Genomic surveillance of invasive meningococcal disease in the Czech Republic, 2015-2017

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Introduction & Aims

The high resolution power of whole genome sequencing (WGS) provides new possibilities for the analysis of *N. meningitidis* for public health purposes. The aim of this study is to present the results of the genomic surveillance of IMD in the Czech Republic for the period 2015–2017, which will improve molecular surveillance achieved by classical sequencing. The reason for investigating IMD isolates from this period by WGS was the increase of MenC which started in the country recently.

Material and Methods

The study set includes all available IMD isolates recovered in the Czech Republic and referred to the National Reference Laboratory for Meningococcal Infections in 2015-2017, a total of 89 *N. meningitidis* isolates – from 2015 (n = 20), 2016 (n = 27), and from 2017 (n = 42). The isolates were assigned to serogroups by conventional serological methods (Pastorex Meningitidis Bio-RAD, antisera *N. meningitidis* ITEST, Bio-RAD).

The next step was the isolation of DNA, using the QIAamp DNA MiniKit. All isolates were studied by WGS, which was conducted by the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, using the Illumina MiSeq platform. WGS data was subsequently processed and optimised, using the Velvet *de novo* Assembler software. Genomes were then analysed and compared using the BIGSdb Genome Comparator tool using the core genome cgMLST scheme v1.0 for *N. meningitidis* (1605 loci). Distance matrices were generated automatically and phylogenetic networks constructed and edited using the SplitsTree4 software and the Inkscape tool.

Results

Serogroup B was the most common (n = 48), followed by serogroups C, W, and Y. Altogether 17 clonal complexes were identified, the most common of which was hypervirulent complex cc11 (n = 25), followed by complexes cc32, cc41/44, cc269, and cc865. Over the three study years, complex cc11 (MenC) showed an upward trend (**Tab.1**).

Almost all MenC cc11 isolates, were assigned to sequence type ST-11. Most C: P1.5,2:F3-3:ST-11 (cc11) isolates formed two genetically close but clearly separated clusters (**Fig.1**). Cluster 1 grouped nine isolates, all of which were from 2017. Cluster 2 included five isolates from 2016 and eight isolates from 2017. It is evident from the table of molecular characteristics (**Tab.2**) that isolates of two largest and highly related clusters shared nearly all characteristics. Their genetic discordance reflected by their distribution into two separated clusters was illustrated by the *nadA* allele. Cluster 1 grouping exclusively isolates from 2017 is characterised by allele 117 producing peptide 121. Cluster 2 isolates were carrying NadA peptide variant 3 (allele 3). The **Table 2** also shows that there is the link with the region where the isolates were detected. Six of nine cluster 1 isolates came from the CZ031 region (South Bohemian region; south). Cluster 2 (n = 13) contained 10 isolates from the neighbouring CZ032 region (Pilsen region; southwest). Thus, two clusters of C: P1.5,2:F3-3:ST-11 (cc11) isolates represent two regionally specific populations of *N. meningitidis* C.

Table 1: Serogroups and clonal complexes of *N. meningitidis* isolates from IMD cases collected in the Czech Republic from 2015 to 2017.

Serogroup	2015	2016	2017	Total
MenB	**14**	**14**	**20**	**48**
cc32	3	5	6	14
cc269	2	0	7	9
cc41/44	2	3	3	8
cc18	1	1	1	3
cc35	2	0	0	2
cc162	1	0	0	1
cc60	1	0	0	1
cc213	1	0	0	1
cc334	0	1	0	1
cc174	0	1	0	1
cc1157	0	0	1	1
ccUA	1	3	2	6
MenC	**4**	**8**	**19**	**31**
cc11	2	6	17	25
cc41/44	2	1	1	4
cc269	0	1	0	1
cc103	0	0	1	1
MenW	**1**	**4**	**1**	**6**
cc865	0	3	1	4
cc11	0	1	0	1
cc22	1	0	0	1
MenY	**0**	**1**	**1**	**2**
cc167	0	1	1	2
MenNG	**1**	**0**	**1**	**2**
cc41/44	1	0	0	1
cc750	0	0	1	1
Total	**20**	**27**	**42**	**89**

Figure 1: Genetic relationship of *N. meningitidis* C isolates from IMD cases collected in the Czech Republic from 2015 to 2017, (n = 31).

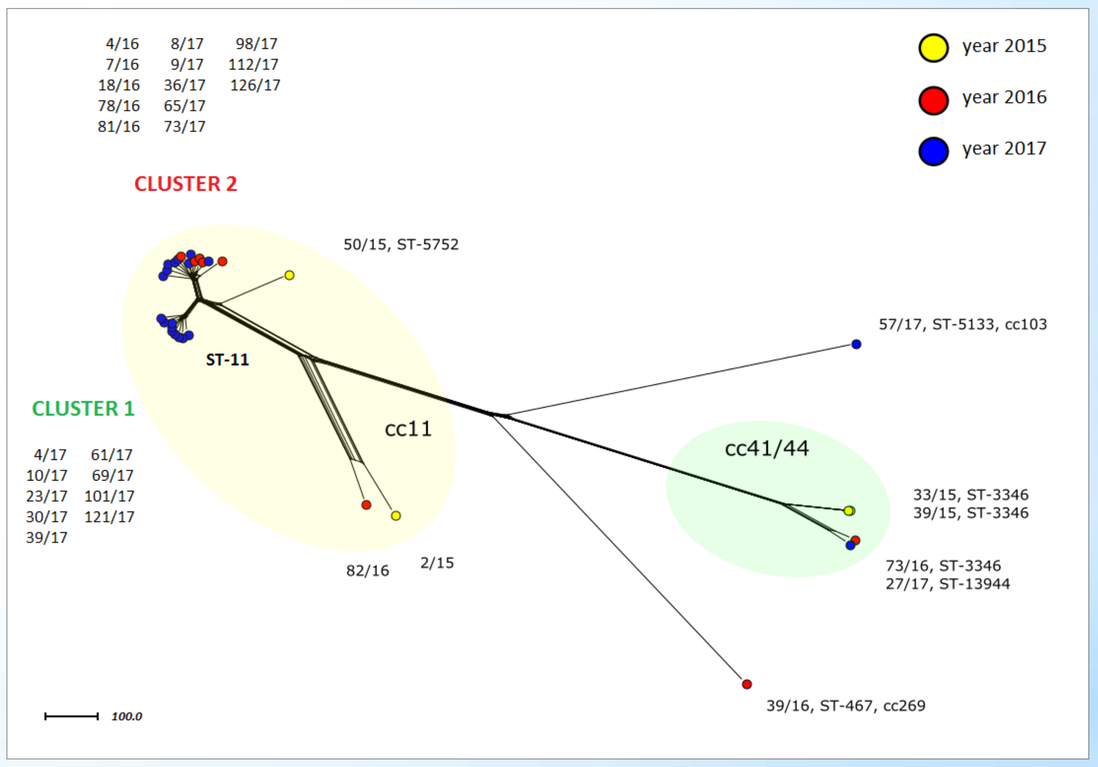
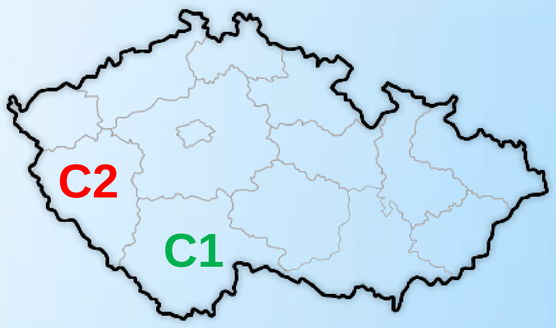


Table 2: Molecular characterization C: P1.5,2:F3-3:ST-11 (cc11) isolates from IMD cases collected in the Czech Republic from 2015 to 2017.

No. of strain	CC	ST	*porA* VR1	*porA* VR2	*fetA* VR	NHBA peptide	*nadA*	NadA peptide	fHbp peptide	BAST type	Region
4/16	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
7/16	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
18/16	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
78/16	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
81/16	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
4/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
8/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
9/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	CZ072
10/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
23/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
30/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
36/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
39/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
61/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	CZ010
65/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
69/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	**CZ031**
73/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**
98/17	**11**	**11**	-	-	F3-3	29	**3**	**3**	22	830	CZ010
101/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	CZ010
112/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	CZ072
121/17	**11**	**11**	5	2	F3-3	29	**117**	**121**	22	8	CZ051
126/17	**11**	**11**	5	2	F3-3	29	**3**	**3**	22	3	**CZ032**

Conclusions

- **The genomic surveillance of IMD in the Czech Republic provides data needed to update immunisation guidelines for this disease.**
- **WGS showed a higher discrimination power and provided more accurate data on molecular characteristics and genetic relationships among invasive *N. meningitidis* isolates.**
- **WGS method proved two regionally specific populations of *N. meningitidis* C: P1.5,2:F3-3:ST-11 (cc11) in the Czech republic.**



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