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Multilocus sequence typing of *Neisseria meningitidis* directly from cerebrospinal fluid

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SUMMARY

MLST typing of *Neisseria meningitidis* directly from clinical material was introduced in the National Reference Laboratory for Meningococcal Infections in Prague. Four cerebrospinal fluid samples were obtained from patients with suspected meningococcal invasive disease. In all samples, all classical laboratory methods gave negative results and the only positive method was PCR, which revealed *Neisseria meningitidis* group C (two specimens) and group B (two specimens), respectively. MLST performed directly from cerebrospinal fluid revealed that the strains causing the two group C infections were of sequence type (ST) 11, while the two group B infections were characterized as ST-32 and ST-33 respectively. Multi-locus sequence typing of meningococci directly from clinical material offers the opportunity to improve further the surveillance of meningococcal disease and has now been introduced into the routine portfolio of tests employed at the national reference laboratory of the Czech Republic.

INTRODUCTION

Two new molecular methods were introduced in the National Reference Laboratory for Meningococcal Infections in Prague in 2000: the polymerase chain reaction (PCR) for non-culture diagnosis of invasive meningococcal disease [1–4] and multilocus sequence typing (MLST) for characterization of meningococcal strains [5, 6]. MLST is considered to be one of the most valuable methods for the unequivocal definition of bacterial strains. When applied to meningococci, it facilitates a better understanding of the epidemiology of these infections and of the evolution of meningococcal populations [7–10].

Recently, two deaths occurred (one of a 13 year old Czech girl and one of a 14 year old Danish girl), caused most probably by *Neisseria meningitidis*, but all classical laboratory methods gave negative results. Polymerase chain reaction amplification of meningococcus-specific DNA (PCR) revealed *N. meningitidis* C from cerebrospinal fluid (CSF) of the Czech girl and *N. meningitidis* B from the CSF of the Danish girl. Following a recent report of molecular typing of *S. pneumoniae* directly from CSF [11], we attempted meningococcal MLST directly from CSF. To test the validity of this approach we also performed MLST on CSF using two other samples where *N. meningitidis* strains were not cultivated and PCR gave positive results.

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METHODS AND RESULTS

Four CSF samples were obtained from patients with suspected meningococcal invasive disease. CSF samples were investigated by PCR using the primers

Table 1. *Oligonucleotides*

Oligonucleotide	Sequence	Target
98-19	5′-ggatcatttcagtgttttccacca-3′	*N. meningitidis* B
98-20	5′-gcatgctggaggaataagcattaa-3′	
98-17	5′-tcaaatgagtttgcgaatagaaggt-3′	*N. meningitidis* C
98-18	5′-caatcacgatttgcccaattgac-3′	
98-6	5′-gctggcgccgctggcaacaaaattc-3′	*N. meningitidis*
98-10	5′-cttctgcagattgcggcgtgccgt-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	*H. influenzae* b
HI	5′-cctaagaagagctcagag-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	*S. pneumoniae*
SP	5′-gctgtggcttaaccatagtag-3′	
NM	5′-tgttgggcaacctgattg-3′	*N. meningitidis*
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	Bacteria
U3	5′-aact(c/a)cgtgccagcagccgcggtaa-3′	

Table 2. *CSF samples*

ID	Year	Age (years)	Nationality	Sex	Outcome	Cultivation	Direct latex-agglutination	PCR
03/01	2001	14	Czech	F	Died	Negative	Negative	*N. meningitidis* C
05/01	2001	14	Danish	F	Died	Negative	Negative	*N. meningitidis* B
159/00	2000	49	Czech	M	Survived	Negative	Negative	*N. meningitidis* C
162/00	2000	2	Czech	M	Survived	Negative	Negative	*N. meningitidis* B

Table 3. *MLST results of PCR products from CSF*

ID	PCR	ST	*abcZ*	*adk*	*aroE*	*fumC*	*gdh*	*pdhC*	*pgm*
03/01	*N. meningitidis* C	11	2	3	8	3	8	4	
05/01	*N. meningitidis* B	32	4	10	5	4	6	3	8
159/00	*N. meningitidis* C	11	2	3	4	3	8	4	6
162/00	*N. meningitidis* B	33	8	10	5	4	6	3	8

summarized in Table 1 for detection of common bacterial DNA, and DNA specific for *N. meningitidis*, *N. meningitidis* B, *N. meningitidis* C, *H. influenzae* b and *S. pneumoniae*. In all CSF samples all classical laboratory methods gave negative results and the only positive method was PCR, which revealed *N. meningitidis* C (samples 03/01 and 159/00) and *N. meningitidis* B (samples 05/01 and 162/00) (Table 2). Samples 03/01 and 05/01 were investigated immediately on receipt in the laboratory while samples 159/00 and 162/00 were stored at −70 °C for 11 and 9 months respectively.

CSF samples were centrifuged at 10000 r.p.m. for 10 min. DNA was extracted from the supernatant using the QIAamp kit (QIAGEN, Germany). For direct MLST from CSF two amplifications were performed.

The first amplification of seven alleles (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC* and *pgm*) was performed in an Amplitrone II thermocycler using Hot start (98 °C for 5 min) under the following conditions: first cycle of denaturation at 94 °C for 2 min, subsequent 35 cycles – denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, polymerization at 72 °C for 1 min and final cycle of polymerization at 72 °C for 1 min. The primer pairs used for the PCR amplification of internal fragments of these genes were identical to those presented on the MLST website (http://neisseria.mlst.net) and were prepared by GENERI BIOTECH, Czech Republic. For the first

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amplification from CSF more DNA (25 μl) was used than for 'classical' MLST of meningococcal strains (10 μl). Second amplification of the same alleles was performed from 1 μl of amplified products and after this a purification of the product was performed with 20% PEG.

The sequencing reactions were performed in PCR tubes with the BigDye terminator cycle sequencing kit (PE Biosystems) and subsequently analysed with an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). The final sequence of each locus was determined with the LASERGENE software package (DNASTAR, Madison, WI, USA). Housekeeping alleles and sequence types (STs) were assigned by reference to the MLST website [12].

Sample No. 03/01

The amplification and sequencing of the *pgm* allele failed (Table 3), but interrogation of the MLST database allowed assignment to ST-11. This finding is consistent with the current epidemiological situation in the Czech Republic, where the majority of deaths from invasive meningococcal disease are caused by group C meningococci of the ET-15/37 complex, and belonging to ST-11.

Sample No. 05/01

MLST results (Table 3) revealed ST-32, of which there are details of 26 meningococcal strains in the MLST database. Of these 26 strains, 24 were isolated from cases of invasive meningococcal disease in the period 1976–99. The majority of the strains originated from Israel (15) and from Norway (5). All these strains are group B meningococci and belong to the ET-5 complex.

Sample No. 159/00

MLST results revealed ST-11 (Table 3). In the Czech Republic, 90% of *N. meningitidis* C causing invasive meningococcal disease belonged to the ET-15/37 complex, and ST-11 in the year 2000.

Sample No. 162/00

MLST results (Table 3) revealed ST-33, of which there are details of four meningococcal strains in the MLST database. All were isolates from cases of invasive meningococcal disease in Brazil, Canada, Cuba and Greece in the period 1989–99. All these strains are group B meningococci and three of them belong to the ET-5 complex.

ST-32 and ST-33 are related and their ancestral ST is ST-30 from Norway.

DISCUSSION

These first results of MLST directly from CSF have encouraged us to adopt a strategy to perform this technique routinely in all suspected meningococcal cases and deaths. It may be of particular value in cases where antibiotics have been administered and/or specimens have not been collected early in the course of the illness. MLST directly from CSF should improve the surveillance of invasive meningococcal disease in the Czech Republic.

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