Prevalence of Nasal Carriage of \textit{Neisseria meningitidis} among Umrah Visitors and Pilgrims during Umrah and Hajj Season.


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A total of 3081 specimens collected from 2412 Umrah visitors and pilgrims to survey and surveillance \textit{Neisseria meningitidis}. About 15 nationalities contributed in this study; the greatest number was from India (481) and the lowest one was from Jordan (1). Turkish and British recorded significant results (p= value 0.04 and 0.006, respectively) among Umrah visitors and Indian (p= value 0.0003) in pilgrims colonized by \textit{Neisseria meningitidis}. Out of 232 \textit{Neisseria meningitidis} isolated from Umrah visitors and pilgrims, 0 and 2 of serotype W135 were isolated before and, 2 and 5 were isolated after Umrah and Hajj respectively. \textit{Neisseria meningitides} showed (8.3%) before Hajj and (10.3%) after Hajj resistant to Azithromycin antibiotic.

Keywords: Umrah, Hajj, \textit{Neisseria meningitidis}, serogroup.

INTRODUCTION

Hajj is the largest annual gathering of its kind in the world that bring over two million people together in a small, geographically-confining area. All adult Muslims who are physically and financially able to do so have a religious obligation to make the pilgrimage once in their lifetime and over two million from around the world gather in Mecca each year. Similarly, many Muslims like to come each year to Mecca during Umrah season between Rabi I and Ramadan to perform Umrah rituals (Month of Ramadan usually shows the highest peak of crowding).

The congregation of so many people from different parts of the world in unavoidably overcrowded conditions within a confined area for a defined period of time presents many public health challenges and health risks are greatly increased with potential for both local and international consequences. One of the main health problems correlated with crowding is respiratory tract and meningitis infections due to its ease of transmission by air droplets. These infections can be transmitted from infected people and more significantly from asymptomatic carriers due to absence of symptoms (Qureshi et al., 2000, and Wilder-Smith and Memish, 2003).

The human nasopharynx and nares are densely colonized by a broad variety of microorganisms including commensal bacteria as well as potentially pathogenic bacteria (PPB) mainly: \textit{Neisseria meningitidis}. Studies
have shown that colonization with PPB is a major contributing factor for respiratory and extra-respiratory infections including pneumonia, sepsis, and meningitis in PPB carriers as well as their closed contacts (Tenover and Gaynes, 2000; Peacock et al., 2001; Ruoff et al., 2003; Cardozo et al., 2006; Musher, 2005 and Kadioglu et al., 2008).

The reported rates of PPB colonization acquisition and carriage vary extensively between different studies and geographical sites (Fontanals et al., 2000; Peacock et al., 2001 and Melles et al., 2007). These differences have been related to genetic background variables and socio-economic conditions including housing, access to health care, poor hygiene, and overcrowded living conditions (Garcia-Rodriguez and Martinez, 2002).

This manuscript studied *N. meningitidis* in Umrah visitors, pilgrims and carriers during Umrah and Hajj seasons in order to determine colonization of those bacteria in different ethnic groups, to find out any significant correlation between carriage rate and ethnic group, prevalence of *N. meningitidis* serotypes, and antibiotic resistance bacterial isolates.

**SUBJECT AND METHODS**

**Subject of the study**

This study was performed on 979 Umrah visitors from different nationalities including; 129 Turkish, 127 Indonesian, 102 Pakistani, 99 Syrian, 98 Nigerian, 79 Egyptian, 77 Iranian, 71 Indian, 56 British, 56 Iraqi, 39 Malaysian, 27 Libyan, 14 Swedish, 4 American and 1 Jordanian, during the period from Rabi-Al-Awwal to end of Ramadan 1430H and also in 1430H Pilgrims from different nationalities (1433 people) including; 481 Indian, 252 Nigerian, 95 Indonesian, 90 Libyan, 68 Syrian, 46 British, 35 Turkish, 11 Australian, 3 Swedish, 2 Iranian, 150 Pakistani, 100 Cameroon, 50 Mali and 50 South Africa during the period from Zo-Alkaeda to end of Zo-Alhejja 1430H in the Microbiology research Laboratory, Faculty of Medicine, Umm Al-Qura University. Informed consent was obtained from all subjects prior to study enrollment.

**Collection of specimens**

Around 1958/2046 Samples were collected from Umrah visitors/pilgrims, at arrival to King Abdul-Aziz International Airport (KAAIA); Saudi Arabia and before leaving the country. Data forms including; nationality, age, sex, smoking, coughing, antibiotic usage, date of collection, contacts numbers for the group’s leaders, were recorded for each visitors/Pilgrims. The remaining swabs were then collected before departure from (KAAIA) from the same visitors/pilgrims or at least from Umrah visitors/pilgrims on the same group.

**Transportation of the specimens**

All Samples were collected on Amies transport swabs media to insure the survival of pathogens in specimens collected on swabs and transported to the Microbiology research laboratory without any delay.

**Cultivation of specimens**

All samples were collected processed in the research Laboratory according to the standard microbiological methods under complete aseptic conditions. Nasopharyngeal swabs were inoculated on chocolate agar and selective modified Thayer Martin agar (MTM, Sigma) and incubated at 37°C in CO₂ incubator.

**Isolation and Identification of Neisseria meningitidis**

*Neisseria meningitidis* was isolated from Thayer Martin medium which was grown after an overnight incubation in CO₂ incubator. The identification was confirmed microscopically by the characteristic appearance as Gram negative diplococci after being stained by Gram stain. An oxidase test was also performed, in which the organism is characteristic positive. All *Neisseria meningitidis* isolates were then confirmed using VITEK 2 systems compact 15 “biomerieux”.

**Identification of bacteria using VITEK 2 Machine**

About 3.0 ml of sterile saline (aqueous 0.45% NaCl, PH 7.0) was placed into a clear plastic test tube. Then sufficient number of pure bacterial colonies was transferred to the tube containing the saline to make a homogenous suspension with an equivalent density of McFarland (N° 2.9 to3.2) using Calibrated VITEK 2 DENSICHEK. The tube was then placed in the cassette with the identification card and data entry.

**Serotyping of Neisseriameningitidis**

*Neisseria meningitidis*serogrouping was performed by using specific antisera latex slide agglutination (*Neisseria meningitidis* Antisera, BD Difco laboratories, USA):

*Neisseria meningitidis* antiserum poly contain groups (A, B, C and D), *Neisseria meningitidis* antiserum Poly 2 contain groups (X, Y and Z), *Neisseria meningitidis*...
antiserum group B and Neisseria meningitidis antiserum group W135.

Preserved N. meningitidis was subcultured on Thayer Martin medium before being incubated in CO2 incubator for 24 hour to obtain a pure culture. After the 24 hour incubation, one drop of the Difco Neisseria meningitidis antiserum was placed on agglutination slide, and then a loopfull of growth of Neisseria meningitidis was then transferred to the drop of the antiserum and mixed thoroughly. The slide was then rotated for one minute. A positive result was reported if agglutination appeared within one minute, while a negative result was reported if there was a positive result no agglutination within one minute.

### STATISTICAL METHODS

All statistical calculations were done using computer programs. Microsoft excel version 10 and spss (statistica package for the social science version 20.00) statistical program at 0.05, 0.01 and 0.001 level of probability (Snedecor and Cochran, 1982). Data was presented using percentage. Comparison of percentage was done using the chi square tests.

### RESULTS

**Distribution of Neisseria meningitidis prevalence in Umrah visitors during the 1430H season according to different nationalities:**

In this study 80 Neisseria meningitidis isolates were isolated from Umrah visitors, table (1). The carriage rate of Neisseria meningitidis as potentially pathogenic bacteria was the highest among the Umrah visitors (2.5%) before performing Umrah and (5.7%) after performing Umrah. In addition, the carriage rate of all PPB among the Umrah visitors was more after Umrah than before Umrah.

The Iranian Umrah visitors were the most ethnic group carries Neisseria meningitidis before performing Umrah (7.79%). While the American Umrah visitors were the most ethnic group carries Neisseria meningitidis after performing Umrah (75%).

**Distribution of Neisseria meningitidis prevalence in Pilgrims during the Hajj 1430H season according to different nationalities:**

In this study 152 Neisseria meningitidis isolates were isolated from Pilgrims, table (2). The carriage rate of
Table 2. Prevalence of *Neisseria meningitidis* isolated from different nationalities of the Pilgrims during the Hajj 1430H season

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Before Hajj</th>
<th>After Hajj</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N° of Pilgrims tested</td>
<td>+ Pilgrims</td>
<td>- Pilgrims</td>
</tr>
<tr>
<td>Indian</td>
<td>481</td>
<td>15 (3.1)</td>
<td>466 (96.9)</td>
</tr>
<tr>
<td>Nigerian</td>
<td>252</td>
<td>25 (9.9)</td>
<td>227 (90.1)</td>
</tr>
<tr>
<td>Indonesian</td>
<td>95</td>
<td>7 (7.3)</td>
<td>88 (92.6)</td>
</tr>
<tr>
<td>Libyan</td>
<td>90</td>
<td>5 (5.6)</td>
<td>85 (94.4)</td>
</tr>
<tr>
<td>Syrian</td>
<td>68</td>
<td>9 (13.2)</td>
<td>59 (86.8)</td>
</tr>
<tr>
<td>British</td>
<td>46</td>
<td>5 (10.9)</td>
<td>41 (89.1)</td>
</tr>
<tr>
<td>Turkish</td>
<td>35</td>
<td>1 (2.9)</td>
<td>34 (97.1)</td>
</tr>
<tr>
<td>Australian</td>
<td>11</td>
<td>0 (0)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Swedish</td>
<td>3</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>Iranian</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Pakistani</td>
<td>150</td>
<td>7 (4.7)</td>
<td>143 (95.3)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>100</td>
<td>3 (3)</td>
<td>97 (97)</td>
</tr>
<tr>
<td>Mali</td>
<td>50</td>
<td>6 (12)</td>
<td>44 (88)</td>
</tr>
<tr>
<td>South Africa</td>
<td>50</td>
<td>0 (0)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>1433</td>
<td>84 (5.9)</td>
<td>1349 (94.1)</td>
</tr>
</tbody>
</table>

* = significant; ** = highly significant

*Neisseria meningitidis* as potentially pathogenic bacteria was the highest among the pilgrims (7.3%) before performing Hajj and (11.1%) after performing Hajj. However, the Swedish pilgrims were the most ethnic group carries *Neisseria meningitidis* before performing Hajj (33.3%). While the Syrian pilgrims were the most ethnic group carries *Neisseria meningitidis* after performing Hajj (20.6%).

Serotypes of *Neisseria meningitidis* isolated from Umrah and Hajj Visitors during 1430H season:

Out of the 80 *Neisseria meningitidis* isolated from Umrah Visitors during the Umrah season, 20 were of Poly (A, B, C, D) serotypes, 8 were of serotype B, 13 were of serotype Poly 2 (X, Y, Z), 2 were of serotype W135 and 37 were non typable, figure. (1). While, out of 152 *Neisseria meningitidis* isolated from pilgrims during the Hajj season, 42 were of Poly (A, B, C, D) serotypes, 16 were of serotype B , 17 were of serotype Poly 2 (X, Y, Z) and 70 were non typable, figure. (2).

Antimicrobial Susceptibility of *Neisseria meningitidis* isolated from Umrah visitors during 1430H season:

For the 80 *Neisseria meningitidis* isolates isolated from Umrah Visitors in this study, all of them were susceptible to Cefotaxim, Ceftriaxone and Meropenem antibiotics. While, in the other hand, (8.3%) before Umrah and (5.4%) after
Umrah were resistant to Azithromycin antibiotic, table (3).

**Antimicrobial Susceptibility of Neisseria meningitidis isolated from Pilgrims during the Hajj 1430H season:**

For the 152 Neisseria meningitides isolates isolated from Pilgrims in this study, all of them were susceptible to Cefotaxime antibiotic. Most of them [(81%) before Hajj and (97.1%) after Hajj] were sensitive to Ceftriaxone antibiotic, while in the other hand, (8.3%) before Hajj and (10.3%) after Hajj were resistant to Azithromycin antibiotic, table (4).

**DISCUSSION**

The congregation of so many people during Umrah and Hajj seasons from different parts of the world in unavoidably overcrowded conditions within a confined area for a defined period of time presents many public health
Antibiotic profile of N. meningitides isolated from Umrah visitors during 1430H season

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Before Umrah</th>
<th>After Umrah</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*S %</td>
<td>R %</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>22  91.67</td>
<td>2  8.33</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>24  100</td>
<td>0  0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>24  100</td>
<td>0  0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>23  95.83</td>
<td>1  4.17</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>22  91.67</td>
<td>2  8.33</td>
</tr>
<tr>
<td>Meropenem</td>
<td>24  100</td>
<td>0  0</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>23  95.83</td>
<td>1  4.17</td>
</tr>
</tbody>
</table>

*S= Number of sensitive; R= Number of resistant

Antibiotic profile of N. meningitides isolated from Pilgrims during the Hajj 1430H season

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Before Hajj</th>
<th>After Hajj</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*S %</td>
<td>R %</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>77  91.7</td>
<td>7  8.3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>72  85.7</td>
<td>12 14.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>68  81</td>
<td>16 19</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>80  95.2</td>
<td>4  4.8</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>77  91.7</td>
<td>7  8.3</td>
</tr>
<tr>
<td>Meropenem</td>
<td>78  92.9</td>
<td>6  7.1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>82  95.5</td>
<td>2  4.5</td>
</tr>
</tbody>
</table>

*S= Number of sensitive; R= Number of resistant

Challenges and health risks are greatly increased, with potential for both local and international consequences. One of the main health problems correlated with these two seasons crowding is meningitis infection. This infection can be transmitted from infected people and more significantly from asymptomatic carriers with potentially pathogenic bacteria (PPB) such as; Neisseria meningitidis the other factor of concern is the possible spread of PPB strains not included in the current vaccines administered to all pilgrims before coming to Saudi Arabia particularly for N. meningitides. For this reason, 979 Umrah visitors and 1433 pilgrims investigated for such infection that could not include in current vaccines administered in their countries or otherwise they will be acquisition of carriage.

With the advances in molecular techniques, we are now able to link and identify strains from one place to another and it has been found that strains linked to Hajj outbreaks may be circulating for years before the onset of an outbreak (Molding et al., 2001). The latest outbreaks occurred during the Hajj pilgrimages of 2000 and 2001, when a shift from serogroup A disease to serogroup W135 occurred (Wilder-Smith et al., 2003).

The Hajj provides the optimum conditions for exposure and colonization of N. meningitidis in nasopharynx. A high rate of colonization is a known prerequisite for epidemics (Caugant, 2008). That is why we concentrated on nasopharyngeal swabs and used a proper media viz. chocolate agar and MTM.

N. meningitidis, a Gram-negative β-proteobacterium of the family Neisseriaceae, is an exclusive pathogen in humans, carried asymptomatically in the nasopharynx by 5%–10% of adults in nonepidemic periods (Stephens et al., 2007). After Umrah Turkish and Britain were the most ethnic groups carry Neisseria meningitides recorded significant values 6.20 and 14.29% (p=0.04 and 0.006, respectively) although those countries have population highly conserved in hygiene and personal cleanliness. However, performing Umrah increases the carriage rate of PBB, since the carriage rate of N. meningitidis among the Umrah visitors were more after performing Umrah than before performing Umrah. The prevalence of N. meningitidis was 24 before and 56 after Umrah indicated by highly significant value p=0.0003. The transmission of the meningococcus is clearly elevated in close contact and
crowded living conditions (e.g., barracks, dorms, pilgrimages).

Human infections caused by meningococcus (*Neisseria meningitidis*) remain a serious health problem, infecting 500,000 to 1.2 million people and killing between 50,000 and 135,000 per year worldwide (Rouphael et al., 2012). The carriage rate of 75% in this study after Umrah is markedly higher than the 2.6% found in pilgrims returning to the United States after the 2001 Hajj (CDC, 2001). This discrepancy is unlikely to be explained by differences in study methodology alone but probably results from differences in living conditions, degree of overcrowding, or social activities during the Umrah.

An international outbreak of meningococcal disease caused by *Neisseria meningitidis* W135 occurred in association with the Haj pilgrimage in 2000 and 2001 (Taha et al., 2000), with a high attack rate not only among the pilgrims but also among household contacts of returning pilgrims (Hahne et al., 2002; Issack and Ragavoodoo, 2002 and Wilder-Smith et al., 2003). Moreover, in this study non-typable serogroup was detected after Umrah in contrast nil before Umrah, or pilgrims; increased after Hajj by 7.4%.

Previous studies suggest that these meningococcal serogroups were carried to the Middle East/Mediterranean region where meningococcal disease outbreaks subsequently occurred among Hajj pilgrims and across sub-Saharan African countries (Trotter and Greenwood, 2007 and Halperin et al., 2012). Still *Neisseria meningitidis* W135 impose a threat to Umrah visitors; 2(3.6%) cases detected after Umrah in contrast nil before Umrah, or pilgrims; increased after Hajj by 7.4%.

Saudi Arabia and other countries adopted the quadrivalent vaccine, serogroups other than A, C, W135 and Y may emerge. Carriage studies in Saudi Arabia showed that almost one third of the isolates were phenotypically nongroupable, not expressing a capsule, though genogrouping, by use of the capsular operon, of these has not, to date, been performed (Balkhy et al., 2003). Moreover, in this study non-typableserogroup was half of the isolates from Umrah visitors and 54.8% in Hajj. Other serogroups may emerge, like serogroup B, for which no broad coverage vaccine is available or other serogroups such as X which was reported as unprecedented incidence of meningitis in Niger (Boisier et al., 2007). In 1998 during Hajj, few cases of serogroup B meningococcal meningitis were reported (El-Bushra et al., 2000 and Balkhy et al., 2004). But in 1998, serogroup B caused 60% (25 out of 42) of disease. Countries with large Muslim populations have started to show a trend of increased serogroup B meningococcal disease; Umrah visitors increased from 2 isolates before to 6 after Umrah. Our findings describing the meningococcal serogroup distribution are inconsistent with previous studies (Al-Mazrou et al., 2004; Memish et al., 2010; Caesar et al., 2013 and Ceyhan et al., 2013).

In Egypt, which is the leading country regarding visitors to Saudi Arabia for religious reasons (Tourism statistics, 2009), a shift from serogroup A to serogroup B predominance in meningococcal diseases was noted in 1998 (Nakhla et al., 2005). Also in Egypt, in laboratory based surveillance for bacterial meningitis from 1998 to 2004, 35% of meningococcal diseases were belonging to serogroup B (Afifi et al., 2007). In Turkey, a prospective study in children showed that 31% of cases of meningococcal disease were due to serogroup B (Ceyhan et al., 2008). The trends of increased serogroup B as a cause of meningococcal disease in neighboring countries in addition to the high mobility related Hajj and Umra should increase our preparedness to expect a sudden change in the etiology of meningococcal disease during the Hajj. Close and intensified efforts for monitoring carriage states of visitors and local inhabitants should be emphasized. Preventive measures against meningococcal disease should be evaluated according to pilgrimage country of origin. Saudi Arabia should be an active member of a global meningococcal network, which allows the flow of information and experience across the globe. As a part of this network, a reference lab should be established in the western region of Saudi Arabia where the holy cities are located.

Two important factors of concern complicate the clinical outcome of PPB carriage during Umrah season. First, the emergence and spread of several antimicrobial resistant strains among PPB in particular the emergence of antibiotic-resistant *S. pneumoniae* (such as; penicillin, macrolide, quinolone), ampicillin-resistant *H. influenzae*, macrolide-resistant *S. pyogenes*, and methicillin resistant *S. aureus*. The other factor of concern is the possible spread of PPB strains not included in the current vaccines administered to all pilgrims before coming to Saudi Arabia particularly for *N. meningitidis* and *S. pneumoniae*.

Laboratory surveillance data are critical to tracking the spread of less susceptible strains and to providing guidance in the empirical selection of antimicrobial agents. For all three bacterial meningitis pathogens, antimicrobial resistance has been identified, affecting the treatment of patients and chemoprophylaxis of close contacts. *N. meningitidis* isolates resistant to sulfonamides are common in many countries. Isolates resistant to rifampicin, penicillin, chloramphenicol, cotrimoxazole, ceftriaxone, and ciprofloxacin have also been identified (Wu et al., 2009).

The most effective antibiotics against *Neisseria meningitides* isolated from Umrah visitors were Cefotaxime, Ceftriaxone and Meropenem antibiotics. While the most effective antibiotics against *Neisseria meningitidis* isolated from pilgrims was Cefotaxime antibiotic. So, chemoprophylaxis with Cefotaxime, Ceftriaxone and Meropenem to eradicate nasopharyngeal carriage of the meningococcus is recommended for close contacts of patients to protect susceptible individuals and prevent further transmission. However, the same finding was also reported by Chang et al. (2012).
REFERENCES


