Epidemiology of invasive meningococcal disease in Portugal in the last decade, 2007-2016

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Introduction & Objectives

The clinical notification of meningococcal disease (MD) has been carried out in Portugal since 1939. In October 2002 the General-Directorate of Health implemented a laboratory based surveillance of MD turning mandatory the clinical and laboratory notification of all cases. The National Reference Laboratory (NRL) of *Neisseria meningitidis* at the National Institute of Health Dr. Ricardo Jorge, Lisbon (NIH) has been managing a hospital laboratory network implemented throughout the country in 2002, which supports the laboratory component of the surveillance. Laboratories should send meningococcal isolates as well as negative culture clinical samples from suspected cases for lab confirmation and genotyping to the NRL. Voluntary vaccination against MenC started in 2002. In 2006 the MenC vaccine was introduced in the national immunization programme, addressed to children under one year of age (3 doses). During 2006 and 2007 a catch-up campaign was addressed to children under 18 years old. In 2012 the MenC vaccination schedule changed to one dose at 12 months of age. Since 2007 the number of invasive C strains became residual. In April 2014 the multi-component vaccine 4CMenB was introduced in the Portuguese market. Since January 2017 this vaccine has been freely given to individuals with increased risk of MD.

The aim of this work is to present data of MD surveillance referring to the period 2007-2016.

Methods

Probable and possible cases were confirmed by real time PCR targeting *ctrA* and *sodC* with Taqman probes, according to published protocols (1,2). Genotyping of *N. meningitidis*, either isolated in culture or DNA present in clinical samples, included: group, *por*A variable regions and, since 2009, MLST. (3,5) Characterization of *fetA* variable regions was introduced at the NIH in 2011. Cluster analysis of the MLST data was performed using an extension of the goeBURST rules algorithm, implemented in the PHYLOViZ platform and displayed in a minimum spanning tree (6).

Results

In the last decade 764 cases of MD were reported in Portugal (677 confirmed and 87 possible/probable). Incidence rate of MD decreased from 1.11/100,000 inhabitants in 2007 to 0.41/100,000 in 2016 (Figure 1). The highest incidence rate occurred in children younger than one year old, decreasing significantly for the 1-4 years age group, decreasing even further and maintaining at very low levels for the other age groups. Although this pattern was observed since 2007-2010, the decrease observed in the 1-4 years age group was particularly significant for the past two years, greatly contributing to the overall decrease in the incidence.

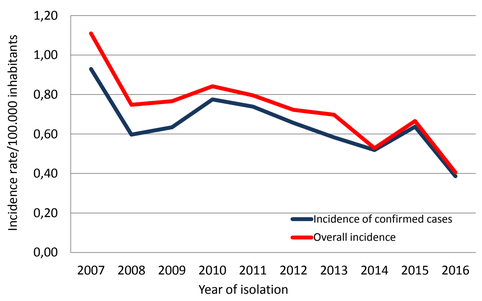
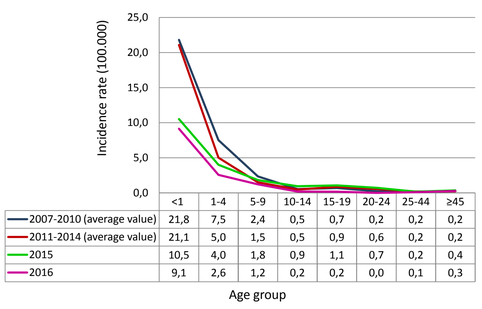


Figure 1 – Incidence rate of MD in Portugal, 2007-2016



	<1	1-4	5-9	10-14	15-19	20-24	25-44	≥45
2007-2010 (average value)	21,8	7,5	2,4	0,5	0,7	0,2	0,2	0,2
2011-2014 (average value)	21,1	5,0	1,5	0,5	0,9	0,6	0,2	0,2
2015	10,5	4,0	1,8	0,9	1,1	0,7	0,2	0,4
2016	9,1	2,6	1,2	0,2	0,2	0,0	0,1	0,3

Figure 2 – Incidence rate of MD by age group in Portugal, 2007-2010, 2011-2014, 2015 and 2016

Cases due to serogroup B were the most frequent in the last ten years of MD surveillance (74.6% of all confirmed cases) followed by cases due to serogroup Y whose incidence is increasing since 2011 (Table 1, Figure 3). Cases due to serogroup C rarely occurred (3.5% of all confirmed cases) and were mostly reported in adults and/or in non-residents. In 2014 one case of MD in an unvaccinated baby under one year of age was reported (Table 2).

Table 1 – Number of invasive meningococcal strains by serogroup and year of isolation, Portugal 2007-2016

Year of isolation	Serogroup A	Serogroup B	Serogroup C	Serogroup W	Serogroup Y	Non-groupable	Unknown	**Total**
2007	0	82	4	1	3	1	7	98
2008	1	57	0	0	3	1	1	63
2009	1	58	1	0	2	0	5	67
2010	2	49	6	0	0	3	22	82
2011	0	55	2	0	10	1	10	78
2012	0	45	3	1	4	2	14	69
2013	0	48	2	1	3	0	7	61
2014	0	33	2	0	5	4	10	54
2015	0	48	4	0	7	2	5	66
2016	0	31	0	1	6	0	2	40
Total	**4 (0.6%)**	**506 (74.6%)**	**24 (3.5%)**	**4 (0.6%)**	**43 (6.3%)**	**14 (2.1%)**	**83 (12.2%)**	**678 (100%)**

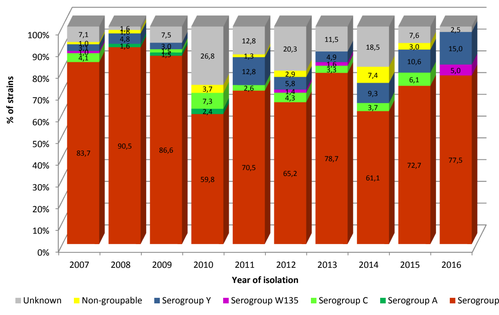


Figure 3 – Percentage of confirmed cases by serogroup per year, Portugal, 2007-2016

Table 2 – Number of invasive serogroup C meningococcal strains by age group per year of isolation, Portugal 2007-2016

Age groups (years)	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
0	0	0	0	0	0	0	0	1	0	0	1
1-4	1	0	0	0	0	0	0	0	0	0	1
5-9	1	0	0	2	0	0	0	0	0	0	3
10-14	0	0	0	0	1	0	0	0	0	0	1
15-19	0	0	0	0	0	0	0	0	0	0	0
20-24	1	0	0	0	0	1	1	0	2	0	5
25-44	0	0	1	1	0	2	0	0	0	0	4
≥45	1	0	0	3	1	0	1	1	2	0	9
Total	**4**	**0**	**1**	**6**	**2**	**3**	**2**	**2**	**4**	**0**	**24**

Serogroup C meningococci

Serogroup C strains exhibited a clonal character with a clear association between ST, subtype and fetA. The most frequent genotypes were C:P1.5-1,10-8:F3-6:ST11(cc11) (43,6%) and C:P1.5,2:ST11(cc11) (31,3%) (Figure 4).

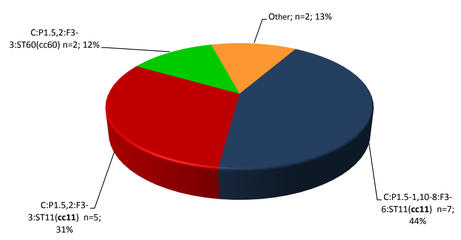


Figure 4 – Serogroup C genotypes of invasive strains, Portugal 2011-2016

Serogroup B meningococci

From 2009 to 2016, 236 out of 367 invasive B strains (64.3%) were genotyped. They presented great genotype diversity (Figure 5) .The most common clonal complexes (cc) were cc41/44 (28.1%), cc213 (13.6%) cc269 (11.9%), cc162 (8.5%) and cc461 (6.8%) (Figure 6). A particular attention was given to clonal complexes cc461 and cc213 since they have been identified only in B strains and they're estimated coverage by meningococcal multicomponent vaccine (4CMenB) was low (0% and 20%, respectively) (7). An increasing trend in the number of strains from these clonal complexes was observed (Figure 7).

Proteins PorA from families 22 and 7-2 were present in 57.2% of B strains isolated between 2009 and 2016, and were mostly associated with cc213, cc269 and cc41/44. These proteins remained the most frequent in the last two years (Figure 8).

Protein FetA presented a great genetic diversity, the most predominantly being F1-5 (22.1%) and F5-5 (15.3%). A weak association between FetA variants and ST was observed, exception to cc461 associated to F3-9 and cc32 associated to F5-1 (Figure 9).

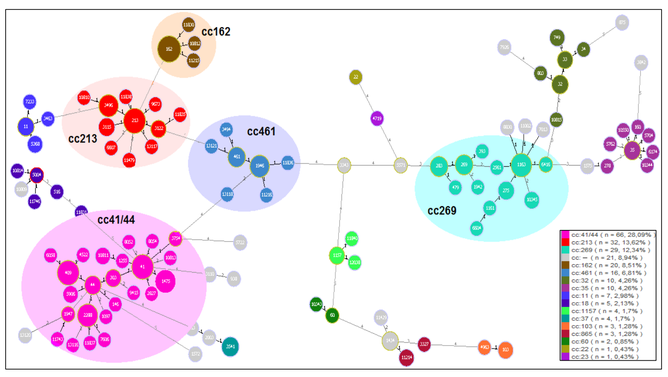


Figure 5 – Minimum spanning tree from MLST sequence typing profile data of serogroup B strains, Portugal 2009-2016. MLST sequence types (STs) are indicated by numbers within circles, where the size of the circles is proportional to the number of isolates from each ST and the different colors mean different clonal complexes (cc) in each ST. Connecting lines are labeled with the number of allelic differences between STs

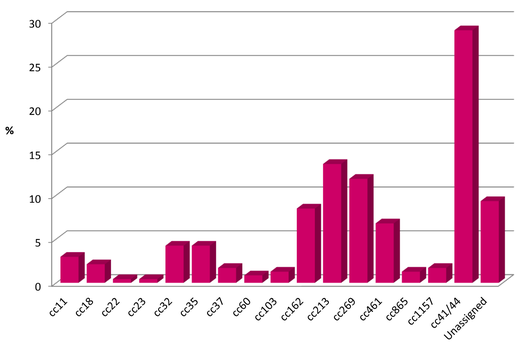


Figure 6 – Proportion of clonal complexes of B strains, Portugal 2009-2016

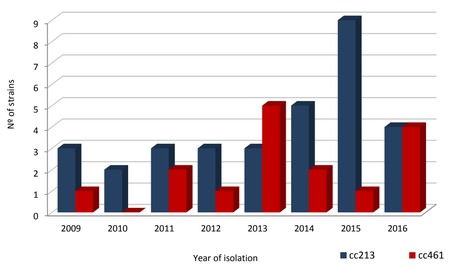


Figure 7 – Number of B strains of cc213 and cc461, Portugal 2009-2016

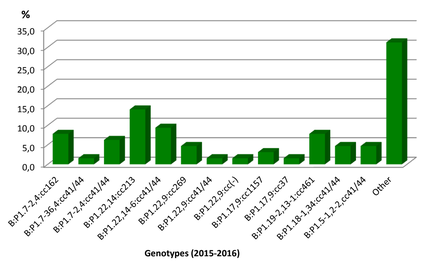


Figure 8 – Most frequent B strains genotypes, Portugal 2015-2016

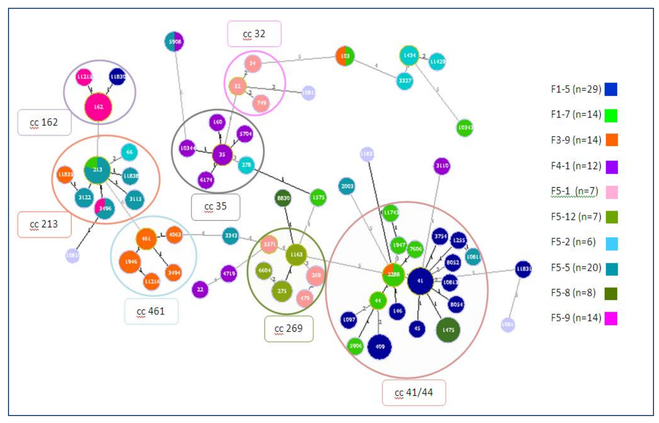


Figure 9 – Minimum spanning tree from MLST sequence typing profile data of serogroup B strains and association of FetA variants to clonal complexes, Portugal 2011-2016

Serogroup Y meningococci

Serogroup Y strains were mostly characterized as cc23 (59.4%) (Figure 10).

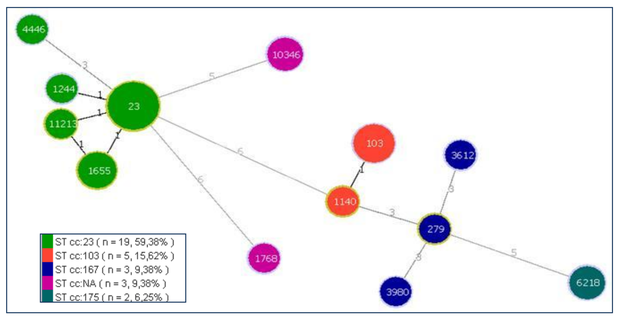


Figure 10 – Minimum spanning tree from MLST sequence typing of 32 serogroup Y meningococci, Portugal 2009-2016

Conclusions

- There was a decreasing trend in the incidence rate of MD over the ten-year period 2007-2016, mostly due to the decreasing number of cases in children up to 4 years old;
- Serogroup B strains were the most frequent, followed by serogroup Y which has been increasingly detected since 2011;
- Serogroup B strains presented great genotype diversity, although they are well structured in different clonal complexes. The complexity of the tree reflects the rapid diversification of clones;
- The compulsory national surveillance of MD remains necessary since 1) serogroup B strains remain the most frequent in spite of the use of the multi-component vaccine 4CMenB, 2) and to continue monitoring the circulating patterns of the different genotypes;

Whole genome sequencing has recently been implemented at the NRL and will contribute for a more rapid and comprehensive identification and characterization of the strains and, consequently, a better understanding of the disease.

References

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