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Molecular surveillance of *Neisseria meningitidis* capsular switching in Portugal, 2002–2006

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SUMMARY

Neisseria meningitidis capsular switching has been reported in several countries. In order to establish the genetic relationship within group B and C strains expressing subtypes 2a or 2b, and to evaluate whether C to B capsular switching occurred in Portugal, 64 meningococci (56 serogroup C and 8 serogroup B) isolated from invasive meningococcal disease were typed using molecular methods. The studied phenotypes, 2b:P1.5,2 and 2a:P1.5-1,10-8, were the most frequent among serogroup C, but were uncommon among serogroup B strains. The multi-locus sequence typing (MLST) allelic profile and the pulsed-field gel electrophoresis (PFGE) fingerprints showed that seven serogroup B strains were genotypically identical to C strains, suggesting that capsular switching occurred. Active laboratory surveillance to find evidence of capsule switching is a now priority as MenC was introduced in the Portuguese vaccination schedule in January 2006.

Key words: Capsular switching, *Neisseria meningitidis*.

INTRODUCTION

Invasive meningococcal disease (IMD) has been a compulsory notifiable disease in Portugal since 1939. The incidence of notified IMD in the 1990s ranged between 1·82 and 3·23/100 000 inhabitants and was, in 2006, 1·19/100 000 [1]. Laboratory notification and further characterization of isolates obtained from IMD was introduced in 2002, with the implementation of a laboratory-based surveillance system of IMD named VigLab-Doença Meningocócica Network (VigLab-DM).

It was concluded from the Portuguese data report that serogroup C was the most frequent cause of IMD in the epidemiological year 2002–2003, accounting for 49% (127/257) of reported cases. Its frequency decreased after mass vaccination of children and teenagers was started in the winter of 2002, and carried on by family doctors or private paediatricians following a public alarm. Cohorts born between 2002 and 2004 showed, at the end of 2005, an estimated vaccine coverage ranging from 60·9% to 69·2% [1]. In January 2006 the meningococcal group C (MenC) conjugate vaccine was introduced in the national vaccination programme, including a first immunization at age 3 months and two boosters at ages 5 and 15 months. Moreover, in this year all children aged <10 years were also targeted and in 2007 the vaccine was offered to teenagers aged <18 years. In 2006 isolates from group B represented 77% (102/132 cases) of the invasive meningococci reported whilst 15% (20/132) were serogroup C. During these >4

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years of laboratory-based surveillance nearly 100 different phenotypes were identified among serogroup B strains isolated in Portugal. Among serogroup C isolates phenotypes C:2b:P1.5,2 (58%, 46/79) and C:2a:P1.5-1,10-8 (14%, 11/79) were the most frequent.

Types 2a and 2b are uncommon among serogroup B meningococci, but have occasionally been isolated in Portugal and other European countries, Canada and the United States [2–5]. They can result from serogroup C strains by a process of recombination whereby a horizontal exchange of chromosomal DNA and the acquisition of alleles encoding capsule biosynthesis genes takes place. This event of capsular switching may occur as a result of a natural selective pressure during the nasopharyngeal colonization or as a consequence of stress resulting from immunization [6].

In order to establish the genetic relationship among group B and C strains expressing subtypes 2a or 2b, and to evaluate whether C to B capsular switching occurred in Portugal, molecular methods were used for typing meningococci strains isolated from IMD cases.

MATERIAL AND METHODS

The VigLab Network requires culture-negative clinical samples suspected of bacterial infection, as well as isolates from IMD cases from all hospital laboratories, to be sent to the reference laboratory at the National Institute of Health (INSA) for DNA identification and further characterization.

Serogroup determination was made either by agglutination methods in the hospital laboratories or by PCR in the reference laboratory. Typing was done by whole-cell ELISA test [7] using monoclonal antibodies from the National Institute for Biological Standards and Control (UK). Subtyping was done by sequencing the two variable regions VR1 and VR2 of *porA*, as previously described by Molling *et al*. [8].

To evaluate whether capsular switching occurred, meningococcal strains isolated from IMD cases obtained through the VigLab-DM network laboratories between October 2002 and December 2006 were typed by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). The characterized strains enclosed all the serogroup B and C isolates typed 2a or 2b.

All isolates were typed by MLST, performed as described by Maiden *et al*. [9] with minor modification. Immolase (Bioline, London, UK) was used to amplify the seven loci; PCR products were purified with Jetquick PCR Product Purification Spin kit (Genomed, Löhne, Germany) and sequenced with ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The sequences of each of the seven loci (*abcZ* 433 bp, *adK* 465 bp, *aroE* 490 bp, *fumC* 465 bp, *gdh* 501 bp, *pdhC* 480 bp, *pgm* 450 bp), were analysed with BioEdit Sequence Alignment Editor (Isis Pharmaceuticals Inc., Carlsbad, CA, USA) and then compared with previously submitted allelic sequences at the Neisseria MLST Database (http://pubmlst.org/neisseria/) to determine the allelic number. The sequence type (ST) and the clonal complex of each isolate were established according to the allelic profile.

The eight serogroup B strains and 29 (randomly selected) of the total 56 serogroup C meningococcal strains were analysed on a PFGE system from Bio-Rad (Chef Mapper XA, Bio-Rad, Hercules, CA, USA) after restriction with *Bgl*II (Roche Diagnostics, Mannheim, Germany). Restriction patterns were photographed with a Gel Doc 2000 camera (Bio-Rad) and analysed with BioNumerics version 3.5 (Applied Maths, Kortrijk, Belgium). Different patterns were assigned the prefix Bg in sequential numeration. A dendrogram was created using the Unweighted Pair Group Method with Arithmetic mean (UPGMA; Bionumerics) with a 5% position tolerance.

RESULTS

During the study period (October 2002 to December 2006), 64 isolates with phenotypes 2a or 2b were typed [group B: 8/334 (2·4%); group C: 56/110 (50·9%)]. Forty-six of the 50 isolates with subtype P1.5,2 were from serogroup C and the remaining four were from serogroup B. From the isolates showing the subtype P1.5-1,10-8, the second most frequent among serogroup C strains, 10 were from serogroup C and four were serogroup B strains.

These 64 isolates were characterized into 12 different sequence types. All typed strains were mainly clustered in two different clonal complexes (Table 1): the ST-11 complex/ET-37 complex, showing only two different allelic profiles, either ST-11 or ST-5368, and the ST-8 complex/cluster A4, occurring mainly as ST-8 and ST-2289.

Five sequence types, resulting from new alleles or a new allelic combination, were submitted to

Table 1. *Molecular typing results of invasive meningococci isolated in Portugal, from October 2002 to December 2006*

ST	ST complex	Phenotype	Type frequency	PFGE	PFGE frequency
8	ST-8 complex/Cluster A4	B:2b:P1.5,2	3	Bg3	3
8	ST-8 complex/Cluster A4	C:2b:P1.5,2	30	Bg3	7
				Bg3.1	2
				Bg3.2	2
				ND	19
2711	ST-8 complex/cluster A4	C:2b:P1.5,2	1	Bg3	1
5716	ST-8 complex/cluster A4	C:2b:P1.5,2	1	Bg3	1
5714	ST-8 complex/cluster A4	C:2b:P1.5,2	1	Bg3.1	1
1713	ST-8 complex/cluster A4	C:2b:P1.5,2	1	Bg3.3	1
66	ST-8 complex/cluster A4	C:2b:P1.5,2	1	ND	1
760	ST-8 complex/cluster A4	C:2b:P1.5,2	1	ND	1
2289	ST-8 complex/cluster A4	C:2b:P1.5,2	8	Bg2	3
				Bg2.1	1
				ND	4
5712	ST-8 complex/cluster A4	C:2b:P1.5,2	1	Bg2.1	1
5368	ST-11 complex/ET-37 complex	C:2a:P1.5-1,10-8	4	Bg11	4
11	ST-11 complex/ET-37 complex	C:2a:P1.5-1,10-8	6	Bg11	5
				ND	1
11	ST-11 complex/ET-37 complex	B:2a:P1.5-1,10-8	4	Bg11.1	4
33	ST-32 complex/ET-5 complex	B:2b:P1.5,2	1	Bg17	1

ST, Sequence type; PFGE, pulsed-field gel electrophoresis; ND, not done.

the Neisseria MLST Database (ST-5368, ST-5712, ST-5713, ST-5714, ST-5716).

The first of the five strains from the newly identified ST-5368 was isolated in October 2002 (the first year of the laboratory-based surveillance system). No further ST-5368 strains have been found in Portugal since October 2003, nor were any reported by other countries in the database.

Fingerprints resulting from PFGE were grouped into three major branches, i.e. Bg2, Bg3 and Bg11 (Fig. 1).

Three of the four studied strains with phenotype B:2b:P1.5,2 were ST-8 and Bg3, as well as most of the group C strains with the same phenotype. The four strains typed B:2a:P1.5-1,10-8 shared the same ST-11 and had slight differences in the pulsed-field pattern when compared with the corresponding C strains.

From the seven recombinant strains identified, three were from children aged 5–12 months whilst the other four cases occurred in adults, aged between 30 and 41 years. None of these patients were vaccinated with MenC. No epidemiologically or geographically linked clusters could be identified among the 64 cases investigated.

CONCLUSIONS

The allelic profile obtained through MLST together with the fingerprint resulting from PFGE of the studied meningococci showed that the three B2b strains had genetic similarities with C2b strains. This strongly suggests that capsular switching from C to B occurred. Moreover, four B2a strains studied had the same sequence type with just a very small difference in one fragment in the restriction pattern, indicating that they were closely related to C2a strains, according to Tenover's criteria [10]. This is also suggestive of capsule switching.

The new ST-5368 belonging to the ST-11 complex, which seems to be restricted to Portugal, is closely related to ST-11, having just a single locus variant in the allele pdhC (pdhC-64 instead of pdhC-4). This single locus variant, based on the large number of nucleotide changes and their gene dispersion, undoubtedly corresponds to a recombination event [11].

Capsule replacement in meningococcal strains as a result of a genetic mechanism has been reported in several countries, with or without previously implemented mass vaccination [2–5]. In the present study we report the first cases of meningococci capsular

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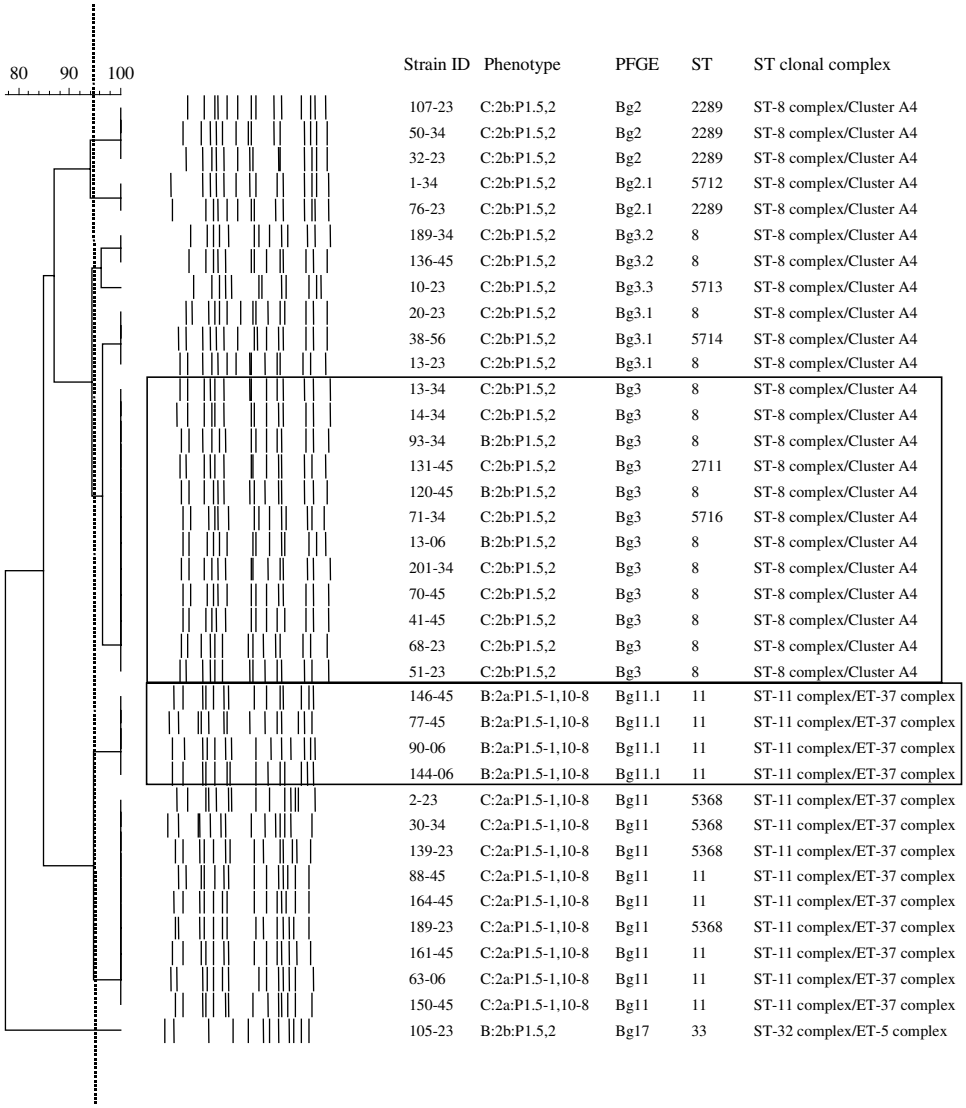

Fig. 1. Clustering of pulsed-field gel electrophoresis (PFGE) fingerprints from invasive meningococci isolated in Portugal, from October 2002 to December 2006. PFGE profiles were compared using the Dice similarity coefficient using a 5% band position tolerance and clustered in an Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram. The grey vertical dotted line in the dendrogram indicates the 95% cut-off value used to discriminate the major PFGE groups

switching in Portugal. The absence of a laboratory-based surveillance system in Portugal before 2002 has hitherto restricted analysis of recombinant strains.

The selective pressure by vaccine-induced immunity is not the only explanation for the phenomenon of capsular switching. The nasopharyngeal competition during co-colonization in carriers, mostly with serogroup B meningococci, should be also considered.

The switching capacity of *Neisseria meningitidis* avoids MenC vaccine-induced immunity [6]. As MenC was introduced in the Portuguese vaccination schedule in January 2006, active laboratory molecular surveillance of capsular switching events is therefore one of our priorities in order to assess the occurrence of recombinant meningococci for which vaccines are still unavailable.

The proximity of Spain, where a significant number of B:2b:P1.5,2 recombinant strains have been found after the 1996–1997 epidemic wave [2], also justifies this surveillance in order to detect and evaluate the extent of a possible dispersion of these recombinant strains in Portugal

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DECLARATION OF INTEREST

None.

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