Irish strains of *Neisseria meningitidis*: characterisation using multilocus sequence typing

K.M. MURPHY, K.A. O'DONNELL, A.B. HIGGINS, C. O'NEILL and M.T. CAFFERKEY*

*Irish Meningococcal and Meningitis Reference Laboratory, The Children's University Hospital, Temple Street, Dublin 1; and *Royal College of Surgeons in Ireland, York Street, Dublin 2, Ireland.*

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ABSTRACT

A total of 56 *Neisseria meningitidis* strains are analysed using multilocus sequence typing (MLST). Twenty-nine distinct sequence types (STs) were identified, eight of which were new. Four known hypervirulent clones – ST-11 (electrophoretic type [ET]-37) complex, ST-44 complex (lineage 3), ST-32 (ET-5) complex and ST-8 complex (cluster A4) – were identified by MLST in 35 disease-associated and four carrier strains. Two other clones (ST-22 complex and ST-269 complex) were identified in nine disease-associated and one carrier strain. The remaining strains were heterogeneous. Additional sequencing within the *FumC* gene further distinguished the ET-15 clone within the ST-11 (ET-37) clonal complex. This resolution of isolates into genetic clones by MLST enhances the more traditional techniques of serotyping and serosubtyping. The data obtained established that hyperendemic meningococcal disease in Ireland could be attributed to strains belonging to four major hypervirulent clones, all of which account for elevated levels of disease worldwide. The extra information provided by MLST will be used to study the population structure and epidemiology of *N. meningitidis* and will allow a comparison of Irish strains with those circulating globally.

KEY WORDS: Bacterial typing techniques.
Meningococcal infection.
Molecular sequence data.
Neisseria meningitidis.
Sequence analysis.

Introduction

Neisseria meningitidis is a significant global pathogen that causes invasive infection. Invasive meningococcal disease (IMD) is hyperendemic in Ireland, with a laboratory-confirmed invasive infection rate of 13/100,00 population in the epidemiological year 1999/2000.[1] This represents one of the highest rates of meningococcal disease in western Europe, where the disease is usually associated with a small number of hypervirulent lineages of serogroup B or C.

Analysis by multilocus enzyme electrophoresis (MLEE) has been used to subdivide meningococcal isolates of all groups into electrophoretic types (ETs).[2,3] In particular, most cases of serogroup C disease are caused by ET-37 complex meningococci and derivatives of the cluster A4, whereas serogroup B disease is most frequently caused by meningococci of the lineage 3 or ET-5 complex.[4]

IMD has many epidemiological manifestations, each of which can be associated with different meningococcal clones. Epidemics and outbreaks are usually associated with organisms from uniform clonal groups, whereas sporadic disease is more likely to be associated with a more diverse group of organisms. Detailed characterisation of *N. meningitidis* isolates into their correct clonal groups is essential in order to investigate outbreaks, to identify virulent strains, and to trace the genetic links between isolates.

The typing methods currently in use, such as serological typing, MLEE and pulsed-field gel electrophoresis (PFGE), have proved unsuitable for rapid and sensitive typing of meningococci, highlighting the need for improved molecular typing methods. Multilocus sequence typing (MLST), an adaptation of MLEE, has become a widely used method that unambiguously defines relatedness among disease and carriage isolates.[5-10] In addition, MLST has identified differences between strains that other molecular methods have failed to detect.[11]

MLST is based on DNA sequence variation in specific regions of seven housekeeping genes that range from 433–501 bp in length. For each gene fragment, different sequences are assigned as distinct alleles, and each isolate is defined by the combination of alleles at each of seven housekeeping loci. This is known as the allelic profile or sequence type (ST). The STs were assigned to lineages using the BURST programme (http://neisseria.mlst.net).

Within a clonal complex, additional sequencing may be required to further distinguish between clones. One group C clone of the ST-11 (ET-37) complex, referred to as ET-15, is a particularly virulent clone that shows high attack and fatality rates.[12,13] MLEE identifies ET-15 meningococci by detecting a point mutation at position 640 within the *FumC* gene.[14] The original *FumC* gene fragment commonly sequenced by MLST fails to include this mutation; therefore, further sequencing is required to detect the $fumC_{640}$ A substitution.

This study aims to utilise MLST to identify and differentiate between the major clones of *N. meningitidis* circulating in Ireland. In the case of the ET-37 complex, additional sequencing to detect the $fumC_{640}$ A substitution is performed. This is the first comprehensive, standardised molecular characterisation of Irish meningococcal isolates.

Correspondence to: Ms. K.M. Murphy
Email: karen.murphy@tsch.ie

Materials and methods

Bacterial strains

In total, 56 *N. meningitidis* isolates were selected for inclusion in this study. All had been received at the Irish Meningococcal and Meningitis Reference Laboratory (IMMRL) between 1997 and 2000. The study included 50 isolates from cases of epidemiologically unrelated IMD and six carrier isolates.

The disease-associated strains comprised 22 patient isolates of group C, 23 group B, three group W135, and one each of group X and group Y. The carrier isolates were four group B, one group C and one non-groupable. Details of these isolates are shown in Table 1.

The case isolates were selected to represent the most common phenotypes causing disease in Ireland. The majority of disease-associated isolates (43/50) included in the study were from one year (1999), accounting for 31% of disease-associated isolates submitted to the IMMRL in that year. This included 25%, and 37% of disease-associated group B and group C isolates, respectively. The additional isolates were chosen to cover the range of disease-associated phenotypes.

Isolates with these phenotypes have caused increased levels of disease worldwide and consequently many have been characterised by MLST. This permitted direct comparison of the results obtained with data already published and available on the website database (http://neisseria.mlst.net).

Among the six carrier isolates, three (two B:4:P1.4 and one B:NT:P1.9 phenotype) were collected from three family members who were related to, and in contact with, a polymerase chain reaction (PCR)-confirmed case (group B) where no isolate was available. The remaining three carrier isolates were phenotypically diverse and were epidemiologically unrelated to the case isolates.

Two well-characterised reference strains, H44/76[6] (of known MLEE and MLST type) and C11 (http://neisseria.mlst.net), were used to establish the MLST technique.[15]

Serological typing

Serogroups were determined by slide agglutination and co-agglutination[16] with A, B, C, W135, X, and Y monoclonal antisera (Murex Biotech). The types and subtypes were determined by a modified dot-blot method using a set of serotype- and serosubtype-specific antibodies (NIBSC, Potters Bar, UK), as described previously.[17]

Multilocus sequence typing

Chromosomal DNA was extracted from *N. meningitidis* grown overnight on chocolate agar at 37°C in 5% CO_2 by the Isoquick NA extraction procedure (Orca Research Inc). The primers and protocols for the amplification and sequencing of the seven housekeeping genes (*Abc-Z, Adk, AroE, FumC, Gdh, PdhC* and *Pgm*) were as listed on the MLST website (http://neisseria.mlst.net).

Additional sequencing was carried out at the fumarase locus on all ST-11 (ET-37) complex strains using the following primer: 5′-CGTAAAAGCCCTGCGCGAC-3′. This primer detects the G to A substitution at position 640 of the *FumC* gene[14], a clone-specific characteristic that permits the distinction of ET-15 from other ET-37 complex strains.

PCR products of the seven housekeeping genes were prepared for sequencing using the High Pure PCR product purification kit (Roche Diagnostics), following the manufacturer's instructions, or using a standard polyethylene glycol/ethanol PCR product purification method (PE Applied Biosystems ABI 310 manual).

Sequencing reactions were performed with the BigDye terminator cycle sequencing kit (PE Applied Biosystems), and the extension products were separated and detected with an ABI Prism 310 automated sequencer (PE Applied Biosystems).

Sequence assembly and analysis

Forward and reverse sequences were determined for each gene and assembled using the Sequencher (Genecode Corp) sequence analysis package. Consensus sequences were submitted to the MLST website where allele numbers were assigned.

Once an allelic number was available for each gene, the allelic profile was submitted and a multilocus sequence type was obtained automatically from the MLST database. Where a new allele or allelic profile was described, the authors contacted the curator of the MLST website to obtain a new allele or ST number. Complexes are named after the central genotype, as described previously.[5,18] The MLST database uses an automated script to assign STs to clonal complexes, based on similarity to previously identified 'central genotypes' at five or more loci.

The STs were further analysed and assigned to lineages using the BURST (Based Upon Related Sequence Types) algorithm (E.J. Feil) available within the START (Sequence Type Analysis And Recombinational Tests) software package (K. Jolley, University of Oxford, UK – available from http://neisseria.mlst.net). This contains a selection of tools that can be used for analysis of MLST data.

BURST is a special clustering algorithm for use with microbial MLST data that examines the relationships within clonal complexes, while ignoring the relationships between different clonal complexes. In this instance, BURST defines a lineage or clone as a group of strains where the members share at least four alleles with at least one other member of that lineage.

Results

Sequence type and lineage assignment

Case isolates: Among the 50 isolates studied, 26 STs were identified, including five new ones. Database analysis grouped the isolates into six clonal complexes and six single STs. Thirty-five of these strains were confirmed as belonging to known major hyperinvasive genetic clones, with 17, seven, eight and three isolates being assigned to the ST-11 (ET-37), ST-32 (ET-5), ST-44 (lineage 3), and ST-8 (cluster A4) complexes, respectively.

The B isolates were divided into three distinct clones, namely the ST-44 (lineage 3), ST-32 (ET-5), and ST-269 complexes. ST-44 (lineage 3) complex contained eight members and included six B:4:P1.4 (STs-154, -41 -482 and -1192) isolates, and one each of phenotype B:4:P1.7 (ST-154) and B:NT:NT (ST-136). In addition, BURST assigned the

Table 1. Molecular and serological typing results of Irish *Neisseria meningitidis* strains.

Strain number	Year of isolation	Source	Serological type	Allelic profile							ST	*FumC* variant	MLEE	MLST classification‡
				abc	adk	aro	fum	gdh	pdh	pgm				
3098	99	Case	B:4:P1.4	118	6	9	5	11	6	9	1192*		Lineage 3	ST-44 complex
3100	99	Case	B:4:P1.4	3	6	9	17	9	6	9	482		Lineage 3	ST-44 complex
3122	99	Case	B:4:P1.4	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3140	99	Case	B:4:P1.4	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3148	99	Case	B:4:P1.4	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3150	99	Case	B:4:P1.4	3	6	9	5	9	6	9	41		Lineage 3	ST-44 complex
3134	99	Case	B:4P1.7	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3014	99	Case	B:NT:NT	27	6	9	3	9	6	16	136		Lineage 3	ST-44 complex
3081	99	Case	B:4:P1.3,6	3	6	34	5	11	6	9	1194†			
1170	97	Case	B:15:P1.7,16	4	10	5	40	6	3	8	259		ET-5 complex	ST-32 complex
3071	99	Case	B:15:P1.7,16	4	10	5	4	6	3	8	32		ET-5 complex	ST-32 complex
3086	99	Case	B:4:P1.15	8	10	5	4	6	3	8	33		ET-5 complex	ST-32 complex
3125	99	Case	B:4:P1.15	8	10	5	4	6	3	8	33		ET-5 complex	ST-32 complex
3089	99	Case	B:4:P1.15	8	10	5	4	5	3	8	34		ET-5 complex	ST-32 complex
3082	99	Case	B:NT:P1.9	4	10	15	9	8	5	9	1195			ST-269 complex
3017	99	Case	B:NT:P1.15	4	10	15	9	8	11	9	269			ST-269 complex
3152	99	Case	B:NT:P1.15	4	10	15	9	8	11	9	269			ST-269 complex
3155	99	Case	B:NT:P1.15	4	10	15	9	8	11	9	269			ST-269 complex
3047	99	Case	B:NT:P1.15	4	10	15	9	8	11	17	283*			ST-269 complex
3016	99	Case	B:NT:P1.5,2	4	10	15	17	8	11	17	479			ST-269 complex
3078	99	Case	B:1:P1.14	7	5	1	13	36	53	15	213			
3025	99	Case	B:NT:P1.5,2	17	5	19	17	3	26	2	60			
3037	99	Case	B:2b:P1.3,6	2	5	15	17	5	24	16	1193			
3170	99	Case	C:2a:NT	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3191	99	Case	C:2a:NT	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3184	99	Case	C:2a:NT	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex
3142	99	Case	C:2a:NT	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex
12080	2000	Case	C:2a:NT	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3039	99	Case	C:2a:P1.10	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3087	99	Case	C:2a:P1.2	2	3	4	3	8	4	6	11*	non ET-15	ET-37 complex	ST-11 complex
3067	99	Case	C:2a:P1.5	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3185	99	Case	C:2a:p1.5	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3125	98	Case	C:2a:P1.5	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex

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B:4:P1.3,6 (ST-1194) isolate to this group, which differed from most of these isolates at the *FumC* locus alone.

The ST-32 (ET-5) complex contained five members, which included three B:4:P1.15 (ST-33 and -34) and two B:15:P1.7,16 (ST-32 and -259) isolates. The ST-269 complex contained six isolates, which included four B:NT:P1.15 (ST-269 and -283), one B:NT:P1.9 (ST-1195) and one B:NT:P1.5,2 (ST-479) isolate.

The C isolates were assigned to three distinct clones, namely the ST-11 (ET-37), ST-8 (cluster A4) and ST-32 (ET-5) complexes. The ST-11 (ET-37) complex contained 16 isolates with an identical allelic profile, which included 15 C:2a and one C:NT:NT strain. Additional sequencing of the *FumC* locus revealed that nine of the 17 (53%) isolates had the $fumC_{640}$ substitution and were therefore designated as ET-15 variant isolates.

Three C:2b:P1.5,2 isolates of three different STs (-1210, -48 and -8) were assigned to the ST-8 complex (cluster A4). In addition, two C:4:P1.10 isolates were identified as ST-34, which is usually associated with the ST-32 (ET-5) complex. BURST analysis indicated that they were more closely related to the group B isolates.

Three W135 isolates with two different STs (-1158 and -22) were assigned to the ST-22 complex. Of the five remaining isolates, four had diverse phenotypic characteristics (one each of B:1:P1.14; B:2b:P1.3,6; X:21:P1.16 and Y:NT:P1.5) and were not expected to be related to any of the other isolates.

Finally, ST-60 had the same B:NT:P1.5,2 phenotype as ST-479, which was assigned to the ST-269 complex; however, these two isolates shared only one identical allele (at the *FumC* loci) and are unlikely to be related.

Carrier isolates: The two B:4:P1.4 (ST-154) and one B:NT:NT (ST-1213) isolates were grouped into the ST-44 complex (lineage 3) and the B:NT:P1.9 (ST-1273) isolate was assigned

Table 1 (continued). Molecular and serological typing results of Irish *Neisseria meningitidis* strains.

Strain number	Year of isolation	Source	Serological type	Allelic profile							ST	*FumC* variant	MLEE	MLST classification‡
				abc	adk	aro	fum	gdh	pdh	pgm				
Continued														
3049	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex
3073	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex
3092	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	non ET-15	ET-37 complex	ST-11 complex
3124	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3126	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3144	99	Case	C:2a:P1.5,2	2	3	4	3	8	4	6	11	ET-15	ET-37 complex	ST-11 complex
3047	98	Case	C:NT:NT	2	3	4	3	8	3	6	574	non ET-15	ET-37 complex	ST-11 complex
3048	99	Case	C:2b:P1.5,2	3	3	7	17	8	5	2	1210		Cluster A4	ST-8 complex
3154	98	Case	C:2b:P1.5,2	2	3	7	9	8	5	2	487		Cluster A4	ST-8 complex
12151	2000	Case	C:2b:P1.5,2	2	3	7	2	8	5	2	8		Cluster A4	ST-8 complex
3085	99	Case	C:4:P1.10	8	10	5	4	5	3	8	34		ET-5 complex	ST-32 complex
3096	99	Case	C:4:P1.10	8	10	5	4	5	3	8	34		ET-5 complex	ST-32 complex
3090	99	Case	W135:NT:P1.3,6	11	5	18	15	11	24	21	1158			ST-22 complex
3136	99	Case	W135:NT:NT	11	5	18	15	11	24	21	1158			ST-22 complex
3182	99	Case	W135:NT:P1.3,6	11	5	18	8	11	24	21	22			ST-22 complex
3035	98	Case	X:21:P1.16	2	5	2	9	15	20	5	750			
3147	99	Case	Y:NT:P1.5	2	7	6	17	16	18	8	167			
3118	99	Carrier	B:4:P1.4	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3108	99	Carrier	B:4:P1.4	3	6	9	5	11	6	9	154		Lineage 3	ST-44 complex
3176	99	Carrier	B:NT:NT	27	6	9	9	9	6	16	1213		Lineage 3	ST-44 complex
3109	99	Carrier	B:NT:P1.9	4	10	64	5	38	11	9	1273			ST-269 complex
3183	99	Carrier	C:2a:P1.5,2	2	3	4	3	8	4	6	11	non ET-15	ET-37complex	ST-11 complex
3007	99	Carrier	NG:NT:P1.5	2	7	6	31	16	18	8	1212			
H44/76		Control	B:15:P1.7,16	4	10	5	4	6	3	8	32		ET-5 complex	ST-32 complex
C11		Control	C:16:P1.7,1	7	5	2	72	3	75	21	344			

* These cases grew *N. meningitidis* with a similar phenotype and identical ST to the initial isolate at a 2nd site.

They were tested separately but considered as one isolate for the purpose of this study

† BURST assigned this B:4:P1.3,6 (ST-1194) isolate to the ST-44 complex (lineage 3).

‡ MLST database classification.

to the ST-269 complex. The C2a:P1.5,2 (ST-11) isolate was assigned to the ST-11 (ET-37) complex (non ET-15). The remaining NG:NT:P1.5 isolate was assigned a new single ST (-1212). No link was observed between the B:4:P1.4 and B:NT:P1.9 isolates collected from the family that had been in contact with the PCR-confirmed case.

Discussion

This is the first report to present molecular typing results on disease-associated and carrier Irish *N. meningitidis* isolates. To date, routine serological typing is performed at the IMMRL on all strains, with no further genetic characterisation routinely undertaken. However, serological typing is of limited value in the case of non-typeable isolates. Overall, there was high concordance between serological type and genetic type for all isolates examined (Table 1).

MLST analysis more accurately measures clonal relationships and genetic relatedness, and allowed further characterisation of strains that appeared either identical or similar phenotypically. The analysis, including 31% of disease-associated isolates from one year (1999), confirmed that four major hypervirulent lineages are present and cause disease in Ireland.

The MLST results confirm that group B disease in Ireland can be attributed to genetically diverse meningococci, which is consistent with endemic disease. The group B:P1.4 strain examined in this study is the most common B phenotype isolated from patients with IMD in Ireland, accounting for 51% of culture-confirmed B disease in the 1999/2000 epidemiological year.[19] All goup B:4 isolates were assigned to the ST-44 complex (lineage 3), but there was considerable variation in allelic profile.

On the database there is much variation within the ST44 complex, which contains a large number of different STs. As a clone becomes widespread in a population, sequence variation begins to occur in its genome and this leads to clonal divergence and a more diverse phenotypic profile within the lineage.[20,21] This can be seen within the ST-44 complex (lineage 3) isolates tested. This complex has been associated with an increased incidence of disease in

countries such as New Zealand, Belgium[21] and The Netherlands.[22]

BURST analysis assigned the B:4:P1.3,6 (ST-1194) isolate to this group, whereas the database did not. However, this ST has a similar allelic profile, sharing many of the same alleles with other members of this complex. STs have been reclassified on the database as more MLST information becomes available; thus, ST-1194 may be reassigned to this complex in the future.

ST-32 (ET-5) complex remains one of the major hypervirulent clones, producing numerous outbreaks worldwide with a tendency to cause hyperendemic disease, especially in teenagers.[23] ST-32 (ET-5) complex strains have a propensity to cause septicaemia and thus there is a high fatality rate. As with ST-44 complex (lineage 3), ST-32 (ET-5) complex strains tend to be highly variable phenotypically, due to microevolution. Although the present study included only five of these isolates, they were phenotypically diverse, exhibiting two main type/subtype combinations.

This clone also contained two serogroup C isolates of 4:P1.10 subtype. This type/subtype combination is more likely to be associated with serogroup B, NG or A organisms.[24] The allelic profile of these two isolates was identical to that of a B:4:P1.15 isolate, suggesting that they are closely related and may have originated from a common ancestor. This finding reinforces a recent report of 15 isolates with unusual C phenotypes (4:P1.4).[25] Eight of these unusual group C isolates displayed STs that had previously been assigned to serogroup B lineage 3 meningococci. The phenomenon of strains with the same genetic background possessing different serogroups is suggestive of capsule switching – an escape mechanism that has been shown to occur in *N. meningitidis* under selective pressure.[26, 27]

There are many isolates listed for the ST-269 complex on the MLST database, with carriers and cases represented in equal numbers. Most are group B, non-typeable strains and include a number of different subtypes and allelic profiles (similar to the profile of the Irish strains tested). There is no documented MLEE designation for this recently described complex and it may represent a new hyperinvasive clone

This study confirms that most of the serogroup C disease in Ireland at this time is caused by ST-11 (ET-37) complex strains. In common with other Western countries, the ET-15 variant is the predominant clone.[12,13,28,29] Previous studies indicate that this clone appears to spread quite rapidly once introduced into a population.[12,29] In addition, possibly due to capsule switching, group B ET-15/37 strains have appeared in a number of countries.[27,30]

This further discrimination is essential to monitor the prevalence of this clone and to aid the epidemiological investigation of outbreaks of ET-37 complex meningococcal disease. This will be particularly important in countries such as Ireland in which a Group C meningococcal conjugate vaccination programme has been introduced.

The C:2b:P1.5,2 type is rare in Ireland, representing 2.2% (four) and 1.8% (three) cases of isolates grown from culture in 1999 and 2000, respectively (unpublished observations). MLST assigned these isolates to the ST-8 complex (A4 cluster), a cluster associated with epidemics and hyperendemic disease worldwide (mainly in young children) since its discovery in the 1960s.[4] The epidemiology and genotypic diversity of this clone requires further investigation.

On the MLST database most ST-22 complex isolates are group W-135:NT:+/-P1.3,6, with slightly more carriers represented than cases. The NT:P1.3,6 and NT:NT serotypes are the only W135 types identified in Ireland, representing 1.8% and 2.1% of culture confirmed cases in 1999 and 2000, respectively.[19] Again this complex has no MLEE designation and is recently described, possibly representing a new hyperinvasive complex. We have not encountered any of the Hajj-related W135:2a:P1.5,2 (ET-37) strains that have caused outbreaks worldwide.[31,32] However, we have identified W135:NT:NT and PCR-positive W135 cases and recognise the need to monitor and genetically type these isolates in order to detect any potential outbreak of this hypervirulent clone.

Six carrier isolates were included in this study. As expected, ST-44 complex (lineage 3) and ST-11 (ET-37) complex were identified in this group according to serotype. The new carrier strain ST-1273 was assigned to the ST-376 complex. More extensive carrier studies are currently in progress to elucidate the population biology of these strains and enable a genetic comparsion of disease and carrier isolates.

IMD has been hyperendemic in Ireland for some eightyears. PCR has had a major impact as it alone accounted for the diagnosis of 61% of all IMD cases in 2002 (unpublished observations). This has resulted in a large number of cases where no isolates are available for characterisation, which highlights the need for a molecular typing method that can be applied directly to clinical specimens.[33-35] MLST can also be extended to include the sequencing of the highly variable PorA and PorB antigen genes, which are compatible with the serological typing system presently in use.[10,11,13,34,35] The ability of MLST to classify strains comprehensively will allow accurate measurement of the biological impact and clinical effectiveness of the serogroup C and any future serogroup B vaccination programmes.

MLST has many advantages over the conventional typing techniques and the results of the present study confirmed that it is a reproducible, accurate and portable typing method. A major advantage of MLST is its ability to compare the results obtained in different laboratories. While sequencing is a relatively expensive technique, particularly compared to traditional serotyping and serosubtyping methods, technical advances continue to reduce the cost, and a number of laboratories have used robotics and high-throughput to substantially reduce the cost.[36]

The MLST data in the present report indicate that while the majority of Irish strains are genetically similar to other Western meningococcal populations, the identification of a number of new STs indicates some genetic diversity. This study allowed us to compare in detail and for the first time the epidemiological pattern and trends of meningococcal disease in Ireland with those occurring worldwide. □

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