

Epidemiological and Population Structure Studies in *Neisseria meningitidis*

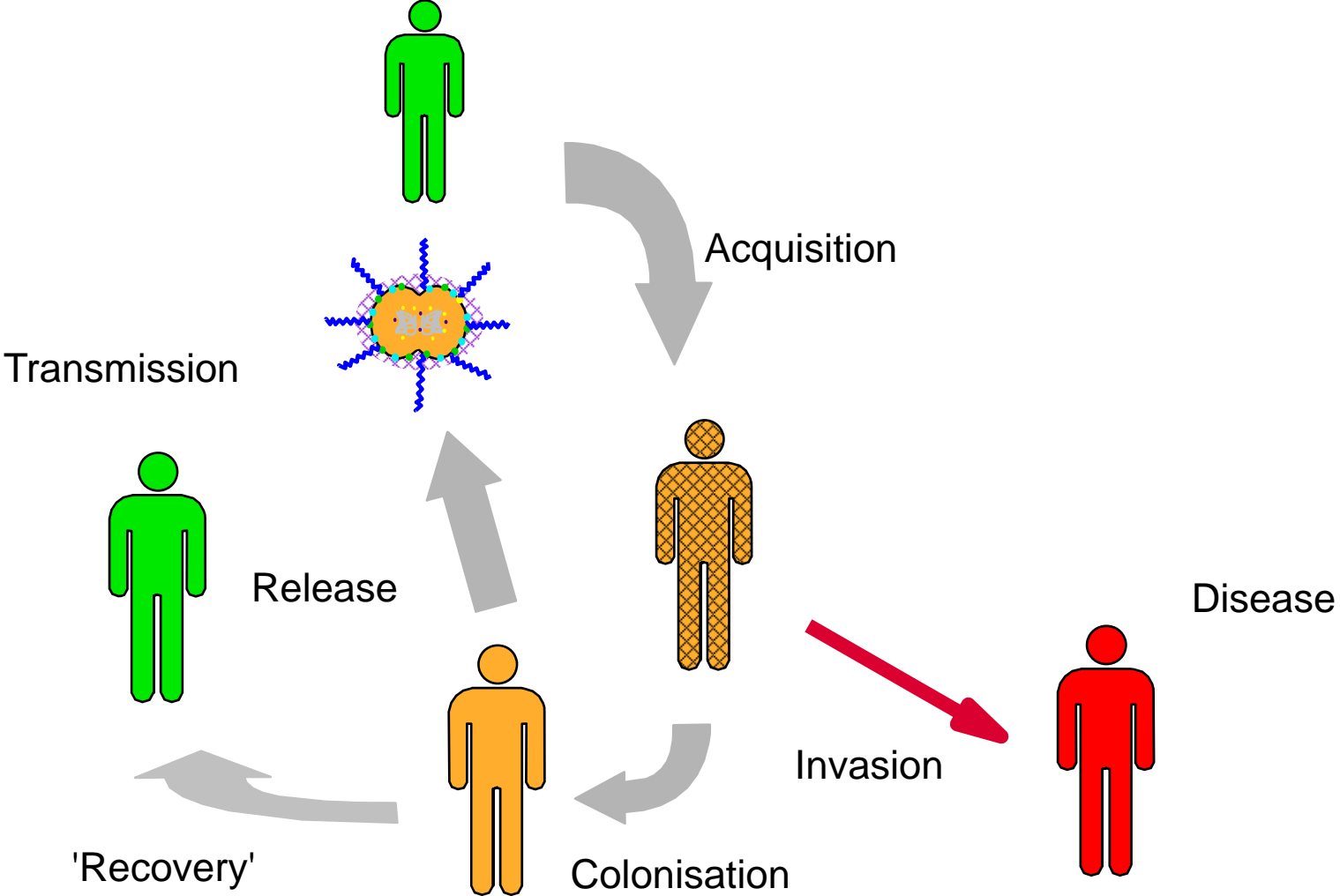
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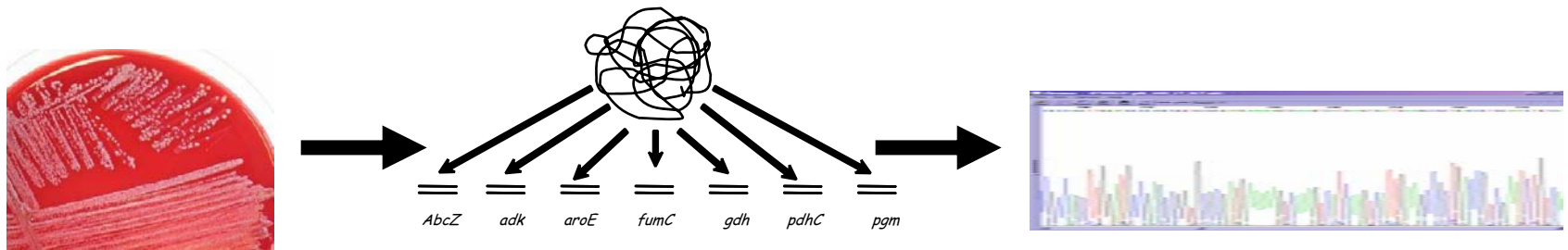
Summary

- 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*



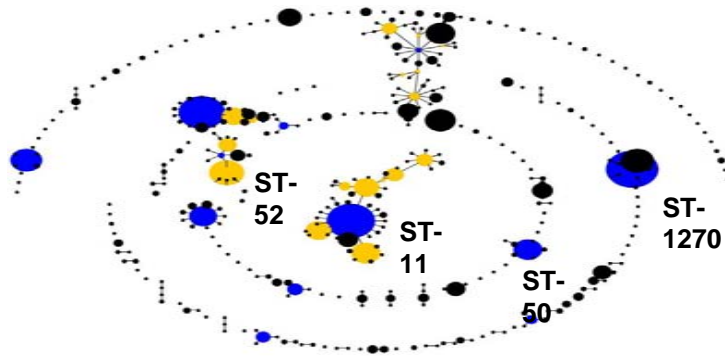
Multilocus Sequence Typing (MLST)



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.

<i>abcZ</i>	<i>adk</i>	<i>aroE</i>	<i>fumC</i>	<i>gdh</i>	<i>pdhC</i>	<i>pgm</i>	
2	3	4	3	8	4	6	ST-11
2	3	19	3	8	4	6	ST-50
7	3	4	3	8	4	6	ST-52
2	3	4	24	8	4	6	ST-67
2	3	4	150	8	4	40	ST-1270

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

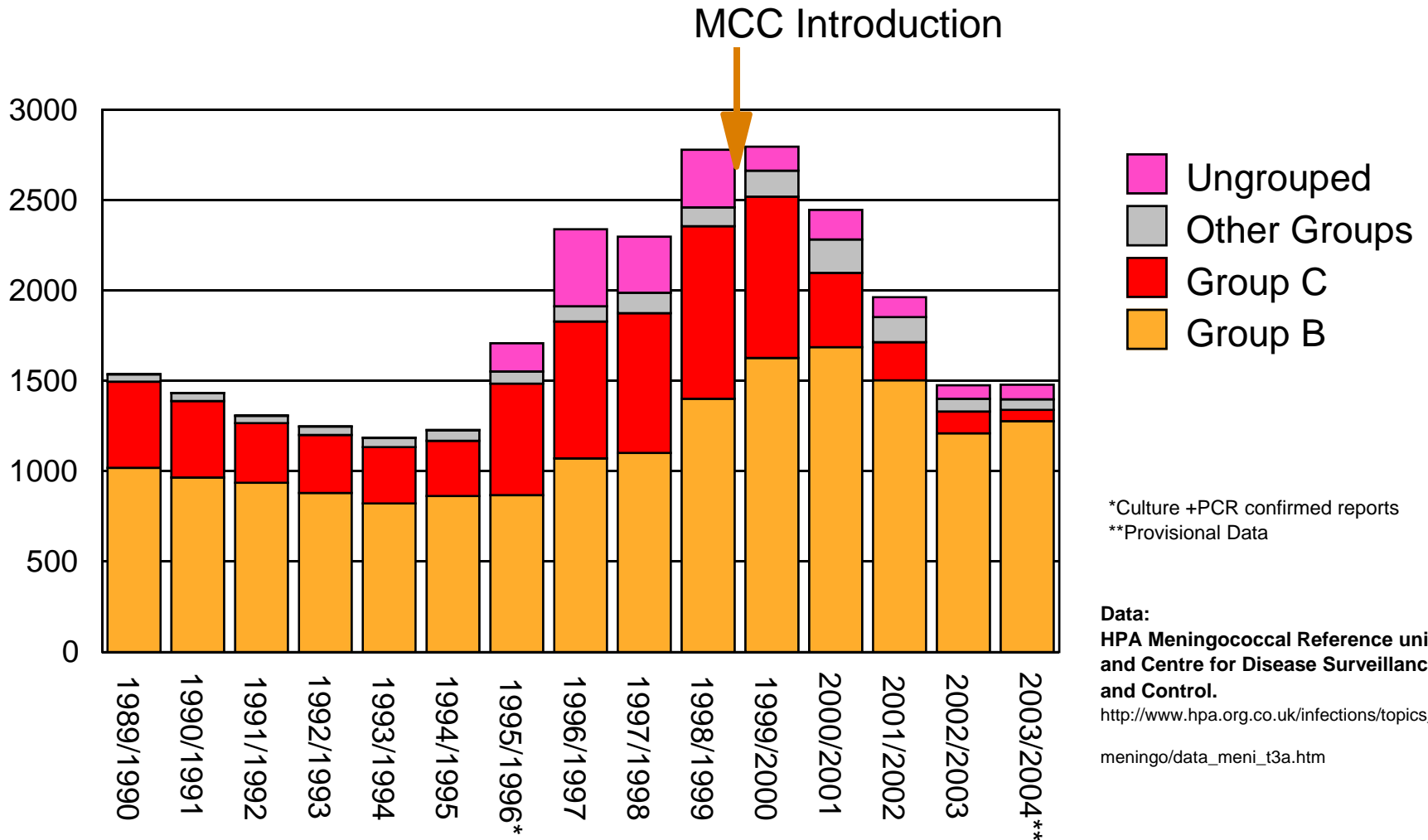
Invasive potential and phenotypes

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

Clonal complex	Disease association OR	B	C	W-135	Y
ST-8	14.7 [6.8 - 31.9]	11	69	0	0
ST-11	28.7 [20.1 - 41.1]	7	525	1	0
ST-22	0.23 [0.17 - 0.32]	5	0	42	1
ST-23	0.14 [0.07 - 0.28]	0	1	0	9
ST-32	2.1 [1.5 - 3.0]	80	1	0	0
ST-35	0.29 [0.16 - 0.53]	13	0	0	0
ST-41/44	1.8 [1.5 - 2.1]	390	7	0	0
ST-213	0.4 [0.28 - 0.57]	39	1	0	0
ST-269	4.4 [3.4 - 5.8]	214	7	0	0
Unassigned	0.28 [0.24 - 0.34]	140	32	2	9

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



Questions Behind the UK Meningococcal 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.



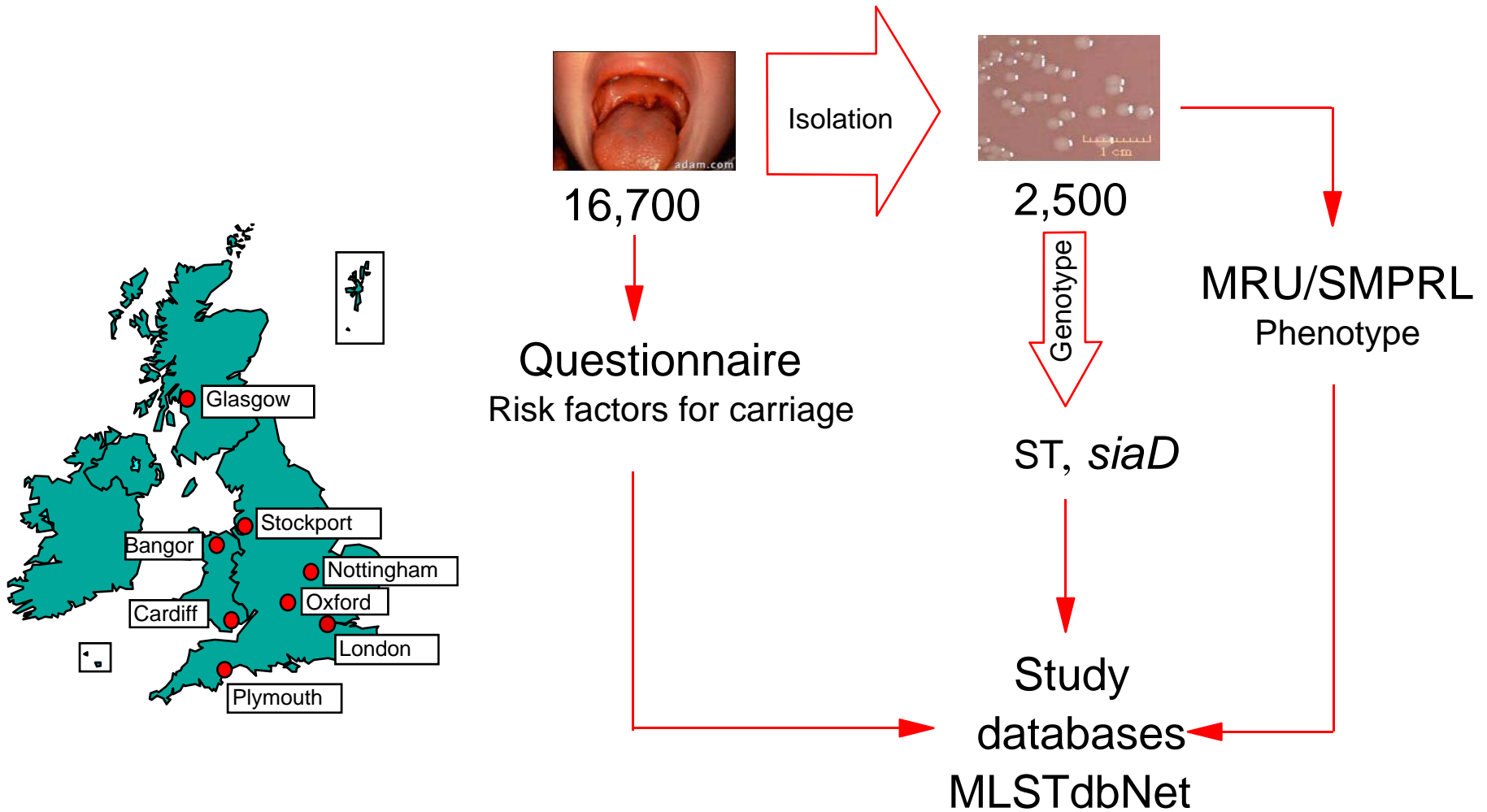
1-Capsule replacement

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

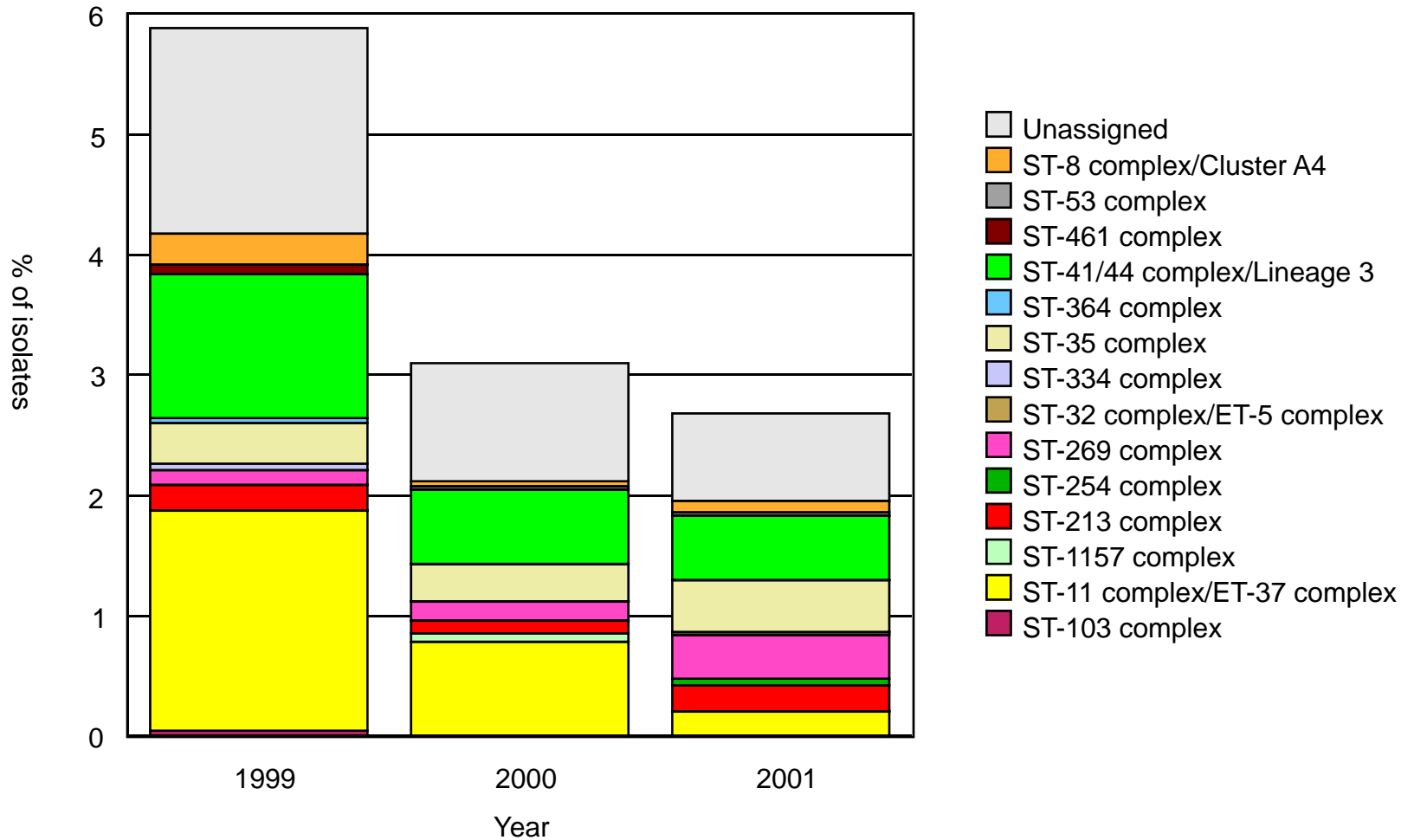
2-Herd immunity

Indirect protection of the unvaccinated individuals

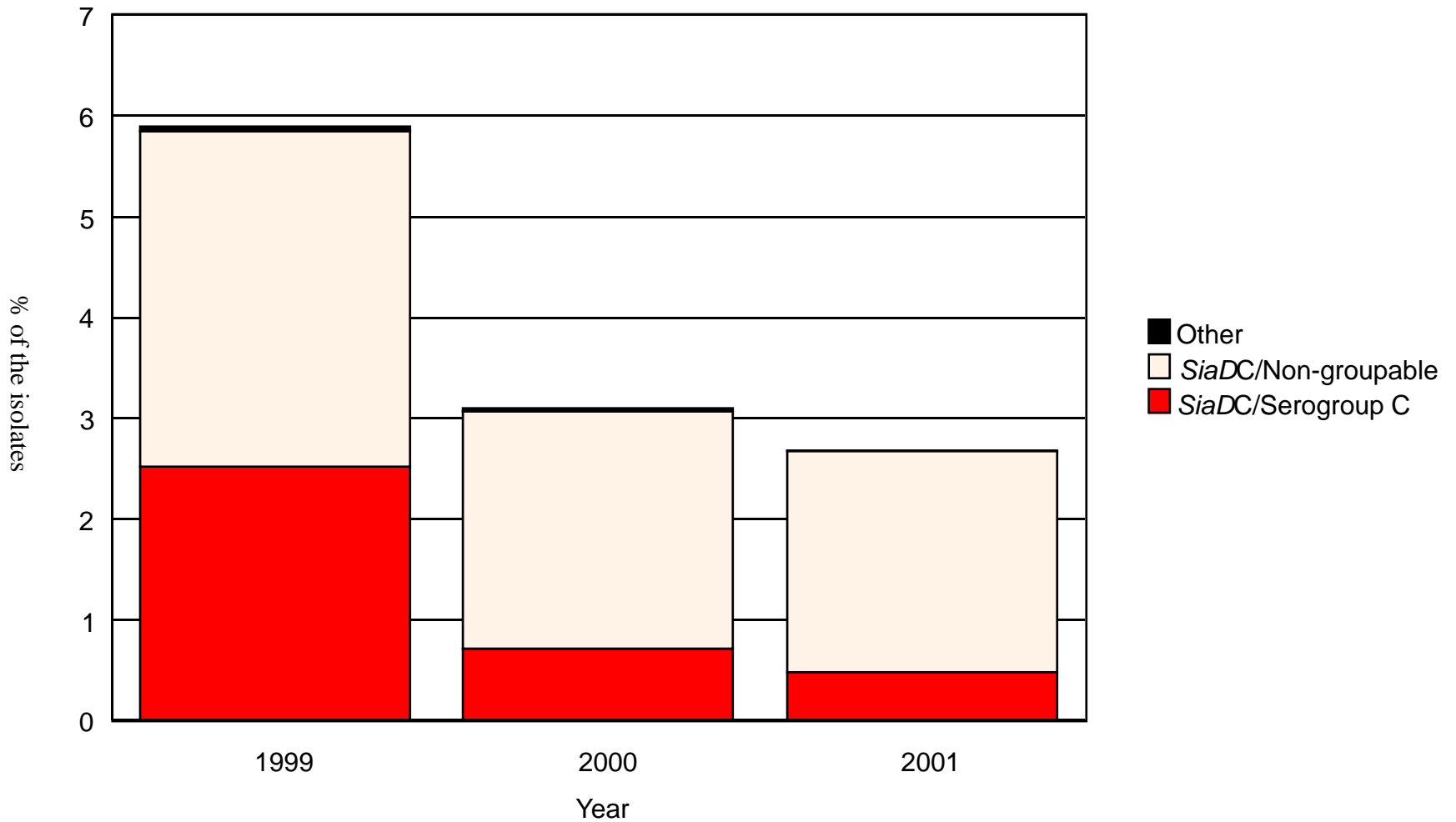
The UK Meningococcal Carriage Study



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



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)

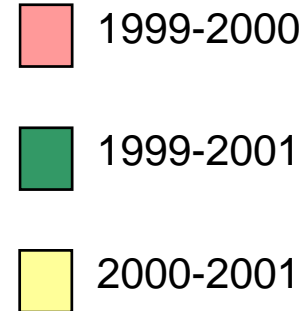
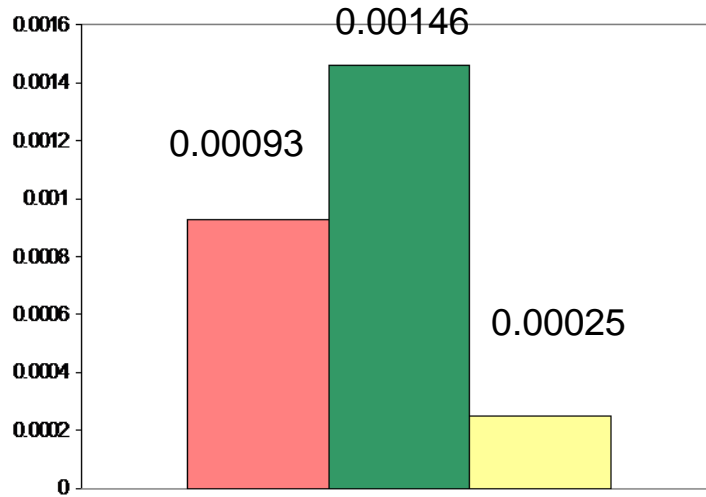
- ANOVA-like approach which partitions the molecular variance into within and among sub-populations components.
 - Renders a p -value using a permutation test.

Definition of “genetic distance”

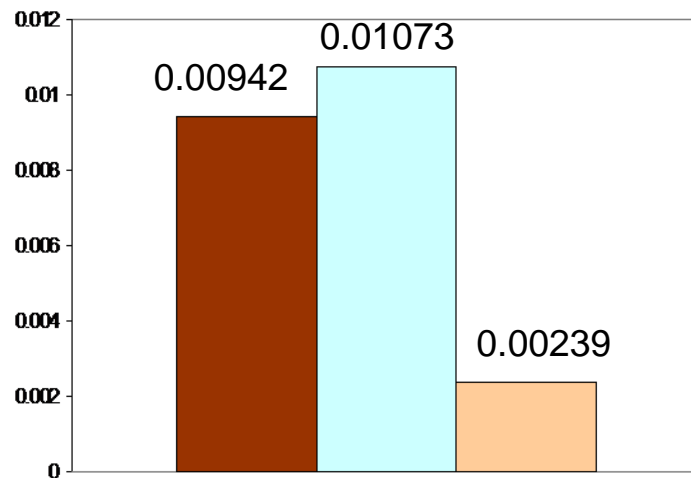
- ST identity: 1 if they are the same; 0 if they are different.

Temporal and Geographic Structuring Among carried *Neisseria meningitidis* in the United Kingdom

Temporal Structuring



Geographic Structuring



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.

Cardiff: MR Evans, J Murray, A Paul.

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...And all the students who took part in the study

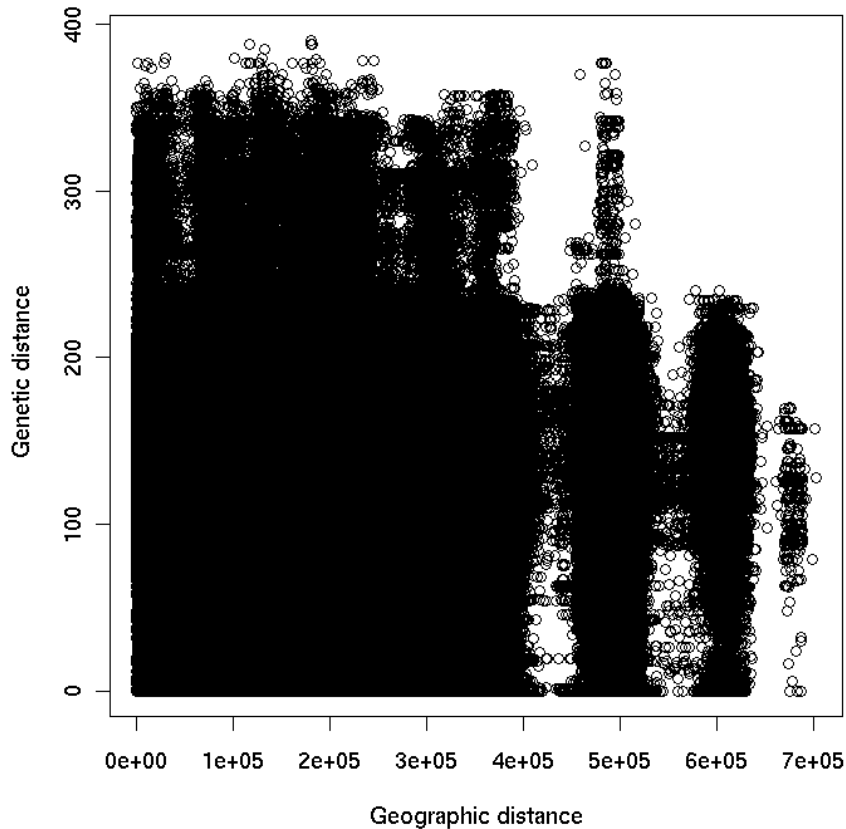
Mantel Test

- 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 or After the Introduction of the MCC

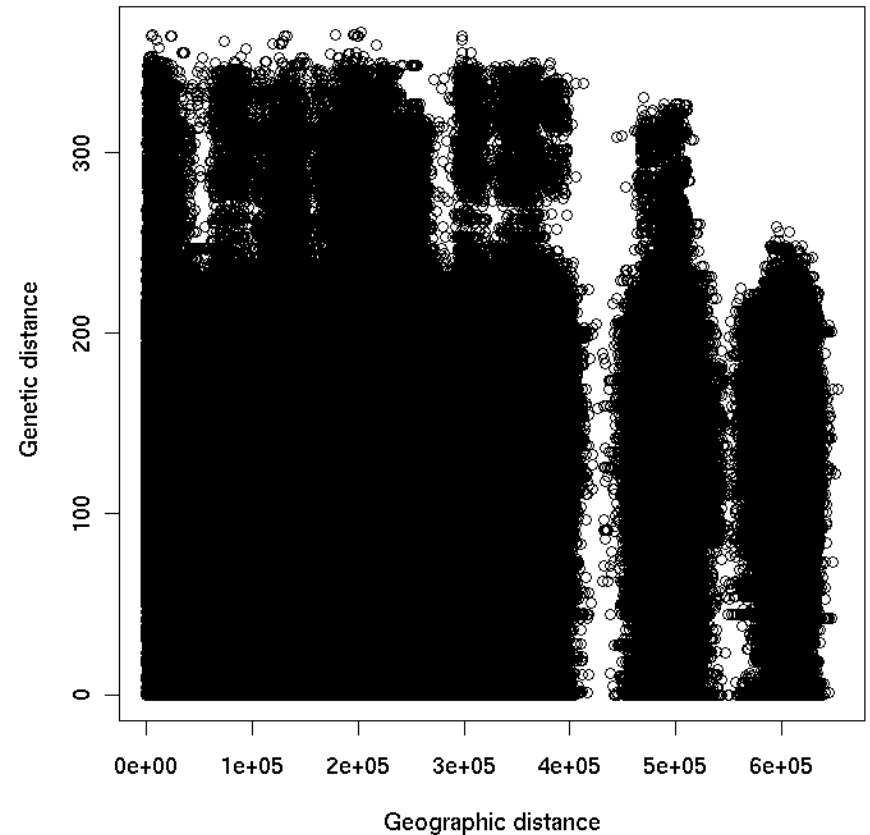
1999

Correlation coefficient = -0.012009

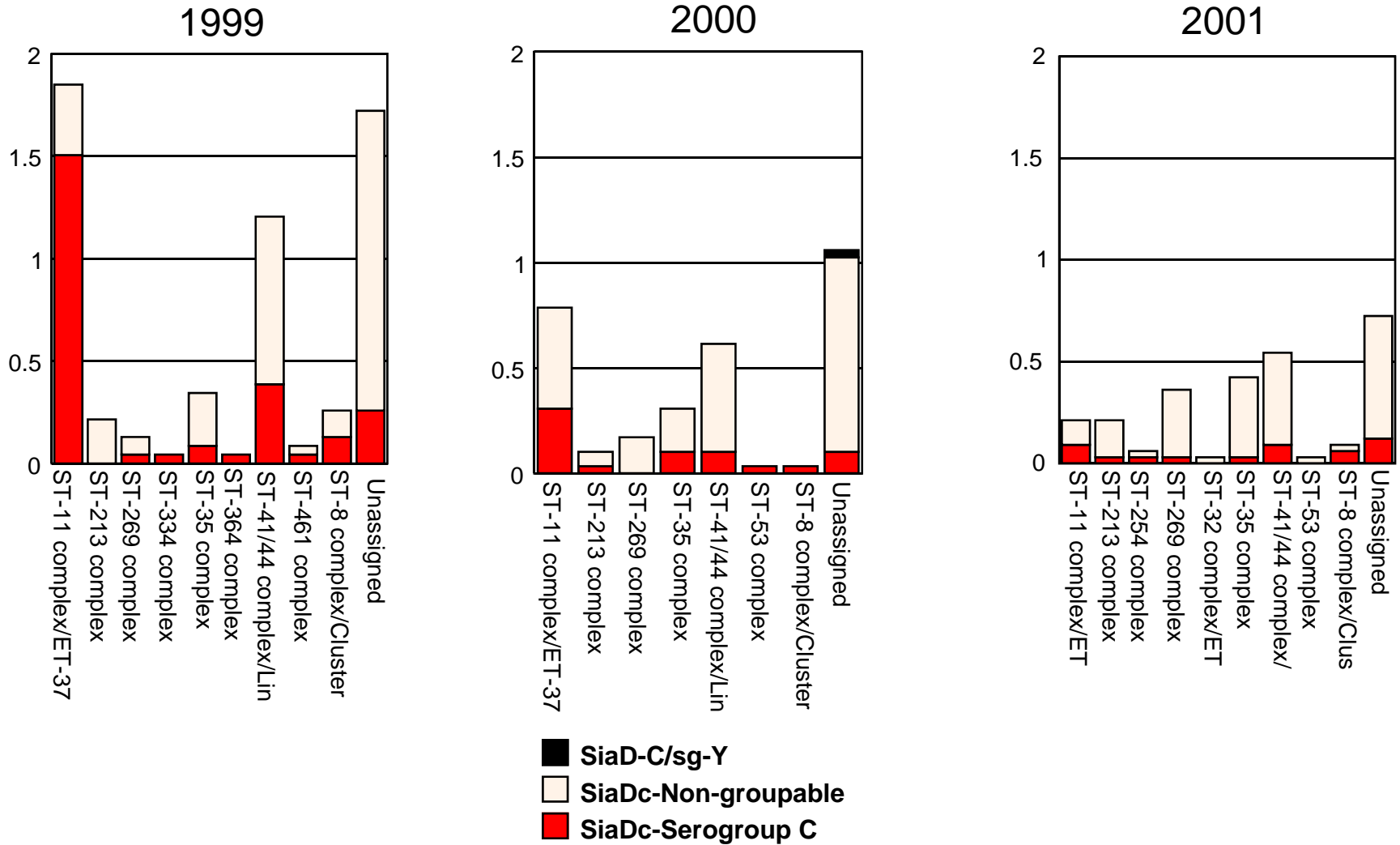


2001

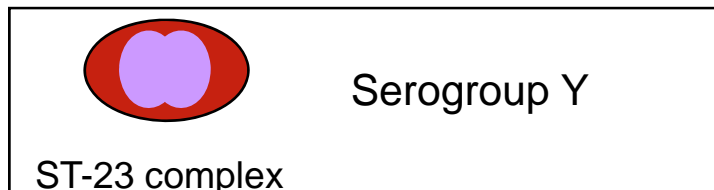
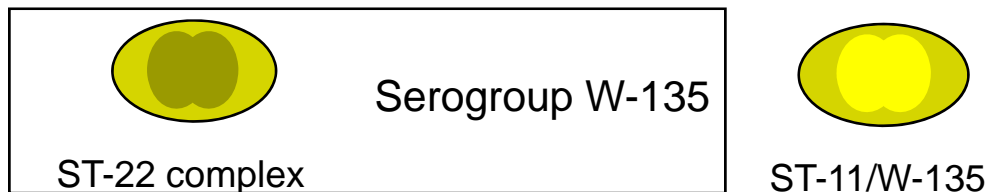
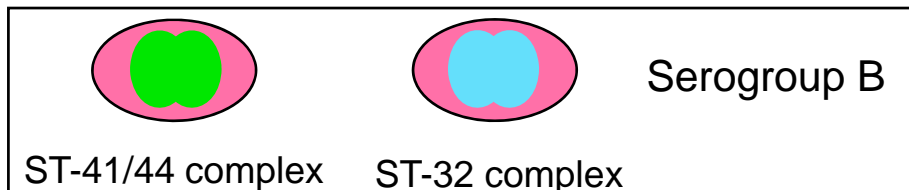
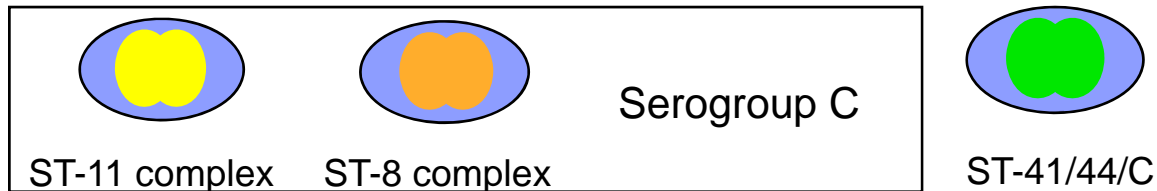
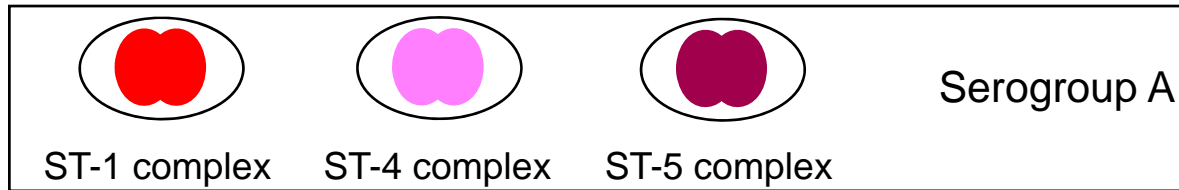
Correlation coefficient = -0.011003



Effects on Capsule Expression by Clonal Complex

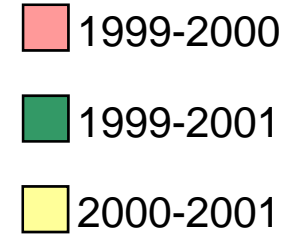
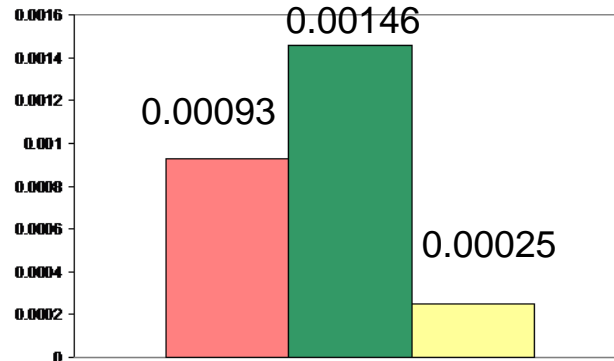


Disease-associated meningococci genotypes and serogroups

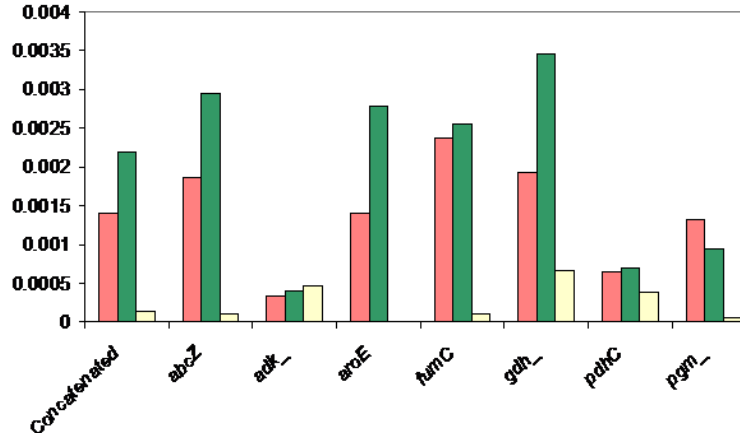


Evidence of Genetic Differentiation Between Pre and Post-Vaccine Populations of *Neisseria meningitidis*

ST Frequency

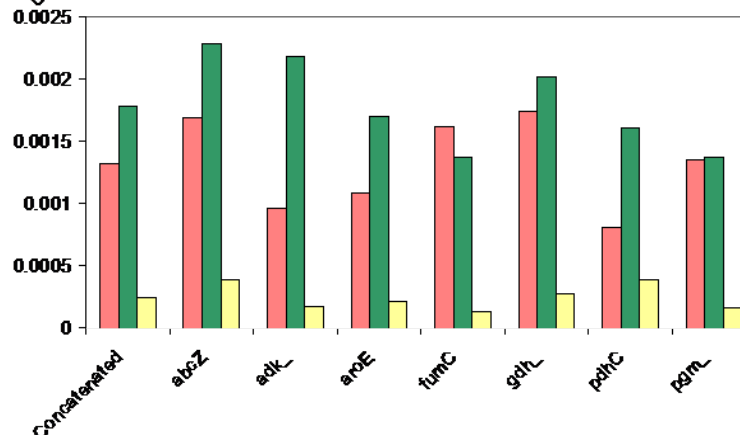


Nucleotide differences



1999-2000: 0.00141
 1999-2001: 0.00220
 2000-2001: 0.00014

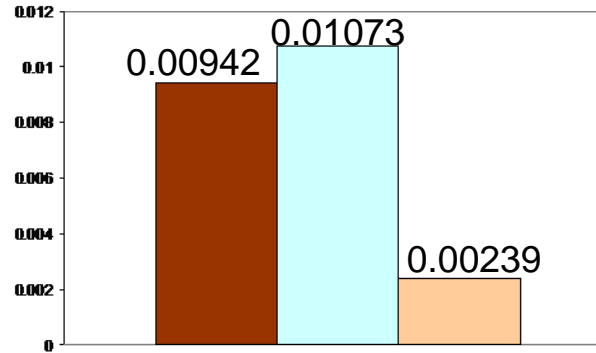
Allelic mismatch



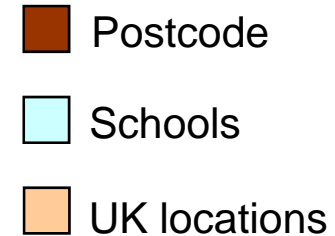
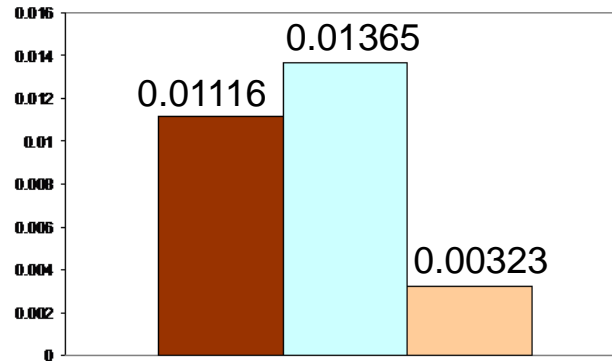
1999-2000: 0.00133
 1999-2001: 0.00179
 2000-2001: 0.00026

Genetic Differentiation Among *Neisseria meningitidis* Collected from Locations, Postal Districts and Schools around the United Kingdom

ST Frequency



Nucleotide differences



Allelic mismatch

