

# Evidence of Genetic structuring among meningococcal strains isolated before and after mass immunization in Cuba.

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**Abstract**  
 Meningococcal disease was endemic in Cuba (10-41 cases per year) in the 70's and was not considered a health problem. Starting from 1962, the reported incidence increased from 0.1, reaching 1.8 cases per 100 000 inhabitants in 1978, with the increased incidence mostly caused by serogroup C. The peak of the epidemic occurred in 1983, reaching a maximum incidence of 14.4 cases per 100 000 inhabitants. This outbreak was largely caused by a single serogroup B clone with the phenotype B:4:P1.19.15 (ET-5 complex, ST 33). In the 1980s research started in Cuba working on a serogroup B-C vaccine (VA-MENGOC-BC®). Starting in 1991 the vaccine was included in the National Immunization Program for all infants and the incidence decreased, reaching 0.3 cases per 100 000 inhabitants in 2003.  
 Meningococcal populations are genetically highly diverse; many genotypes have been identified by examination of housekeeping genes that are subject to selection for conservation of metabolic function. These genotypes have been identified as electrophoretic types by multilocus enzyme electrophoresis and more recently as sequence types (STs) by multilocus sequence typing (MLST). Isolate collections corresponding to populations of asymptotically carried meningococci show the greatest genetic diversity, while most disease-associated meningococci belong to a limited number of clonal complexes known as hyperinvasive lineages.  
 A total of 440 strains isolated from cases and carriers were investigated by conventional and molecular techniques. These strains were collected in Cuba during 22 years (1993 to 2005). The purpose of this study was to obtain a general view of the Cuban meningococcal epidemiology and to try to understand the impact of mass vaccination with VA-MENGOC-BC® on *Neisseria meningitidis* population. We found the ST-32 complex as the predominant in strains isolated either from cases and healthy carriers. The ST-32 complex found in carriers was related to the epidemic period in Cuba. The ST-53 complex was also important among carrier's isolates.

**Methods**

**Growth of meningococci and DNA preparation**  
 A total of 167 meningococcal isolates were propagated on heated blood agar plates in an atmosphere of 5% CO<sub>2</sub> for 8 to 16 h. Genomic DNA was prepared by using an Isoquick nucleic acid extraction kit (Orca Research Inc.).

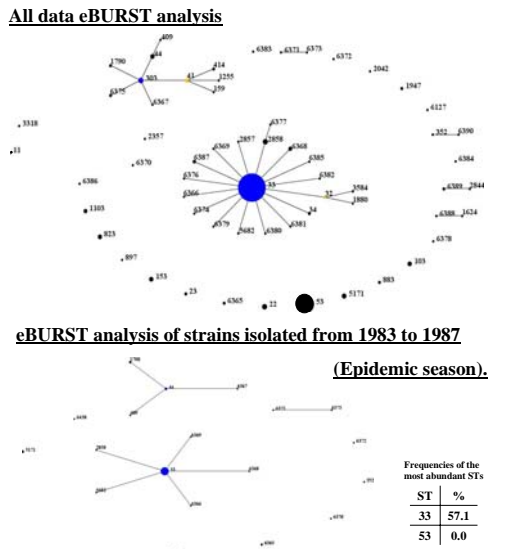
***N. meningitidis* isolates in 1983-2005 in Cuba**

Year of isolation	Cases (No. of Isolates)	Carriers (No. of Isolates)
1983	15	0
1985	12	1
1986	21	0
1987	7	0
1988	14	1
1989	19	24
1990	15	39
1991	15	37
1992	29	31
1993	0	31
1998	3	70
1999	11	26
2001	0	4
2002	1	9
2003	4	0
2005	1	0
<b>Total</b>	<b>167</b>	<b>273</b>

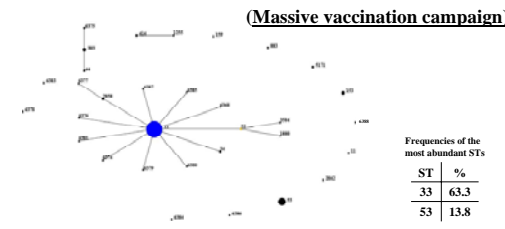
**Multilocus Sequence Typing, General methodology**

**eBURST**  
 The eBURST algorithm is implemented as a Java applet at <http://eburst.mlst.net>, and detailed guidance in its use is available at this website.

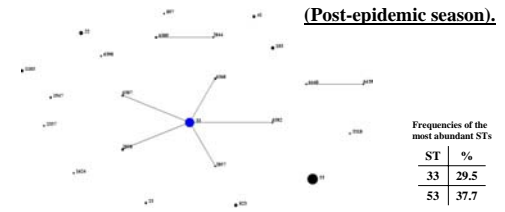
**Results: *Neisseria meningitidis* population snapshots**



**eBURST analysis of strains isolated from 1988 to 1992 (Massive vaccination campaign)**



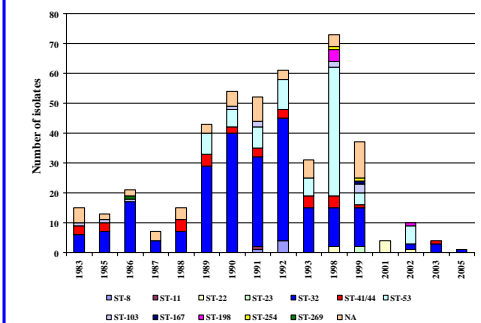
**eBURST analysis of strains isolated from 1993 to 2005 (Post-epidemic season)**



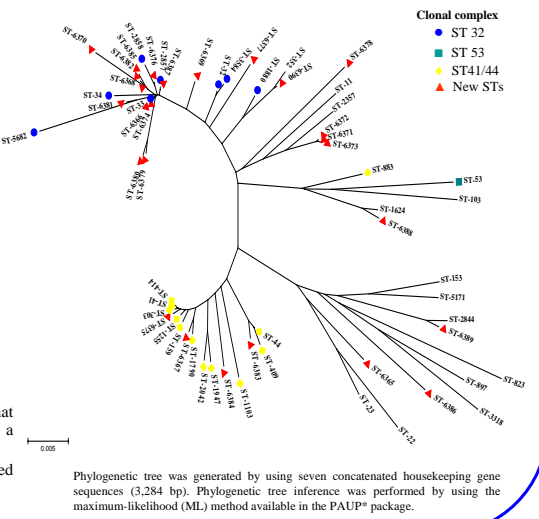
Numbers in the diagram correspond to ST numbers. The area of each circle in the eBURST diagram corresponds to the abundance of the isolates of the ST in the input data; ST-33 and ST-303 are the predicted founders of the groups (represented in blue). The ST-32 and ST-41 are founders of subgroups.

**Results**

**Distribution of meningococcal Clonal Complexes in Cuba, 1983-2005**



**Phylogenetic analysis of ST obtained from the Cuban data**



**Conclusions**

- The analysis of database of strains isolated from cases suggest that coincidentally with VA-MENGOC-BC® vaccine introduction, a significant change in the clonal complex distribution is recorded.
- ST-32 and ST-53 was the predominant clonal complexes obtained from the carriers isolates.

**Conclusions**

- ST-33 is the main responsible for the low, but steady, incidence of meningococcal disease that has occurred since the introduction of the VA-MENGOC-BC® vaccine in Cuba.
- The ST-32 complex found in carriers was related to the epidemic period in Cuba.
- No significant population structuring took place at polymorphisms, or allele distributions.

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