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Semiautomation of Multilocus Sequence Typing for the Characterization of Clinical Isolates of *Neisseria meningitidis*

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The Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) provides a national service for the laboratory confirmation of meningococcal and pneumococcal disease in Scotland. Part of this service includes the serogrouping of meningococcal isolates followed by typing and subtyping. The procedures for this are labor-intensive but important for the identification of linked cases and the surveillance of disease so that effective public health measures can be taken. However, different strains of meningococci, such as those within the electrophoretic type 37 complex, occurring during case clusters of disease are now indistinguishable by current methods. The SMPRL has started using multilocus sequence typing (MLST) as a routine method for the characterization of isolates of *Neisseria meningitidis*. MLST produces nucleotide sequence data of seven housekeeping genes providing results that are useful for public health management. However, the method is laborious and time-consuming and therefore lends itself towards automation. The SMPRL therefore developed a semiautomated method for MLST using a 96-well format liquid handler and an automated DNA sequencer. Semiautomated MLST is now provided as a reference service for Scotland. This work describes the methodology required for the characterization of *N. meningitidis* and highlights its usefulness for public health intervention.

The diagnosis of meningococcal disease (MD) is usually based on clinical presentation and is ideally confirmed with the isolation of *Neisseria meningitidis* from a patient source, usually blood or cerebrospinal fluid (CSF). Rapid and accurate isolate characterization is essential to distinguish meningococcal isolates in clusters of cases. In Scotland, serogroups B and C are responsible for the majority of cases, and there are about 300 requests for the characterization of meningococcal isolates each year (6). Isolates of *N. meningitidis* are traditionally identified and characterized using phenotypic markers. Although serogrouping, serotyping, and sero-subtyping based on outer membrane protein antigen detection are carried out, these are not always adequate, especially for endemic strains. Difficulty may also occur in distinguishing between strains that are part of large groups of genetically similar clones, especially strains of the electrophoretic type 37 (ET-37 complex) (18, 28). The ET-37 complex is one cluster of related clones causing epidemic serogroup C disease in Europe and elsewhere (27). Since 1995, strains of the ET-37 complex have been the most common cause of serogroup C MD in Scotland (6, 23, 24, 25), and meningococci isolated during case clusters have not been easily differentiated.

Multilocus sequence typing (MLST) was therefore introduced as a national DNA sequence typing service for the characterization of isolates of *N. meningitidis*. As MLST generates a lot of sequence information, the analysis of resultant data must be performed with care. It is therefore useful if the number of strains analyzed by MLST are kept to the minimum required for epidemiological surveillance. In Scotland, approximately 300 strains of *N. meningitidis* are characterized from patients and carriers each year. This number can be efficiently processed by the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) for MLST. The method was first described by Maiden and colleagues (16) and is based on the well-tested principle of multilocus enzyme electrophoresis (MLEE). However, MLEE is laborious, and it can be difficult to compare results between laboratories (9, 19, 26), whereas MLST provides data in a digital and therefore portable format, because each gene is sequenced. MLST was first validated using *N. meningitidis* (16) because it is a species in which recombination events are common (8, 12). A collection of 107 meningococcal isolates from patients with invasive disease and healthy carriers that had been previously characterized by MLEE was used. Initially 10 loci were chosen (16), but a subset of 7 loci was chosen on the basis of its discriminatory power. The SMPRL has extended this to incorporate an eighth gene, *porA*, which encodes a class I outer membrane protein (10, 14, 15, 21, 22, 27). *porA* is used for characterizing *N. meningitidis* to sero-subtype level and provides information, along with serogroup and serotype data, for differentiating strains. However, this methodology is currently limited by the use of monoclonal antibodies, whereas *porA* sequencing can provide sequence data relating to a greater number of sero-subtypes. Sequence data from three variable regions (VRs) (VRs1, 2, and 3) can be obtained from within this gene, which further increases the discriminatory power of this method and provides greater res-

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olution within major lineages (11, 20, 22). This method of MLST can therefore be used for fully characterizing strains isolated during case clusters of MD (11, 29).

MLST remains a laborious technique but has been partially automated (5). The method is described as semiautomated because some aspects of sequence analysis are performed manually, but initial gel analysis and assignment of allele numbers for MLST is automatic. Additional benefits of the semiautomation of MLST are the maintenance of high standards of reproducibility and the minimization of cross-contamination. Although no system can completely eliminate PCR contamination, this system reduces the potential during PCR setup, PCR product dilution, cleanup, and sequence labeling setup. This is due to the use of a non-cross-contamination (NCC) platform and consumables whereby all components liable to contamination, including reagents, samples, and tubes, possess an NCC lid which is removed as necessary for pipetting procedures for only short periods of time. Any automated system incorporating a variety of complex stages, including PCR setup, execution, and sequence labeling, would ideally be contained on one workstation. The SMPRL took this into account when developing the system. This unique type of MLST system was introduced as a routine tool for the detection and characterization of meningococcal DNA and allows an effective and efficient identification and tracking system for meningococcal infection in Scotland. The purpose of this paper is therefore to describe the procedures involved in setting up a semiautomated system for MLST which also incorporates sequencing of *porA* for the characterization of *N. meningitidis* strains. We also demonstrate how this is introduced as a national reference laboratory service for public health management and epidemiological purposes.

MATERIALS AND METHODS

Patients and specimens. All *N. meningitidis* C:2a strains, totaling 45 strains, received by the SMPRL during the year 2000 were included. These were chosen first as these strains had become prevalent in Scotland in 1998 and are associated with outbreaks and increased mortality rates. These isolates originated from blood, eye, CSF, throat, and other miscellaneous sites from patients with confirmed meningococcal disease or from carriers. All strains were initially isolated in hospitals throughout Scotland.

Phenotypic characterization. All strains were isolated on horse blood agar (Oxoid, Basingstoke, United Kingdom) at 37°C in an atmosphere of 5% CO_2. Serogrouping of *N. meningitidis* was performed by latex agglutination, coagglutination, and *siaD* PCR as previously described (3, 4, 7). Serotyping, serosubtyping, and antibiotic sensitivity profiling were performed as previously described (13).

Genotypic characterization. This MLST method utilized a robotic liquid-handling system, the RoboAmp-4200 system, and an automated DNA sequencer, the Licor L4200-L2 DNA sequencer (both from MWG-Biotech, Milton Keynes, United Kingdom). This allowed the automation of most of the procedures required for the DNA amplification of the MLST and *porA* genes and subsequent sequence labeling from meningococcal isolates.

PCR sample preparation. Clinical isolates of *N. meningitidis* were cultured on horse blood agar (Oxoid) and incubated overnight in the presence of 5% CO_2 at 37°C. One fresh colony was inoculated into 0.5 ml of 18-MΩ-distilled water and boiled for 1 min. The suspension was centrifuged at 15,000 × *g* for 2 min, and the supernatant was used as a source for the detection of meningococcal DNA.

PCR amplification. Programming of the RoboAmp-4200 liquid handling system was performed according to the manufacturer's instructions. All PCR reagents were maintained at 4°C on the platform. Each PCR was performed in a final volume of 50 μl using 1.1× Reddymix PCR master mix, containing 1.25 U of *Taq* DNA polymerase (Abgene, Epsom, United Kingdom); 75 mM Tris-HCl (pH 8.8 at 25°C); 20 mM $(NH_4)_2$; 1.5 mM $MgCl_2$; 0.01% (vol/vol) Tween 20; a 0.2 mM concentration each of dATP, dCTP, dGTP, and dTTP; and red dye for gel electrophoresis. For a 50-μl reaction mixture, 45 μl of PCR master mix and 1 μl of each MLST or *porA* primer pair (11, 15, 16, 27) (Table 1) were added to produce a master mix volume of 47 μl. These preprepared master mixes were placed on the RoboAmp-4200 refrigerated reagent rack, and the DNA preparation samples were placed on the sample area. Within a refrigerated NCC 96-well plate, 47 μl of master mix was automatically added to the appropriate wells using a washable tip, along with 3 μl of DNA preparation, making a final 50-μl reaction mixture. After each stage of the setup, the washable tip was automatically washed with 2 ml of 18-MΩ-distilled water. The NCC 96-well plate was automatically placed into the integrated MWG-Biotech Primus 96 thermocycler. The PCR conditions were altered from those described by Maiden and colleagues (16) to a step-down PCR method to ensure complete and reproducible amplification of all eight genes. The step-down PCR conditions were 94°C for 2 min; 3 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min; 3 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min; 3 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min; 20 cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min; and finally 72°C for 10 min. After PCR the NCC plate was automatically removed from the thermocycler to a refrigerated block.

PCR product purification. A 5-μl aliquot of each PCR product was subsequently transferred into another NCC 96-well plate. A total of 0.5 U of shrimp alkaline phosphatase (U.S. Biochemical [USB] Corporation, Cleveland, Ohio) and 0.1 U of exonuclease I (USB Corporation) were added to each PCR product and automatically placed in the integrated thermocycler. The cycle conditions were 37°C for 15 min followed by 80°C for 15 min. This process removed unused primers and deoxynucleoside triphosphates that could interfere with sequencing.

PCR sequence labeling. A 3-μl aliquot of each purified PCR product was automatically transferred using the washable tip into an open 96-well microtiter plate. From a refrigerated block on the platform, a predilution was performed by adding 24 μl of 18-MΩ-distilled water and 1.5 μl of both forward and reverse sequencing primer specific for each PCR product (11, 14, 16, 27). All forward-sequencing primers were tagged with 700-nm infrared dye, and all reverse sequencing primers were tagged with 800-nm infrared dye. Four microliters of each prediluted sequence mix was distributed into appropriate wells of another open 96-well plate, containing 2 μl (each) of A, C, G, and T from a Thermo Sequenase fluorescence-labeled primer cycle sequencing kit (Amersham Pharmacia Biotech, Amersham, United Kingdom). Finally, 15 μl of Chill-out 14 liquid wax (Genetic Research Instruments) was added to each well. Appropriate washing of the washable tip occurred with 18-MΩ-distilled water throughout the procedure. The plate was automatically placed into the integrated thermocycler. The sequence cycle conditions were 95°C for 2 min; 30 cycles of 95°C for 15 s, 50°C for 30 s, and 70°C for 30 s, and finally 72°C for 10 min. Afterwards the plate was automatically removed from the thermocycler and placed on a refrigerated block. A 4-μl aliquot of formamide loading dye-stop solution (Amersham Pharmacia Biotech) was automatically added to all 96 wells with the washable tip and subsequently placed into the integrated thermocycler at 65°C for 10 min. The process took approximately 8 h for full automation from start to finish.

DNA sequencing. A 0.2-mm-thick sequencing gel was cast using two 41-cm plates separated by two 0.2-mm strips. The gel matrix contained 7.5 ml of Rapid XL Solution 40% (USB Corporation), 4 ml of formamide, 21 g of urea, 5 ml of 10× TBE (162 g of Tris base, 27.5 g of boric acid, 9.3 g of EDTA in 1 liter of 18-MΩ-distilled water), 28 ml of 18-MΩ-distilled water, 75 μl of *N,N,N′, N′*-tetramethylenediamine and 350 μl of 10% ammonium persulfate (Sigma, Poole, United Kingdom). A 0.2-μl aliquot of each sample was loaded manually onto a 96-well gel using a multichannel gel loading syringe (Hamilton, Carnforth, United Kingdom). The gel was run at 2,000 V, 35 mA, and 45 W with 1 liter of TBE running buffer for 5 h on the Licor L4200-L2 DNA sequencer (MWG-Biotech).

Sequence interpretation for MLST gene fragments. The sequence data was automatically read from the Licor sequencer using the integrated image analysis and data collection software. Each gene sequence was downloaded onto the BLAST nucleotide sequence search engine (http://www.ncbi.nlm.nih.gov/BLAST/) (1). After sequence comparisons and appropriate editing, the MLST fragment was downloaded onto the MLST website (http://mlst.zoo.ox.ac.uk/), where a sequence type was recovered from a combination of allelic numbers from each gene. When sequence data resulted in a new allele or new combination of alleles, data were sent to the Centre for the Epidemiology of Infectious Disease at the University of Oxford. A new allele number was provided for the appropriate gene sequence and a new sequence type number was assigned to the new allele combination. The MLST database was updated with all new variants.

Sequence interpretation of *porA* gene fragments. The sequence data were automatically read from the Licor sequencer as described above. Each sequence was downloaded onto the BLAST nucleotide search engine (http://www.ncbi.nlm.nih.gov/BLAST/) (1). After sequence confirmation and editing, the *porA*

TABLE 1. PCR amplification and sequencing primers used for MLST

Associated gene	Primer sequence	Reference
Amplification primers		
PorA-F	5′-ATGCGAAAAAAACTTACCGCCCTC-3′	27
PorA-R	5′-AATGAAGGCAAGCCGTCAAAAACA-3′	14
AbcZ-F	5′-AATCGTTTATGTACCGCAGG-3′	16
AbcZ-R	5′-GTTGATTTCTGCCTGTTCGG-3′	16
Adk-F	5′-ATGGCAGTTTGTGCAGTTGG-3′	16
Adk-R	5′-GATTTAAACAGCGATTGCCC-3′	16
AroE-F	5′-ACGCATTTGCGCCGACATC-3′	16
AroE-R	5′-ATCAGGGCTTTTTTCAGGTT-3′	16
FumC-F	5′-CACCGAACACGACACGATGG-3′	10
FumC-R	5′-ACGACCAGTTCGTCAAACTC-3′	10
Gdh-F	5′-ATCAATACCGATGTGGCGCGT-3′	16
Gdh-R	5′-GGTTTTCATCTGCGTATAGAG-3′	16
PdhC-F	5′-GGTTTCCAACGTATCGGCGAC-3′	16
PdhC-R	5′-ATCGGCTTTGATGCCGTATTT-3′	16
Pgm-F	5′-CTTCAAAGCCTACGACATCCG-3′	16
Pgm-R	5′-CGGATTGCTTTCGATGACGGC-3′	16
Sequencing primers		
PorA-F	5′-AACGGATACGTCTTGCTC-3′	27
PorA-R	5′-TCCGTACGCTACGATTCTCC-3′	14
AbcZ-F	5′-GAGAACGAGCCGGGATAGGA-3′	16
AbcZ-R	5′-GAGAACGAGCCGGGATAGGA-3′	16
Adk-F	5′-AGGCTGGCACGCCCTTGG-3′	16
Adk-R	5′-CAATACTTCGGCTTTCACGG-3′	16
AroE-F	5′-GCGGTCAACYTACGCTGATT-3′	16
AroE-R	5′-ATGATGTTGCCGTACACATA-3′	16
FumC-F	5′-TCGGCACGGGTTTGAACAGC-3′	10
FumC-R	5′-CAACGGCGGTTTCGCGCAAC-3′	10
Gdh-F	5′-CCTTGGCAAAGAAAGCCTGC-3′	16
Gdh-R	5′-GCGCACGGATTCATATGG-3′	16
PdhC-F	5′-TCTACTACATCACCCTGATG-3′	16
PdhC-R	5′-ATCGGCTTTGATGCCGTATTT-3′	16
Pgm-F	5′-CGGCGATGCCGACCGCTTGG-3′	16
Pgm-R	5′-GGTGATGATTTCGGTTGCGCC-3′	16

gene sequence was converted from its nucleotide sequence into an amino acid sequence by using the Translate program (http://expasy.cbr.nrc.cu/tools/dna.html). The VRs were identified, and VRs 1 and 2 were downloaded onto the *porA* website (http://mlst.zoo.ox.ac.uk/porA-vr/), where a variant number was assigned. New variants were again sent to the Centre for the Epidemiology of Infectious Disease at the University of Oxford. The *porA* database was updated with all new variants only after detailed checks of all data collected and appropriate tests were repeated. The variant number for VR3 was compared to data generated by Riesbeck et al. to provide the variant number (20).

RESULTS

Phenotypic characterization. All 45 isolates were confirmed as *N. meningitidis*, and phenotypic results showed that these were characterized as serogroup C, serotype 2a (Table 2). The sero-subtypes of the strains varied according to the expressed PorA subtype but included P1.2, P1.5, P1.2,5 and P1.10. Four strains were not sero-subtypeable.

Genotypic characterization. (i) Automation. The process using the RoboAmp-4200 system with a washable tip took approximately 8 h starting from culture supernatant to labeled sequence product. The sequence data created with the Licor L-4200 system took a further 6 h from gel loading to complete sequence analysis and production of sequence and variant types. The semiautomated MLST method was found to be efficient and reproducible and removed the laborious nature of this technique. Typically, the PCR setup and sequence labeling procedures were performed overnight by the liquid-handling robot. Gel loading, electrophoresis, and sequence analysis were then performed during the day, such that full MLST data could be completed within a 24-h period. When and if required, the liquid-handling robot could set up a maximum of 12 cultures on one run so that samples were available for running two sequencing gels per day, thereby providing MLST results at a maximum output of six cultures per day.

(ii) MLST. The automated MLST and *porA* gene sequencing procedure, which consists of the sequencing of eight independent genes, was applied for the first time to all *N. meningitidis* C:2a strains received by the SMPRL during the year 2000. Genotypic data are shown in Table 2. The STs gained from MLST were reflective of the phenotypic results for all isolates. In fact, all strains, irrespective of sero-subtype, were ST 11, and this matched with the phenotypic data, as many C:2a strains are known to be of the ST 11 lineage. Further discrimination among the cases was apparent from the nucleotide sequences of VRs 1, 2, and 3 of the *porA* gene, which determine the sero-subtype of the organism. *porA* analysis enabled differentiation of strains that appeared similar by standard phenotypic methods. For example, C:2a:P1.5 strains identified phenotypically were of VR types 5-1, 10-4, 36b or 5-1, 10-8, 36b. PorA analysis also led to the identification of one new sero-subtype (Table 2).

TABLE 2. MLST and *porA* gene sequencing results for 45 *N. meningitidis* C:2a isolates

Isolate no.	Isolate type	Phenotypic characterization			Allelic profile	MLST type	VR		
		Serogroup	Serotype	Sero-subtype			1	2	3
1	Blood	C	2A	P1.5	2343846	11	5-1	10-8	LLGSGSDG[b]
2	Blood	C	2A	P1.10	2343846	11	5-1	10	36b
3	Blood	C	2A	NST[a]	2343846	11	5-4	10-8	36b
4	CSF	C	2A	P1.2,5	2343846	11	5	2	36b
5	CSF	C	2A	P1.5	2343846	11	5	2	36b
6	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
7	Throat	C	2A	P1.5	2343846	11	5-1	10-4	36b
8	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
9	Eye	C	2A	P1.5	2343846	11	5-1	10-4	36b
10	Blood	C	2A	P1.2	2343846	11	5	2	36b
11	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
12	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
13	CSF	C	2A	P1.5	2343846	11	5-1	10-4	36b
14	CSF	C	2A	P1.5	2343846	11	5-1	10-4	36b
15	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
16	Blood	C	2A	P1.2,5	2343846	11	5	2	36b
17	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
18	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
19	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
20	Blood	C	2A	NST[a]	2343846	11	5-1	10-4	36b
21	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
22	Eye	C	2A	P1.5	2343846	11	5-1	10-4	36b
23	Blood	C	2A	P1.2	2343846	11	5	2	36b
24	Blood	C	2A	P1.2,5	2343846	11	5	2	36b
25	Blood	C	2A	P1.2,5	2343846	11	5	2	36b
26	Skin	C	2A	P1.2, P1.5	2343846	11	5	2	36b
27	Blood	C	2A	P1.5	2343846	11	5-1	10-8	36b
28	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
29	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
30	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
31	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
32	Throat	C	2A	P1.5	2343846	11	5-1	10-4	36b
33	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
34	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
35	CSF	C	2A	P1.2,5	2343846	11	5	2	36b
36	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
37	Blood	C	2A	NST[a]	2343846	11	5-1	10-4	36b
38	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
39	Sputum	C	2A	P1.2,5	2343846	11	5	2	36b
40	Shoulder	C	2A	P1.5	2343846	11	5-1	10-4	36b
41	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
42	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
43	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
44	Blood	C	2A	P1.5	2343846	11	5-1	10-4	36b
45	Blood	C	2A	NST[a]	2343846	11	5-1	10-4	36b

[a] NST, nonsubtypeable with monoclonal antibodies.
[b] This VR3 is a new variant not previously documented.

DISCUSSION

Both short-term and long-term epidemiological studies depend on reliable typing methods that can be used to determine bacterial spread between individuals (2, 30). Outbreaks of infectious disease often result from exposure to a common source of the etiologic agent, and generally this source causing the outbreak is derived from a single cell whose progeny are genetically identical or closely related to the source organism (19). Because of this, different outbreaks from different sources at different times and in different geographical locations may be differentiated using modern sequence typing methods. Previous studies have highlighted the reliability of MLST in comparison with such techniques as MLEE. The

SMPRL has taken this one step further and incorporated a rigid, reliable, and reproducible semiautomated MLST service to sequence all routine isolates referred to the reference laboratory from Scotland.

There have been many advantages highlighted from using a sequence-based system for typing organisms such as *N. meningitidis* (9, 16, 26). The use of MLST and *porA* gene sequencing integrated within a semiautomated setup gives greater attractiveness to the method than the equivalent manual system. Manual setup of MLST is time-consuming, laborious, and tedious and must be performed during normal working hours unless some form of automation or shift working is used. Due to the repetitive pipetting, the potential for errors in liquid handling is increased. Automation of MLST provides an efficient, accurate, reproducible, and faster method than the manual procedures. This allows time to be spent analyzing data for immediate public health management and long-term epidemiology of MD.

Although DNA sequencing and therefore MLST are currently relatively expensive (17), DNA sequencing can be used effectively for the national surveillance of meningococcal disease and other important bacterial infections (5). However, due to the cost of such testing, automated MLST is probably only cost-effective in national reference laboratories or large clinical laboratories where a high throughput can justify the cost. In the future though, it is expected that routine clinical laboratories will gain access to DNA sequencing as costs continue to fall.

Although MLST and *porA* sequencing have been used to differentiate meningococcal strains in case clusters (10, 11, 20), this is the first time to our knowledge that a semiautomated sequencing system has been described and subsequently used as a national service on clinical isolates of *N. meningitidis*. This semiautomated setup can be used to characterize isolates in a timely manner so that results can be obtained within the time scale required for public health management. It can produce an ST based on all seven MLST gene fragments plus all three variable regions within the *porA* gene within a 24-h period. The throughput capacity with our current system allows six bacterial isolates to be typed by MLST per day, although higher-throughput liquid-handling robots and DNA sequencers are available.

While it is not the purpose of this work to analyze the data gained from performing MLST on all samples received by a national reference laboratory in 1 year, already this method has indicated the sequence variability of strains that appear identical by traditional phenotypic typing methods. This study has demonstrated that MLST and *porA* gene sequencing can be automated and introduced as a national typing service for both short- and long-term public health intervention.

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REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Andraghetti, R., M. George, S. Clarke, P. Christie, and I. G. Jones.** 2000. Investigation of an apparent outbreak of meningitis in the Western Isles. SCIEH Wkly. Rep. **34:**106–108.
3. **Borrow, R., H. Claus, U. Chaudhry, M. Guiver, E. B. Kaczmarski, M. Frosch, and A. J. Fox.** 1998. *SiaD* PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections. FEMS Microbiol. Lett. **159:**209–214.
4. **Borrow, R., H. Claus, M. Guiver, L. Smart, D. M. Jones, E. B. Kaczmarski, M. Frosch, and A. J. Fox.** 1997. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialyltransferase (*SiaD*) PCR ELISA. Epidemiol. Infect. **118:**111–117.
5. **Clarke, S. C., M. A. Diggle, J. A. Reid, L. Thom, and G. F. S. Edwards.** 2001. Introduction of an automated service for the laboratory confirmation of meningococcal disease in Scotland. J. Clin. Pathol. **54:**556–557.
6. **Clarke, S. C., S. Brownlie, B. C. Denham, J. A. Reid, L. Thom, P. Christie, and G. F. S. Edwards.** 2001. Meningococcal disease in Scotland 1999. SCIEH Wkly. Rep. **35:**130–133.
7. **Eldridge, J., E. M. Sutcliffe, J. D. Abbott, and D. M. Jones.** 1978. Serological grouping of meningococci and detection of antigen in cerebrospinal fluid by coagglutination. Med. Lab. Sci. **35:**63–66.
8. **Ende, A. V. D., C. T. P. Hopman, and J. Dankert.** 1999. Detection of *porA* by recombination between clusters of repetetive extragenic palindromic sequences in *Neisseria meningitidis*. Infect. Immun. **67:**2928–2934.
9. **Enright, C. E., and B. G. Spratt.** 1999. Multilocus sequence typing. Trends Microbiol. **7:**482–487.
10. **Feavers, I. M., A. J. Fox, S. Gray, D. M. Jones, and M. C. J. Maiden.** 1999. Antigenic diversity of meningococcal outer membrane protein *PorA* has implications for epidemiology analysis and vaccine design. Clin. Diagn. Lab. Immunol. **3:**444–450.
11. **Feavers, I. M., S. J. Gray, R. Urwin, J. E. Russel, J. A. Bygraves, E. B. Kaczmarski, and M. C. J. Maiden.** 1999. Multilocus sequence typing and antigen sequencing in the investigation of a meningococcal disease outbreak. J. Clin. Microbiol. **37:**3883–3887.
12. **Feil, E. J., M. C. J. Maiden, M. Achtman, and B. G. Spratt.** 1999. The relative contributions of recombination and mutation to the divergence of clones of *Neisseria meningitidis*. J. Mol. Biol. Evol. **11:**1496–1502.
13. **Frasch, C. E., W. D. Zollinger, and J. T. Poolman.** 1985. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev. Infect. Dis. **7:**504–509.
14. **Maiden, M. C., J. A. Bygraves, J. McCarvil, and I. M. Feavers.** 1992. Identification of meingococcal serosubtypes by polymerase chain reaction. J. Clin. Microbiol. **30:**2835–2841.
15. **Maiden, M. C. J., J. Suker, A. J. McKenna, J. A. Bygraves, and I. M. Feavers.** 1991. Comparison of the class 1 outer membrane proteins of eight serological reference strains of *Neisseria meningitidis*. Mol. Microbiol. **5:**727–736.
16. **Maiden, M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russel, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
17. **Olive, M. D., and P. Bean.** 1999. Principles and applications of methods for DNA-based typing of microbial organisms. J. Clin. Microbiol. **37:**1661–1669.
18. **Popovic, T., C. T. Sacchi, M. W. Reeves, A. M. Whitney, L. W. Mayer, C. A. Nobel, G. W. Ajello, F. Mostashari, N. Bendana, J. Lingappa, R. Hajjeh, and N. E. Rosenstein.** 2000. *Neisseria meningitidis* serogroup W135 associated with ET-37 complex. Emerg. Infect. Dis. **4:**428–429.
19. **Reeves, M. W., A. P. Bradley, M. Diermayer, and J. D. Wenger.** 1995. Epidemic-associated *Neisseria meningitidis* detected by multilocus enzyme electrophoresis. Emerg. Infect. Dis. **2:**53–54.
20. **Riesbeck, K., P. Orvelid-Mölling, H. Fredlund, and P. Olcén.** 2000. Long-term persistence of a discotheque-associated invasive *Neisseria meningitidis* group C strain as proven by pulse-field gel electrophoresis and *porA* gene sequencing. J. Clin. Microbiol. **38:**1638–1640.
21. **Sacchi, C. T., A. P. S. Lemos, M. E. Brandt, A. M. Whitney, C. E. A. Melles, C. A. Solari, C. E. Frasch, and L. W. Mayer.** 1998. Proposed standardization of *Neisseria meningitidis PorA* variable-region typing nomenclature. Clin. Diagn. Lab. Immun. **5:**845–855.
22. **Saunders, N. B., W. D. Zollinger, and V. B. Rao.** 1993. A rapid and sensitive PCR employed for amplification and sequencing of *porA* from a single colony-forming unit of *Neisseria meningitidis*. Gene **137:**153–162.
23. **Smart, L. E., P. Christie, S. C. Clarke, J. A. Reid, L. Thom, B. C. Denham, and G. F. S. Edwards.** 1999. Meningococcal disease in Scotland 1997. SCIEH Wkly. Rep. **33:**2–6.
24. **Smart, L. E., S. J. O'Brien, J. A. Reid, L. Thom, and R. W. A. Girdwood.** 1996. Meningococcal disease in Scotland 1995. SCIEH Wkly. Rep. **30:**138–140.
25. **Smart, L. E., S. J. O'Brien, J. A. Reid, L. Thom, and R. W. A. Girdwood.**

1996. Meningococcal disease in Scotland 1996. SCIEH Wkly. Rep. **31:**248–253.
26. **Spratt, B. G.** 1999. Multilocus sequence typing: molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the internet. Curr. Opin. Microbiol. **2:**312–316.
27. **Suker, J., I. M. Feavers, M. Achtman, G. Morelli, J.-F. Wang, and M. C. J. Maiden.** 1994. The *porA* gene in serogroup A meningococci: Evolutionary stability and mechanism of genetic variation. Mol. Microbiol. **12:**253–265.
28. **Vogel, U., H. Claus, M. Frosch, and D. A. Caugant.** 2000. Molecular basis for detection of the ET-15 clone within the ET-37 complex of *Neisseria meningitidis*. J. Clin. Microbiol. **38:**941–942.
29. **Vogel, U., G. Morelli, K. Zurth, H. Claus, E. Kriener, M. Achtman, and M. Frosch.** 1998. Necessity of molecular techniques to distinguish between *Neisseria meningitidis* strains isolated from patients with meningococcal disease and from their healthy contacts. J. Clin. Microbiol. **36:**2465–2470.
30. **Williams, D. W., M. J. Wilson, and M. A. O. Lewis.** 1999. Deoxyribonucleic acid typing methods for medically important microorganisms. Br. J. Biomed. Sci. **56:**56–65.