccinated without a vaccination card (self-reported). | 12·6 |
| Unvaccinated with a fake vaccination card (self-reported). | 11 |
| Bivalent (A and C) vaccine. | 0·5 |
| Quadrivalent polysaccharide vaccine | 54·7 |
| Quadrivalent conjugale vaccine. | 16 |
| Unknown meningococcal vaccine. | 6·2 |
| **Time of vaccination before arrival in Saudi Arabia** | |
| ≤ 60 days | 64·3 |
| ≥ 61 days | 35·7 |
| **Antibiotic use** | |
| Yes | 55·8 |
| No | 44·2 |
| **Smoking status** | |
| Smoker | 10·6 |
| Non-smoker | 89·4 |
| **Number of pilgrims inside a tent** | |
| 6 – 8 | 16·4 |
| 10 – 20 | 13·9 |
| 50 – 100 | 39·5 |
| ≥ 100 | 30·3 |

Table 3: Risk factors for carriage of *Neisseria meningitidis* ACWY among pilgrims to Mecca, 2017

| Exposure | Carriage of *Neisseria meningitides* ACWY | | | |
|---|---|---|---|---|
| | Crude Odds ratio (95% CI) | P value | Adjusted Odds ratio * (95% CI) | P value |
| **Time of meningococcal vaccination** | | | | |
| ≤ 60 days | Reference | | | |
| ≥ 61 | 1 (0·34,3·26) | 0·92 | 1·6 (0·55 –4·8) | 0·36 |
| **Type of meningococcal vaccine** | | | | |
| Bivalent \ quadrivalent polysaccharide | Reference | | | 0·67# |
| Quadrivalent Conjugate | 0·73 (0·16,3·27) | 0·8 | 0.8 (0.08 – 7.7) | 0·8 |
| Unknown | 0·37 (0·05,2·77) | 0·33 | 0·4 (0·05,4.3) | 0·5 |
| **Sex** | | | | |
| Female | Reference | | | |
| Male | 0·72 (0·26 - 2) | 0·53 | 0·6 (0·22 –1·4) | 0·25 |
| **Age in years** | | | | |
| ≤ 34 | Reference | | | 0·81# |
| 35 – 44 | 0·77 (0·11-5·11) | 0·78 | 0·44 (0·04-4·2) | 0·4 |
| 45 – 54 | 0·95 (0·14 –6·1) | 0·95 | 0·7(0·08 – 6) | 0·7 |
| 55 – 64 | 1·8 (0·32- 10·5) | 0·47 | 1 (0·16- 6·4) | 0·9 |
| ≥ 65 | 1·6 (0·31 – 8·3) | 0·55 | 1·1 (0·25 -5·4) | 0·8 |
| **Education level** | | | | |
| Low | Reference | | | 0·83# |
| Middle | 1 (0·20 – 5·6) | 0·93 | 0·77 (0·15 - 4.) | 0·7 |
| High | 0·5 (0·08 – 2·9) | 0·44 | 0·53 (0·08-3·6) | 0·5 |
| **Country classification by income** | | | | |
| Low/lower middle income | Reference | | | 0·97# |
| Upper middle income | 0·89 (0·24 –3·2) | 0·85 | 1·1 (0·32,4·1) | 0·8 |
| High income | 0·54 (0·06 –4·7) | 0·57 | 2.3 (0·04,128) | 0·6 |
| **Smoking status** | | | | |
| Non- smoker | Reference | | | |
| Smoker | 1·3 (0·25 – 6·5) | 0·74 | 1 (0·10 - 10) | 0·9 |
| **Antibiotic use** | | | | |

| | | | | |
|---|---|---|---|---|
| No | Reference | | | |
| Yes | 1·2 (0·38 – 3·8) | 0·72 | 1 (0·33-3.1) | 0·9 |
| **Marital status** | | | | |
| Married | Reference | | | |
| Unmarried | 0·57(0·07 – 4·2) | 0·58 | 0·57 (0·07-4·4) | 0·5 |
| **Length of stay in KSA** | 1 (0.9 – 1) | 0.24 | 1 (0.9 – 1) | 0.12 |
| **Number of pilgrims inside the tent** | | | | |
| 6- 8 | Reference | | | 0·82# |
| 10-20 | 0·66 (0·05 –8·3) | 0·74 | 0·6(0·05-8·4) | 0·7 |
| 50-100 | 0·74 (0·10 – 5) | 0·76 | 0·8 (0·11, 6) | 0·8 |
| ≥ 100 | 1·5 (0·29 – 7·6) | 0·61 | 1·4 (0·3,7·3) | 0·6 |

*The model was adjusted for variables (age, sex, education, type of meningococcal vaccine, timing of meningococcal vaccination, smoking status, marital status, and antibiotic use, length of stay in KSA, country classification by income and number of pilgrims inside the tents).

# Overall Wald test