Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit

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The laboratory confirmation of meningococcal disease and characterization of *Neisseria meningitidis* isolates was improved considerably in England and Wales by the Meningococcal Reference Unit between epidemiological years 1993/94 and 2003/04 to meet the challenge of increasing numbers of cases of clinical disease and the requirement for enhanced surveillance. Improved case ascertainment was made possible by the rapid introduction of an innovative centralized reference service for non-culture PCR-based DNA detection of meningococci utilizing the *ctrA* and *siaD* PCR assays, complemented by consistent phenotypic characterization of submitted isolates from culture-proven cases. This allowed the increased prevalence of serogroup C disease in specific age groups and the apparent associated increase in mortality from 1995/96 to 1999/00 to be defined, thereby prompting accelerated intervention with the newly licensed meningococcal serogroup C conjugate (MCC) vaccines into the under-25-year UK population (in November 1999). The continued increase in and predominance of serogroup B cases (1993/94 to 2000/01) were observed in conjunction with their diverse and changing phenotypic characteristics. Trends observed to be associated with the predominant phenotypic combinations of serogroup, serotype and sero-subtype were: a decline of both C : 2b and B : 2b meningococci, and a decline of B : 15 : P1.7,16 with a concomitant increase of B : 4 : P1.4 over the 11-year period. Detailed routine surveillance rapidly confirmed the introduction of W135 : 2a : P1.5,2 meningococci into the UK during 2000 and 2001. The importance of continued detailed surveillance of this important pathogen cannot be overestimated, both to monitor the effectiveness of the MCC vaccine and to identify changes within the meningococcal population that can inform the design of anti-serogroup B vaccines.

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INTRODUCTION

Isolates and clinical specimens from cases of meningococcal disease referred to the Meningococcal Reference Unit (MRU) of the Health Protection Agency (HPA) form an essential component in the surveillance of meningococcal disease in England and Wales. Although meningococcal disease is a relatively rare infection, the presentation and high case fatality continue to keep the disease prominent in the public perception. Surveillance of confirmed meningococcal disease, including surveillance of the diversity of

causative strains, is essential to aid cluster management and to inform vaccine development. Over the past decade there have been important changes in the methods for diagnosis and characterization of the organism, and also in the epidemiology of the disease itself. We review the changes in laboratory surveillance methods and the trends in epidemiology detected by the HPA MRU over an 11-epidemiological-year period from 1993/94 to 2003/04.

METHODS

Laboratory surveillance

Laboratory confirmation of meningococcal disease classically requires the isolation of the organism from one or more normally

Abbreviations: CFR, case fatality ratio; CSF, cerebrospinal fluid; HPA, Health Protection Agency; MCC, meningococcal serogroup C conjugate; MRU, Meningococcal Reference Unit; NT, non-typeable; OMP, outer-membrane protein.

sterile body sites, primarily cerebrospinal fluid (CSF) or blood. *Neisseria meningitidis* isolates are received at the MRU from laboratories in England and Wales for species confirmation and phenotypic characterization using biochemical and serological techniques (Rosenqvist *et al.*, 1990; Wedege *et al.*, 1990; Kuipers *et al.*, 2001; <http://neisseria.org/nm/typing/mabs/panel.shtml>). In the early 1990s, the number of clinically diagnosed cases of meningococcal disease reported by statutory notifications began to exceed the number of laboratory-confirmed cases. The problem of reduced case ascertainment by culture confirmation (Ramsay *et al.*, 1997), probably due to changes in clinical practice, such as a decrease in the number of lumbar punctures being performed (Richards & Towu-Aghantse, 1986) and the administration of antibiotics to patients before their admission to hospital (Bohr *et al.*, 1983; Cartwright *et al.*, 1992), was addressed by the development of PCR-based assays for the detection of meningococcal DNA (Guiver & Borrow, 2001). PCR testing was introduced as a confirmation test, to augment laboratory surveillance of meningococcal disease, in October 1996 (Davison *et al.*, 1996; Kaczmarek *et al.*, 1998). Detailed methods are given below.

(a) *N. meningitidis* isolate confirmation and characterization methods.

N. meningitidis isolates were received at the MRU from laboratories in England and Wales for species confirmation and phenotypic characterization using biochemical and serological techniques. Following subculture, the organisms were biochemically confirmed by means of cysteine tripticase agar oxidative carbohydrate utilization tests (glucose, maltose, lactose and sucrose). The capsular polysaccharide serogroup was determined by means of co-agglutination using in-house-produced rabbit polyclonal antibodies absorbed onto killed *Staphylococcus aureus* suspensions (Rosenqvist *et al.*, 1990; Wedege *et al.*, 1990; Kuipers *et al.*, 2001). The serogrouping reagent panel comprised serogroups A, B, C, X, Y, Z, 29E and W135. The in-house co-agglutination results were occasionally supplemented with commercial latex antigen kits (Meningite-5, bioMérieux, Wellcogen ACYW135 or B/*Escherichia coli* KI, BioStat). From July 1999, isolates were also screened for serogroups A, B and C by using murine mAbs supplied by the National Institute for Biological Standards and Control (NIBSC), South Mimms, UK, in a dot-blot ELISA format (Rosenqvist *et al.*, 1990; Wedege *et al.*, 1990; Kuipers *et al.*, 2001; <http://neisseria.org/nm/typing/mabs/panel.shtml>). The serotype and sero-subtype of isolates were also determined in the dot-blot ELISA (Rosenqvist *et al.*, 1990; Wedege *et al.*, 1990) using a NIBSC meningococcal mAb panel that included serotypes P3.1, P2.2a, P2.2b, P3.4, P3.11, P3.14, P3.15, P3.21 and P2.22, and sero-subtypes P1.1, P1.2, P1.3, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P1.14, P1.15, P1.16 and P1.19, where the class 1 outer-membrane protein (OMP) PorA (sero-subtype) is designated P1.* and the class 2 or class 3 OMP PorB (serotype) is designated P2.* or P3.*, respectively. All the mAbs were available for the period 1993 to 2003 except P1.13 (used from September 1993), P1.19 (used from July 2002) and P2.16 (discontinued from November 1994). Serotype P3.4 was most often characterized by reactions with a serotype 4 variant mAb initially sourced from W. Zollinger, Walter Reed Army Institute of Research, Silver Spring, MD, from December 1995, although some isolates were also reactive with another less sensitive variant serotype 4 mAb supplied by NIBSC from 1993 until July 2001, and initially obtained from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands (Urwin *et al.*, 1998).

(b) Non-culture case confirmation of meningococcal disease by PCR methods.

In 1995/96, the MRU introduced DNA-based non-culture detection of meningococci by means of a PCR ELISA. The rapid expansion of the PCR ELISA service offered to England and Wales from October 1996 increased the number of samples to

be handled and necessitated a transfer in 1998 to an automated system, the Perkin-Elmer Applied Biosystems (ABI) 7700 real-time PCR system using Taqman chemistry (Guiver *et al.*, 2002). PCR assays using primers and probes were designed to detect IS1106 (an insertion sequence) (Davison *et al.*, 1996; Ni *et al.*, 1992), *ctrA* (part of the meningococcal capsular biosynthesis locus) (Borrow *et al.*, 1998; Guiver & Borrow, 2001) and *siaD* (encoding a sialyl-transferase responsible for the addition of sialic acid residues to the polysialic acid chain of the capsule polysaccharide) (Guiver & Borrow 2001; Borrow *et al.*, 1997). Initially, because of the increased sensitivity achieved as a consequence of the multiple copies carried within the genome, the insertion sequence IS1106 was used in the PCR ELISA to screen samples for the presence of meningococcal DNA (Davison *et al.*, 1996; Ni *et al.*, 1992), but its use was discontinued after 1996 when IS1106 was detected in other genera (Guiver & Borrow, 2001). From 1997, all samples received at the MRU were screened for the presence of meningococcal DNA using *ctrA*, which detects meningococcal DNA from clinically significant serogroups (A, B, C, Y, W135, X and 29E) (Guiver & Borrow 2001). *siaD* assays were used to confirm the serogroup of the sialic-acid-containing capsules for serogroups B and C (from 1997 to present) (Guiver & Borrow 2001; Borrow *et al.*, 1997) and serogroups Y and W135 (from 1999) (Guiver & Borrow 2001; Borrow *et al.*, 1998). Serogroup A was confirmed by the *mynA* PCR assay from 2000 (Overlid *et al.*, 1999; Diggle *et al.*, 2003).

Improvements in DNA extraction procedures since 1995 have increased the efficacy of the non-culture confirmation methods and allowed the application of automated procedures. From 1997 to 2002, DNA extraction from EDTA whole blood samples was by capture column systems such as those supplied by Qiagen and Gentra (Flowgen) (Guiver & Borrow, 2001), and for CSF, plasma or serum, the preferred method was DNAzol (Molecular Research Centre) (Guiver & Borrow, 2001). Since 2002, the MRU has used the MagNApure (Roche Diagnostics) automated DNA extraction system to improve the quality and reproducibility of DNA extracts. Samples accepted for PCR testing include CSF, joint fluids, serum, plasma and EDTA whole blood.

Data. Information on laboratory-confirmed cases of meningococcal disease was stored on a database, and included information on the demographics of the cases (age, sex and region) and the characteristics of the infecting organism (for cultured isolates this included serogroup, serotype and sero-subtype, but for those cases that were identified by non-culture methods from clinical specimens, usually only the serogroup was recorded). Only cases confirmed by culture were assigned a phenotype (serogroup, serotype and sero-subtype), and among these, many were serotype or sero-subtype non-typeable (NT), because specific typing reagents were unavailable. Data on laboratory-confirmed cases of meningococcal disease referred to the MRU over the 11 epidemiological years 1993/94 to 2003/04 (July 1 1993 to June 30 2004) are presented. Analysing the data by epidemiological year takes into account the seasonal nature of meningococcal disease by centring the peak incidence, which occurs in the winter months. Deaths registered with the UK Office for National Statistics (ONS) attributed to meningococcal disease are routinely linked to laboratory-confirmed cases, and this information is also presented here. Completeness of ascertainment was improved by the routine follow up of confirmed cases of meningococcal disease reported to the HPA Centre for Infections by laboratories in England and Wales, requesting referral to the MRU. Since 2000, the National Public Health Service for Wales has also been offering a diagnostic PCR, although positive specimens should be referred to the MRU.

RESULTS AND DISCUSSION

Disease incidence

In the early 1990s, the incidence of meningococcal disease was fairly stable, with around 1200 cases reported each year up to 1994/95, corresponding to an incidence of around 2.3 per 100 000 (Table 1). In 1995/96, the reported incidence of disease increased by 39 % to 3.3 per 100 000, by a further 37 % to 4.6 per 100 000 in 1996/97, and still further by 19 % to peak at 5.4 per 100 000 in 1998/99 (test for trend in disease incidence by year, $P < 0.001$). The incidence of laboratory-confirmed meningococcal disease caused by all serogroups declined to less than 3.0 per 100 000 in 2002/03 and 2003/04 (Table 1). The age distribution for all serogroups during this period was typical of meningococcal disease, with a high incidence in infants and young children and a small secondary peak in older teenagers. An age shift was discerned between 1994/95 and 1995/96, which persisted through the 1990s, with proportionately more cases occurring in older age groups, particularly in the 15–19 year age group (Fig. 1).

Method of confirmation

The excess of notifications over laboratory-confirmed cases decreased after 1995/96 (Fig. 2), following the introduction of direct PCR-based testing of clinical samples (Kaczmarek *et al.*, 1998) (Table 2). It was therefore possible to explain the increase in disease incidence at this time (Table 1) by improved case ascertainment rather than by a real change in disease incidence. However, Ramsay *et al.* (1997) found that while there were indeed improvements in ascertainment through laboratory reporting, shifts in the age and serogroup distribution, together with an increasing number of notifications (Fig. 1), provided evidence of real, changing trends in disease. The introduction and provision of a free nationwide (UK) service for the non-culture (PCR-based) confirmation of meningococcal disease undoubtedly

improved the quality of epidemiological information available. In 2002/03 and 2003/04, 47 and 45 %, respectively, of meningococcal cases were confirmed by PCR alone (Table 2).

Increase in serogroup C disease

The increased incidence of laboratory-confirmed meningococcal disease after 1994/95 was mostly attributable to an increase in serogroup C cases. In particular, between 1994/95 and 1995/96, the number of cases of serogroup C disease more than doubled (Table 3), and the proportion of cases directly attributable to serogroup C infection increased from 25 to 36 % ($P < 0.0001$). After adjusting for the ungrouped cases (those cases confirmed as *N. meningitidis* by *ctrA* PCR testing but for which no serogroup was identified) that were likely to be due to serogroup C, the proportion of serogroup C cases increased to 40 % in 1995/96. Most of this sustained increase in serogroup C cases over the period 1993/94 to 1998/99 was due to the phenotypic combination of serotype 2a (Table 4), serosubtype P1.5,2 (Table 5). The C:2a:P1.5 phenotype increased as a proportion of serogroup C case isolates from 16 % in 1993/94 to a peak of 48 % in 2001/02 (Table 5). This hyperinvasive clone had previously been identified as a cause of hyperendemic disease in Canada (Whalen *et al.*, 1995) and is associated with multilocus sequence type 11 (ST-11) (previously identified by multilocus enzyme electrophoresis as ET-37 complex). C:2a strains are also associated with a higher fatality rate in the UK (Trotter *et al.*, 2002a).

Serogroup C conjugate vaccination

Introduction of meningococcal serogroup C conjugate (MCC) vaccines. As a result of this sustained increase in serogroup C disease, the UK accelerated its programme of clinical trials, and in 1999 the new MCC vaccines were licensed on the basis of safety and immunogenicity data (Miller *et al.*, 2001). In November of the same year, the MCC vaccines were incorporated into the routine infant

Table 1. Incidence (cases per 100 000 population) of laboratory-confirmed meningococcal disease (all serogroups) by age group, epidemiological years 1993/94 to 2003/04

Age group (years)	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
<1	40.7	42.3	43.7	66.2	69.0	82.0	80.5	74.5	73.0	56.7	58.1
1–4	12.6	11.4	13.6	22.2	22.8	28.8	30.9	26.1	21.9	18.6	18.8
5–9	2.7	2.4	3.8	5.7	5.9	7.3	8.2	6.1	4.6	3.5	3.1
10–14	1.5	1.5	3.2	4.7	4.7	6.0	5.1	4.4	3.4	2.4	2.3
15–19	4.5	4.4	8.3	11.5	10.2	12.6	10.5	8.0	5.9	4.4	4.1
20–24	1.5	1.8	2.9	4.2	3.5	4.0	4.1	5.0	2.9	1.7	2.2
25–44	0.5	0.6	0.8	1.2	1.3	1.4	1.7	1.8	1.1	0.7	0.7
45–64	0.5	0.4	0.8	1.3	1.0	1.6	1.7	1.6	1.2	0.8	0.8
>65	0.7	0.6	0.8	1.1	1.3	1.3	1.4	1.2	1.3	0.9	1.0
Overall incidence	2.3	2.4	3.3	4.6	4.5	5.4	5.4	4.7	3.8	2.8	2.8

Fig. 1. Age distribution of cases of laboratory-confirmed meningococcal disease, 1993/94 to 2003/04.

immunization schedule at 2, 3 and 4 months, and a catch-up campaign was launched to offer vaccine to everyone under the age of 18 (Chief Medical Officer *et al.*, 1999; Miller *et al.*, 2001). This catch-up campaign ran until September 2000 and was phased by age group, those at highest risk being targeted first. The vaccine was well accepted, with coverage above 80% in all but the oldest age groups (Trotter *et al.*, 2002b). Laboratory-confirmed cases of serogroup C in MCC-vaccinated individuals were investigated whenever possible (C. Auckland and others, unpublished results).

Impact of MCC vaccination. The phased introduction of MCC vaccines required accurate case confirmation to determine the effectiveness of vaccine intervention. The

MRU was able to confirm the characterization of serogroup C case isolates, and, via the provision of the non-culture meningococcal PCR diagnosis and grouping service, give an accurate estimate of the levels of serogroup C infection in England and Wales.

The number of cases of serogroup C disease declined each year after the introduction of the vaccine from a high of 954 cases in 1998/99 (and 892 cases in 1999/00) to only 64 cases (Table 3) in 2003/04, representing a 93% decline since 1998/99. The proportion of all cases caused by serogroup C fell from 34.4% in 1998/99 to 4.2% in 2003/04 ($P < 0.0001$) (Table 3). Fig. 3 compares the number of cases in those aged under 20 years (those initially targeted in the vaccine campaign) and those aged 20 years or above. Short-term vaccine effectiveness was high in all age groups

Fig. 2. Number of laboratory-confirmed cases (by serogroup) and total number of notifications of meningococcal disease, 1993/94 to 2003/04.

Table 2. Method of confirmation in laboratory-confirmed cases

Values show the number of cases; the proportion as a percentage of the total is shown in parentheses. Note that the HPA MRU routine PCR service was available only from October 1996.

Epidemiological year	Culture	PCR only	PCR and culture	Total cases confirmed
1993/94	1186 (100 %)	0	0	1186
1994/95	1228 (100 %)	0	0	1228
1995/96	1494 (87 %)	159 (9 %)	55 (3 %)	1708
1996/97	1181 (51 %)	790 (34 %)	366 (16 %)	2337
1997/98	993 (43 %)	800 (35 %)	505 (22 %)	2298
1998/99	994 (36 %)	1049 (38 %)	735 (26 %)	2778
1999/00	988 (35 %)	1122 (40 %)	689 (25 %)	2799
2000/01	797 (33 %)	1120 (46 %)	528 (22 %)	2445
2001/02	622 (32 %)	903 (46 %)	436 (22 %)	1961
2002/03	473 (32 %)	699 (47 %)	305 (21 %)	1477
2003/04	442 (30 %)	670 (45 %)	381 (26 %)	1493

(Ramsay *et al.*, 2001; Trotter *et al.*, 2004). After 4 years, effectiveness remains high in children vaccinated when above the age of 5 months, but declines to low levels in children vaccinated routinely at 2, 3 and 4 months (Trotter *et al.*, 2004).

Significant herd immunity effects have resulted (Ramsay *et al.*, 2003) in a lower prevalence of carriage in vaccinated compared to unvaccinated individuals (Maiden *et al.*, 2002), and benefits to older, unvaccinated age groups are now being observed. The incidence of serogroup C disease in 20–24 year olds, who were not initially targeted for vaccination, increased by 50 % (from 44 to 66 cases per year) between 1998/99 and 2000/2001. This led the UK Department of Health to extend the availability of the MCC vaccine to all individuals aged less than 25 years from December 2001 (Chief Medical Officer *et al.*, 2002).

Coverage in this age group is uncertain, but there were only seven cases in 20–24 year olds in 2003/04 (although some individuals in this age group in 2003/04 would have been vaccinated at age 17 or younger in the original catch-up programme). The number of cases in the 25–44 year age group increased from 76 in 1998/99 to 110 in 1999/2000, but subsequently declined to 14 cases in 2002/03. Cases in over 45 year olds also declined, from an average of 117 cases per year over the period 1998/99 to 2000/01 to only 27 in 2003/04.

The distribution of serotypes, for those cases of serogroup C disease that were confirmed using culture methods, are described in Table 4, and the major phenotypes (serogroup, serotype and sero-subtype) are described as the percentage of serogroup C isolates in Table 5. The phenotypic profiles of disease-causing isolates have not changed substantially

Table 3. Number of laboratory-confirmed cases by serogroup, 1993/94 to 2003/04

Serogroup	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
A	2	1		1	1	1	1	3	1	1	2
B	822	863	867	1069	1101	1401	1628	1686	1502	1211	1297
C	312	305	618	758	774	954	892	410	210	121	64
NG*	6	10	18	18	31	27	13	12	2	3	2
W135	12	21	20	42	40	44	96	129	101	46	47
X	3	6	2	2	7	7	3	7	6	2	2
Y	26	21	25	21	32	23	28	28	29	17	28
Z/29E	3	1	2	1	3	1	4	7	0	0	1
Ungrouped†			156	425	309	320	134	163	110	76	50
Total	1186	1228	1708	2337	2298	2778	2799	2446	1961	1477	1493

*NG, non-groupable isolates (due to non-expression of capsule with throat swab isolates from clinically confirmed cases or carrier isolates obtained from possible cases).

†Ungrouped isolates were tested by PCR, and although the presence of meningococcal DNA was confirmed (often in low amounts), it was not possible to determine the serogroup by *siaD* or *myxA* PCR assays.

Table 4. Serotypes of laboratory-confirmed serogroup C meningococcal disease, 1993/94 to 2003/04

Serotype	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
1	1	1	2	4	2	4	2	2	0	0	1
4	3	1	9	5	6	4	4	1	2	2	0
14	0	1	0	1	0	2	0	1	0	0	0
15	2	2	0	1	0	1	0	0	0	0	0
16	2	2	0	0	0	0	0	0	0	0	0
21	0	0	0	1	5	12	5	3	0	0	0
22	0	0	1	1	4	4	2	2	3	1	0
2a	161	173	433	429	379	451	388	211	113	56	32
2b	89	58	125	94	72	78	43	9	8	0	2
NT	54	67	45	60	89	112	108	32	21	15	10
Untyped*	0	0	3	162	217	289	343	150	63	47	19
Total number of isolates	312	305	615	596	557	668	552	261	147	74	45
Total number of cases	312	305	618	758	774	957	895	410	210	121	64

*Serogroup of case determined by PCR only; no isolate available for serotype.

following vaccination. In particular, there is no evidence of an increased frequency of B:2a cases (Table 6) or other phenotypes (serotypes or serosubtype combinations) that were predominantly associated with serogroup C prior to the implementation of vaccination. There is no evidence that the vaccination programme has led to extensive capsular switching.

Serogroup B disease

Throughout the 11-year time period, serogroup B was the most common cause of meningococcal disease. The proportion of laboratory-confirmed serogroup B cases

was 69 % in 1993/94, dropping to 46 % in 1996/97 as the number of serogroup C cases increased, but rising to 87 % in 2003/4 following the introduction of the MCC vaccine. Although not as dramatic as that seen for serogroup C, the number of reported cases of serogroup B disease rose each year from 1995/96 to 2000/01 (Table 3). Again, some of this increase may be explained by improved case ascertainment through PCR testing, although further insights can be gained by looking at the trends in the predominant phenotypes (Tables 6 and 7).

Further subdivision of meningococcal serogroups by means of serological reagents to determine the serotype

Table 5. Selected phenotypes of serogroup C case isolates expressed as a percentage of serogroup C case isolates, 1993/94 to 2003/04

Phenotype	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
C:2a:NT	9.9 %	7.5 %	9.3 %	11.6 %	14.0 %	10.5 %	12.1 %	13.0 %	15.6 %	27.0 %	17.8 %
C:2a:P1.5	15.7 %	25.9 %	25.2 %	32.2 %	31.8 %	36.7 %	37.5 %	44.8 %	48.3 %	41.9 %	40.0 %
C:2a:P1.5,2	14.1 %	18.7 %	32.0 %	23.3 %	18.5 %	17.5 %	15.6 %	20.7 %	10.9 %	2.7 %	11.1 %
C:2a:P1.2	8.0 %	2.0 %	0.7 %	2.2 %	1.8 %	1.2 %	3.3 %	0.8 %	2.0 %	2.7 %	0
C:2b:NT	6.1 %	3.6 %	1.3 %	1.2 %	1.8 %	2.5 %	1.3 %	0.4 %	2.7 %	0	0
C:2b:P1.5	3.2 %	2.6 %	2.0 %	1.5 %	0.9 %	0.4 %	0.2 %	0.0 %	0.0 %	0	0
C:2b:P1.5,2	12.5 %	10.5 %	15.6 %	10.9 %	9.2 %	7.6 %	5.3 %	2.7 %	2.0 %	0	4.4 %
C:2b:P1.2	3.8 %	0.3 %	0.3 %	1.3 %	0.7 %	0.7 %	0.2 %	0	0	0	0.0 %
C:2b:P1.10	1.9 %	1.3 %	0.3 %	0.2 %	0	0.0 %	0	0	0	0	0.0 %
C:NT:P1.5	2.9 %	3.0 %	1.1 %	1.2 %	5.4 %	3.1 %	5.4 %	3.1 %	3.4 %	8.1 %	8.9 %
C:NT:P1.5,2	0.6 %	2.6 %	1.1 %	4.9 %	5.9 %	8.2 %	7.1 %	3.1 %	2.7 %	0.0 %	6.7 %
C:NT:NT	6.1 %	5.6 %	1.8 %	1.7 %	2.3 %	2.8 %	4.0 %	3.4 %	4.8 %	4.1 %	2.2 %
Total number of C isolates*	312	305	615	596	557	668	552	261	147	74	45

*Total number of isolate-confirmed serogroup C cases; cases identified by PCR only were not characterized by serotype and serosubtype.

Fig. 3. Confirmed cases of serogroup C disease, 1993/94 to 2003/04, by age. White bars, <20 years; hatched bars, ≥20 years.

and sero-subtype (based on expressed OMPs) has allowed the discrimination of related cases of serogroup B for outbreak or cluster investigation. In addition, the immunogenic potential of the OMPs has stimulated the development of vaccines that include specific sero-subtypes, continued monitoring of which is necessary to inform the design of potential novel vaccines. The period 1993/94 to 2003/04 was the first in which consistent phenotypic surveillance using a fixed panel of mAbs was applied to all case isolates from England and Wales.

Serogroup B isolates showed greater phenotypic diversity with regard to serotype than serogroup C isolates (Table 6). In terms of serotype, there was a major shift in the proportion of cases attributable to B:4 isolates; the number of cases increased more than tenfold between 1994/95 and 1995/96 ($P < 0.0001$). However, this increase

was in part due to the availability of an additional anti-serotype 4 monoclonal antibody from December 1995 onwards, which was better at discriminating UK strains to serotype 4 than the one originally used. The corollary was a corresponding decrease in the number of B:NT isolates. The number of B:2b isolates decreased steadily, from 12 % (97 isolates) in 1993/94 to only 1 % (11 isolates) in 1997/98 ($P < 0.0001$), maintaining low levels subsequently. A decrease was also observed for B:15 isolates, from 133 to 20 reported cases per year ($P < 0.0001$) over the time period 1993/94 to 2003/04. Phenotype B:16 was not identified after mid-December 1994 due to the unavailability of the serotype 16 reagent. Phenotype B:1 also appeared to increase slightly 1993/94 to 1999/00, while phenotypes B:14 and B:2a persisted at background levels (fewer than 25 cases identified per year) 1993/94 to 2003/04.

Table 6. Serotypes of cases of laboratory-confirmed serogroup B meningococcal disease, 1993/94 to 2003/04

Serotype	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
1	27	40	39	41	52	72	95	85	70	65	77
4	8	27	314	350	326	390	366	320	299	214	215
11	2	0	0	1	0	0	0	0	0	0	1
14	10	7	6	9	18	19	22	15	19	5	22
15	133	102	87	91	73	62	58	49	50	22	20
16	7	10	0	0	0	0	0	0	0	0	0
21	0	0	0	6	7	19	11	14	6	1	6
22	0	0	11	13	17	10	12	14	15	13	12
2a	20	12	14	11	4	7	17	14	12	6	10
2b	97	71	42	26	11	14	4	12	6	3	0
NT	518	594	354	318	319	364	399	394	334	318	349
Untyped*	0	0	0	203	274	444	644	769	692	564	585
Total number of isolates	822	863	867	866	827	957	984	917	811	647	712
Total number of cases	822	863	867	1069	1101	1401	1628	1686	1502	1211	1297

*Serogroup of case determined by PCR only; no isolate available to determine serotype.

Table 7. Selected phenotypes of serogroup B case isolates expressed as a percentage of serogroup B case isolates (culture confirmed), 1993/94 to 2003/04

Phenotype	Epidemiological year										
	1993/94	1994/95	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	2003/04
B:15:P1.7,16	12.7 %	9.0 %	6.9 %	7.7 %	6.3 %	3.9 %	4.1 %	3.4 %	3.8 %	1.7 %	1.4 %
B:NT:P1.4	21.5 %	23.6 %	5.3 %	3.8 %	4.2 %	3.8 %	5.9 %	4.9 %	5.3 %	7.1 %	5.9 %
B:4:P1.4	0.5 %	1.6 %	21.7 %	25.5 %	24.8 %	24.7 %	22.3 %	22.0 %	21.7 %	19.0 %	17.0 %
B:NT:P1.9	2.8 %	3.0 %	3.6 %	3.8 %	5.1 %	5.9 %	6.1 %	7.9 %	10.9 %	9.1 %	12.6 %
B:NT:P1.15	16.2 %	16.7 %	12.7 %	11.5 %	12.8 %	12.9 %	12.2 %	13.0 %	9.6 %	0.5 %	0.1 %
B:NT:P1.5	0.9 %	2.1 %	1.0 %	1.0 %	0.6 %	0.9 %	0.8 %	1.0 %	1.0 %	1.1 %	1.5 %
B:NT:P1.5,2	0.9 %	1.5 %	2.1 %	1.7 %	2.5 %	1.8 %	1.3 %	2.7 %	1.2 %	2.6 %	1.5 %
B:1:P1.14	0.5 %	0.8 %	0.7 %	0.9 %	1.6 %	2.0 %	3.4 %	3.5 %	4.3 %	5.4 %	5.6 %
B:2b:P1.10	8.4 %	5.7 %	3.6 %	2.3 %	0.8 %	0.6 %	0.2 %	0.3 %	0.1 %	0.0 %	0.0 %
B:2a:NT	0.2 %	0.5 %	0.0 %	0.0 %	0.1 %	0.0 %	0.2 %	0.2 %	0.4 %	0.2 %	0.0 %
B:2a:P1.5	0.1 %	0.3 %	0.2 %	0.1 %	0.2 %	0.2 %	1.0 %	1.1 %	0.4 %	0.6 %	1.1 %
B:2a:P1.5,2	1.2 %	0.3 %	0.7 %	0.9 %	0.1 %	0.5 %	0.2 %	0.2 %	0.2 %	0.0 %	0.0 %
B:NT:NT	7.8 %	9.6 %	8.0 %	4.5 %	4.4 %	5.3 %	4.5 %	3.5 %	3.5 %	5.4 %	4.2 %
<i>B:2a*</i>	2.4 %	1.4 %	1.6 %	1.3 %	0.5 %	0.7 %	1.7 %	1.5 %	1.5 %	0.9 %	1.4 %
<i>B:2b*</i>	11.8 %	8.2 %	4.8 %	3.0 %	1.3 %	1.5 %	0.4 %	1.3 %	0.7 %	0.5 %	0.0 %
Total number of B isolates†	822	863	867	866	827	957	984	917	811	647	712

*In italic type: composite serogroup B type 2 phenotypes, including others not specified elsewhere in the table.

†Total number of isolate-confirmed serogroup B cases.

Phenotypic combinations of serogroup:serotype:sero-subtype have been used as surrogate markers for specific strains (Wedegé *et al.*, 1995; Caugant *et al.*, 1987). A number of phenotypic trends (strain population changes) were observed from 1993/94 to 2003/04: B:15:P1.7,16, which was important in the UK (and Europe) in the 1980s declined during the 11-year period, in the absence of any intervention. The increasing predominance of B:4:P1.4 (or B:4/NT:P1.4) case isolates over the 11 years, which has slowed latterly, suggests a gradual accumulation of immunity to this meningococcal phenotype. The continued usefulness of serotyping is shown by the increase in cases attributable to B:NT:P1.9 (from 2.8 % in 1993/94 to 11 % in 2003/04) and B:1:P1.14 (from 0.5 % to 15 %) meningococci. This is important, as it demonstrates a characteristic feature of meningococcal epidemiology, the natural turnover or variation in meningococcal antigens, whereby a particular strain emerges (e.g. B:15:P1.7,16), and then declines to be replaced by a new strain (e.g. B:4:P1.4), possibly in response to herd/host population immune responses. This has implications for vaccines designed around surface proteins such as PorA (sero-subtype) or PorB (serotype) and the timeliness with which they may need to be introduced and used.

Other serogroups

There was a 25 % increase in cases (from 96 to 129) caused by serogroup W135 in all ages between 1999/00 and 2000/01, which was largely due to an outbreak of W135 meningococcal disease among pilgrims returning from Saudi Arabia

and their contacts (Hahne *et al.*, 2002a, b). The effectiveness of the MRU surveillance was demonstrated by the observation of an initially small number of serogroup W135 cases with a phenotype not typical of that seen in the UK, such that the association with the Hajj pilgrimages was rapidly established. The 2000 outbreak led to an official recommendation that Hajj travellers receive meningococcal quadrivalent vaccine (for serogroups A, C, Y and W135) in 2001, which in 2002 became a compulsory visa requirement. Only 47 cases of serogroup W135 disease were laboratory confirmed in 2002/03, none of which was associated with the 2002 Hajj pilgrimage. Removing the effect of the Hajj outbreaks on the serogroup W135 case isolates, a steady increase was observed over the period 1993/94 to 1999/00 (from 12 to 49 cases, respectively) that was maintained up to 2003/04 (at 47 cases).

The number of cases of serogroup Y disease remained stable at around 30 cases per year, and there were only minor fluctuations in the small numbers of cases due to other serogroups (A, 29E, X and Z), which rarely cause disease in the UK. The use of quadrivalent conjugate vaccines rather than the monovalent MCC vaccine may be more suited to countries in which the burden of disease from serogroup W135 and/or serogroup Y is higher.

Mortality

Deaths among laboratory-confirmed cases of meningococcal disease are shown in Table 8. The overall case fatality ratio (CFR) remains around 6 %, despite evidence that death rates can be reduced in specialist centres (Booy *et al.*, 2001;

Table 8. Deaths from meningococcal disease and case fatality ratio, by serogroup

Epidemiological year	Serogroup B			Serogroup C			Other serogroups			All serogroups		
	No. of deaths	No. of cases	CFR	No. of deaths	No. of cases	CFR	No. of deaths	No. of cases	CFR	No. of deaths	No. of cases	CFR
1993/94	49	822	6.0 %	26	312	8.3 %	4	52	7.7 %	79	1186	6.7 %
1994/95	45	863	5.2 %	34	305	11.1 %	6	60	10.0 %	85	1228	6.9 %
1995/96	49	867	5.7 %	39	618	6.3 %	19	223	8.5 %	107	1708	6.3 %
1996/97	61	1069	5.7 %	90	758	11.9 %	27	510	5.3 %	178	2337	7.6 %
1997/98	75	1101	6.8 %	95	774	12.3 %	13	423	3.1 %	183	2298	8.0 %
1998/99	75	1401	5.4 %	118	954	12.4 %	18	423	4.3 %	211	2778	7.6 %
1999/00	61	1628	3.7 %	94	892	10.5 %	14	279	5.0 %	169	2799	6.0 %
2000/01	86	1685	5.1 %	67	411	16.3 %	18	349	5.2 %	171	2445	7.0 %
2001/02	64	1502	4.3 %	35	210	16.7 %	13	249	5.2 %	112	1961	5.7 %
2002/03	64	1211	5.3 %	20	121	16.5 %	7	145	4.8 %	91	1477	6.2 %
2003/04	56	1297	4.3 %	9	64	14.1 %	4	132	3.0 %	69	1493	4.6 %
Total	685	13448	5.1 %	627	5419	11.6 %	143	2845	5.0 %	1455	21712	6.7 %

Thorburn *et al.*, 2001). Earlier work has shown meningococcal phenotype, in particular the C:2a strain, and increasing age of the host to be associated with death (Trotter *et al.*, 2002a). The number of deaths among laboratory-confirmed cases of serogroup C meningococcal disease in 0–19 year olds fell from 78 in 1998/99 to only one in 2003/04 after the introduction of MCC vaccines. The number of deaths in over 20 year olds increased from 40 in 1998/99 to 56 in 2000/01, but subsequently fell to eight in 2003/04.

Future directions

The characterization by serotyping of meningococcal isolates has identified predominant phenotypes and revealed epidemiological trends over the 11-year period. As molecular methods become more widely available and less costly it is possible that DNA-based typing may replace the conventional serological methods in the near future. The development and application of DNA-based typing for meningococci by PorA sequencing and multilocus sequence typing (MLST) (Maiden *et al.*, 1998) is envisaged for routine surveillance within the next 5 years, and will improve the discrimination of isolates and increase their typeability. Surveillance by MLST will also allow the genetic relatedness of disease-causing meningococci to be studied, irrespective of their surface properties. Historical data for meningococcal disease surveillance currently rely on serological typing, and so links between molecular and serological schemes should be well established to enable consistent epidemiological analysis and surveillance.

Conclusions

Ongoing, high-quality laboratory surveillance is essential for monitoring the epidemiology of meningococcal disease; its output will inform the need for and assess the impact of vaccination programmes, and play a critical role in outbreak management.

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