Epidemiological and Population Structure Studies in *Neisseria meningitidis*
Summary

• Life cycle of *N. meningitidis*, typing scheme and disease-associated genotypes.

• Meningococcal disease in the UK and the investigation into meningococcal carriage after the introduction of the serogroup C conjugate vaccine (MCC).

• Geographical, temporal and vaccine-induced population structure.
Life cycle of *N. meningitidis*

- Acquisition
- Transmission
- Release
- Invasion
- Colonisation
- 'Recovery'
- Disease
Multilocus Sequence Typing (MLST)

Sample isolation and DNA extraction.

PCR / sequencing of 7 loci (housekeeping genes).

Each sequence is assigned an arbitrary allele number

STs used to analyse population structure and assign isolates to clonal complexes.

ST-11: 2 3 4 3 8 4 6
ST-50: 2 3 19 3 8 4 6
ST-52: 7 3 4 3 8 4 6
ST-67: 2 3 4 24 8 4 6
ST-1270: 2 3 4 150 8 4 40

7 numbers from 7 loci form an allelic profile or sequence type (ST):

- abcZ
- adk
- aroE
- fumC
- gdh
- pdhC
- pgm
# Invasive potential and phenotypes

**England and Wales, 1999: 1664 disease, 2045 carriage isolates**

<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>Disease association OR</th>
<th>B</th>
<th>C</th>
<th>W-135</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-8</td>
<td>14.7 [6.8 - 31.9]</td>
<td>11</td>
<td>69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST-11</td>
<td>28.7 [20.1 - 41.1]</td>
<td>7</td>
<td>525</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ST-22</td>
<td>0.23 [0.17 - 0.32]</td>
<td>5</td>
<td>0</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>ST-23</td>
<td>0.14 [0.07 - 0.28]</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>ST-32</td>
<td>2.1 [1.5 - 3.0]</td>
<td>80</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST-35</td>
<td>0.29 [0.16 - 0.53]</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST-41/44</td>
<td>1.8 [1.5 - 2.1]</td>
<td>390</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST-213</td>
<td>0.4 [0.28 - 0.57]</td>
<td>39</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST-269</td>
<td>4.4 [3.4 - 5.8]</td>
<td>214</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unassigned</td>
<td>0.28 [0.24 - 0.34]</td>
<td>140</td>
<td>32</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>
1-Meningococcal disease in the UK and the investigation into meningococcal carriage after the introduction of the serogroup C conjugate vaccine (MCC)
Serogroups of Meningococcal Disease Isolates in England and Wales

Data: HPA Meningococcal Reference unit and Centre for Disease Surveillance and Control.
http://www.hpa.org.uk/infections/topics_az/meningo/data_meni_t3a.htm

*Culture +PCR confirmed reports
**Provisional Data
Questions Behind the UK Menincococcal Carriage Study

- Neisseria meningitidis populations are highly diverse and dynamic
- Low prevalence of disease-associated strains

Immunisation with MCC could reduce the carriage of serogroup C disease-associated strains.

1-Capsule replacement
   Emergence of new hypervirulent strains with serogroups other than C (i.e. B, Y, W-135)

2-Herd immunity
   Indirect protection of the unvaccinated individuals
The UK Meningococcal Carriage Study

- Isolation: 16,700
- Genotype: 2,500
- MRU/SMPRL Phenotype
- ST, siaD
- Study databases: MLSTdbNet

Questionnaire: Risk factors for carriage

- Glasgow
- Stockport
- Nottingham
- Oxford
- London
- Plymouth

Bangor

Cardiff
Changes on the Distribution of Serogroup C Clonal Complexes Over Three Years

Year

1999 2000 2001

% of isolates

Unassigned
ST-8 complex/Cluster A4
ST-53 complex
ST-461 complex
ST-41/44 complex/Lineage 3
ST-364 complex
ST-35 complex
ST-334 complex
ST-32 complex/ET-5 complex
ST-269 complex
ST-254 complex
ST-213 complex
ST-1157 complex
ST-11 complex/ET-37 complex
ST-103 complex
Effect of the MCC on Serogroup C Capsule Expression
Conclusions (I)

• The introduction of the MCC vaccine in the UK substantially reduced the prevalence of the disease-causing strains of ST-11 complex among the general population.

• The expression of the capsule among ST-11 complex strains has been significantly reduced, more so than in any other serogroup C associated clonal complexes.

• No vaccine escape variants were detected in this study nor has been any indication of their emergence seven years after the vaccination campaign.

• The reduction on carriage of the ST-11 complex strains is consistent with the observation that herd immunity plays a key role in protecting unvaccinated people and young infants, among whom protection from the vaccine wanes rapidly.
2-Geographical, temporal and vaccine-induced population structure
Aim of the Study

• To detect temporal stratification among meningococcal isolates obtained in three consecutive years following a mass-vaccination campaign with MCC.

• To investigate the geographic structure among bacterial population within a country.

• To investigate the population structure among carried *Neisseria meningitidis* in relation to the boundaries of human communities.
Analyses Implemented

F-Statistics or $F_{ST}$

- Measures the extent of the genetic differentiation among subpopulations.
  - Ranges from 0 (no differentiation) to 1 (complete differentiation).

Analysis of Molecular Variation (AMOVA)

- ANOVA-like approach which partitions the molecular variance into within and among sub-populations components.
  - Renders a $p$-value using a permutation test.

Definition of “genetic distance”

- ST identity: 1 if they are the same; 0 if they are different.
Temporal and Geographic Structuring Among carried *Neisseria meningitidis* in the United Kingdom

**Temporal Structuring**
- 1999-2000: 0.00093
- 1999-2001: 0.00146
- 2000-2001: 0.00025

**Geographic Structuring**
- Postcode: 0.00942
- Schools: 0.01073
- UK locations: 0.00239
Conclusions (II)

- $F_{ST}$ shows evidence of population structuring between pre and post-vaccine isolates.
- Higher levels of gene flow restriction observed among different schools and postal districts than among different cities around the UK.
Acknowledgements

Sample Collection:

Bangor: D Casey, KT Dunkin, C Roberts, AM Walker.
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...And all the students who took part in the study
Mantel Test

• Chi-square test that measures correlation \((r)\) between two inter-related distance matrices of the same rank.
  
  • The matrices used contain pairwise genetic and geographic distances.
  • \(r\) measures the correlation between genetic and geographic distances.

• Null hypothesis assumes no correlation between the matrices \((r=0)\).
  
  • High values of \(r\) indicate genetic differences between populations from different locations.

• Significance assessed as the proportion of the permutations that lead to an \(r>0\)
Mantel Test Shows No Correlation Between Genetic and Geographic Distance Either Before of After the Introduction of the MCC

1999
Correlation coefficient = -0.012009

2001
Correlation coefficient = -0.011003
Effects on Capsule Expression by Clonal Complex

- ST-11 complex/ET-37
- ST-213 complex
- ST-269 complex
- ST-35 complex
- ST-41/44 complex/Lin
- ST-53 complex
- ST-8 complex/Cluster
- Unassigned

1999

2000

2001

- SiaD-C/sg-Y
- SiaDc-Non-groupable
- SiaDc-Serogroup C

- ST-11 complex/ET
- ST-213 complex
- ST-254 complex
- ST-269 complex
- ST-32 complex/ET
- ST-35 complex
- ST-41/44 complex/Lin
- ST-8 complex/Cluster
- Unassigned
Disease-associated meningococci genotypes and serogroups

- **Serogroup A**
  - ST-1 complex
  - ST-4 complex
  - ST-5 complex

- **Serogroup C**
  - ST-11 complex
  - ST-8 complex
  - ST-41/44/C

- **Serogroup B**
  - ST-41/44 complex
  - ST-32 complex

- **Serogroup W-135**
  - ST-22 complex
  - ST-11/W-135

- **Serogroup Y**
  - ST-23 complex
Evidence of Genetic Differentiation Between Pre and Post-Vaccine Populations of *Neisseria meningitidis*

**ST Frequency**
- 1999-2000: 0.00093
- 1999-2001: 0.00146
- 2000-2001: 0.00025

**Nucleotide differences**
- 1999-2000: 0.00133
- 1999-2001: 0.00179
- 2000-2001: 0.00026

**Allelic mismatch**
- 1999-2000: 0.00141
- 1999-2001: 0.00220
- 2000-2001: 0.00014
Genetic Differentiation Among *Neisseria meningitidis* Collected from Locations, Postal Districts and Schools around the United Kingdom

**ST Frequency**

- Postcode: 0.00942, 0.00239
- Schools: 0.01073
- UK locations: 0.01116, 0.01033

**Nucleotide differences**

- Postcode: 0.01116
- Schools: 0.01365, 0.00323

**Allelic mismatch**

- Postcode: 0.01033
- Schools: 0.01262, 0.00322