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# High case-fatality rates of meningococcal disease in Western Norway caused by serogroup C strains belonging to both sequence type (ST)-32 and ST-11 complexes, 1985–2002

I. SMITH[1]*, D. A. CAUGANT[2,3], E. A. HØIBY[2], T. WENTZEL-LARSEN[4] AND A. HALSTENSEN[1,5]

[1] *Institute of Medicine, University of Bergen, Bergen, Norway*
[2] *Department of Airborne Infections, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway*
[3] *Department of Oral Biology, University of Oslo, Oslo, Norway*
[4] *Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway*
[5] *Department of Medicine, Haukeland University Hospital, Bergen, Norway*

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## SUMMARY

A total of 293 meningococcal disease (McD) patients from Western Norway hospitalized during 1985–2002 were examined for risk factors related to death. The case-fatality rate (CFR) increased from 4% during 1985–1993 to 17% during 1994–2002. We analysed the phenotypic and genotypic characteristics of the meningococcal patient isolates, with the aim of identifying whether highly virulent meningococcal strains contributed to the increased CFR. The Norwegian epidemic strain B:15:P1.7,16/ST-32 complex was overall the most common phenotype/genotype ($n=75$) and caused most deaths ($n=9$; CFR 12·0%). However, fatality was significantly associated with disease caused by serogroup C meningococcal strains; C:15:P1.7,16/ST-32 and C:2a/ST-11 complex strains, which had the highest CFRs of 21·1% and 18·2% respectively. Serogroup B strains of the ST-32 complex differing by serotype and/or serosubtype from the epidemic strain had a CFR of 5·1%, while the CFR of disease caused by other strains (all phenotypes and genotypes pooled) was 2·2%. The distribution of phenotypes/clonal complexes varied significantly between 1985–1993 and 1994–2002 ($P<0·001$); B:15/ST-32 complex strains decreased whereas both C:15:P1.7,16/ST-32 complex strains and strains with other phenotypes/clonal complexes increased. Our results indicate that C:15:P1.7,16/ST-32 and C:2a/ST-11 complex strains were highly virulent strains and contributed to the high CFR of McD in patients from Western Norway. To reduce fatality, rapid identification of such virulent strains is necessary. In addition, early and specific measures should include public information, vaccination of populations at risk of disease and carriage eradication, when clustering of patients occurs.

## INTRODUCTION

The Norwegian serogroup B meningococcal epidemic, which commenced in Northern Norway in the mid-1970s, spread rapidly to the rest of the country including Western Norway [1, 2]. A clinical study was performed on meningococcal disease (McD) patients hospitalized during 1976–1984 with the aim of reducing fatality. The results of the study led to a change of focus in public information from meningitis to septicaemia, including colour prints of meningococcal skin rash [3].

We studied risk factors related to death in McD

* Author for correspondence: Dr I. Smith, Institute of Medicine, University of Bergen, Bergen, Norway.
(Email: ingrid.smith@med.uib.no)

patients from Western Norway hospitalized during 1985–2002, and found that the case-fatality rate (CFR) varied substantially during the study period [4]. Many studies have found increased CFRs of McD in association with specific meningococcal serotypes or clonal complexes [5, 6]. Therefore, we examined the relation between characteristics of meningococcal patient strains and fatality, in an attempt to identify whether the circulation of highly virulent meningococcal strains may have contributed to the change in CFR of McD in Western Norway.

## MATERIAL AND METHODS

### Patients

Altogether 293 McD patients hospitalized at Haukeland University Hospital in Bergen, Western Norway in the period 1985–2002 were included in the study [4]. The patients were included based on clinical features, bacteriological confirmation from a normally sterile site and/or microscopy of Gram-negative diplococci in cerebrospinal fluid (CSF).

*Confirmed case*. Isolation of *N. meningitidis* from sterile site ($n=215$). Detection of Gram-negative diplococci in CSF by microscopy ($n=26$). McD in one patient was confirmed by agglutination test.

*Probable case*. Meningitis/back rigidity, fever and meningococcal skin rash, but no systemic isolate ($n=37$). Age $\leqslant 20$ years with severe septicaemia with hypotension and/or ecchymoses, but without meningitis and no systemic isolate ($n=14$).

Patients with a temperature $>37{\cdot}8\,^{\circ}C$ and meningococcal skin rash but no systemic isolate ($n=5$) and patients transferred from other hospitals ($n=11$) were not included. The patients were classified into four disease categories based on clinical symptoms on admission to the hospital [3, 7].

### Patient isolates

The patient isolates were from blood culture, CSF, other normally sterile site and/or tonsillopharynx. Samples were inoculated on blood agar plates and incubated overnight at 37 °C in 5% $CO_2$ and 80% humidity. Meningococcal isolates were identified by oxidase reaction, Gram-stain microscopy, fermentation of glucose, maltose, sucrose and lactose and serogrouping by agglutination with commercial antisera (Difco Laboratories, Detroit, MI, USA). Meningococcal isolates were kept lyophilized or frozen at $-70\,^{\circ}C$ in Greaves solution until required.

### Phenotyping

Further strain characterisation included serogrouping, serotyping and serosubtyping, which was performed using a dot-blot method with monoclonal antibodies (mAbs) [8, 9]. mAbs against the A, B, C, W and Y polysaccharide antigens, the 2a, 2b, 4, and 15 serotype antigens and the P1.2, P1.4, P1.5, P1.7, P1.14, P1.15, and P1.16 serosubtype antigens were used. When isolates did not react with these mAbs, the panel of mAbs was broadened. Strains that did not react with serotype-specific mAbs were designated as non-serotypable (NT). Correspondingly, strains that did not react with serosubtype-specific mAbs were designated as non-serosubtypable (NST). The phenotypes of the patient strains were made up by the combination of the serogroup, serotype and serosubtype (Table 1).

### Genotype identification

Meningococcal strains collected before 1999 were characterized by multilocus enzyme electrophoresis (MLEE) using variation at 14 enzyme loci. Distinctive multilocus genotypes were designated as electrophoretic types (ETs) and genetically closely related ETs were assigned to an ET complex [10, 11]. From 1999, MLEE was substituted by multilocus sequence typing (MLST), which uses the nucleotide sequences of fragments from seven housekeeping genes [12]. Strains with identical sequences of gene fragments are assigned to the same allele number, and the combination of alleles of the seven gene fragments defines the multilocus sequence type (ST) for each strain. Both techniques provide equivalent data that can be fully correlated. The MLST nomenclature will be used in this study (e.g. ST-32 and ST-32 complex) with cross-references to the MLEE nomenclature (e.g. ET-5 and ET-5 complex) where necessary. The distribution of clonal complexes of the genotyped strains is listed in Table 1.

In Norway, there has been a good correlation between phenotypes and genotypes: all C:15:P1.7,16 and B:15:P1.7,16 strains belonged to the ET-5/ST-32 complex, all C:2a strains belonged to the ET-37/ST-11 complex and, with a few exceptions, there was a strong association between B:15 strains and the ET-5/ST-32 complex. This formed the basis for assigning

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Table 1. *The distribution of phenotypes and clonal complexes by time period*

| Strain characterization | 1985–1993 *n* (% of 199) | 1994–2002 *n* (% of 94) | Whole period (% of 293) |
|---|---|---|---|
| Phenotype* | | | |
| Serogroup B | | | |
| B:15:P1.7,16 | 54 (27·1) | 21 (22·3) | 75 (26·0) |
| B:15 with other serosubtypes | 26 (13·1) | 11 (11·7) | 37 (12·6) |
| B:4 | 12 (6·0) | 9 (9·6) | 21 (7·2) |
| B with other serotypes | 10 (5·0) | 14 (14·9) | 24 (8·2) |
| B:NT:NST† strains | 25 (12·6) | 0 (0·0) | 25 (8·5) |
| Serogroup C‡ | | | |
| C:15 | 4 (2·0) | 15 (16·0) | 19 (6·5) |
| C:2a | 12 (6·0) | 9 (9·6) | 21 (7·2) |
| C with other serotypes | 1 (0·5) | 7 (7·5) | 8 (2·7) |
| C:NT:NST strains | 4 (2·0) | 2 (2·1) | 6 (2·1) |
| Other serogroups | | | |
| Y, W-135, NG | 4 (2·0) | 1 (1·1) | 5 (1·7) |
| No isolate | 47 (23·6) | 5 (5·3) | 52 (17·8) |
| Clonal complex (130 genotyped strains) strains | | | |
| ST-32 complex = ET-5 complex | 30 (15·1) | 45 (47·9) | 75 (25·6) |
| ST-11 complex = ET-37 complex | 6 (3·0) | 10 (10·6) | 16 (5·5) |
| ST-8 complex = Cluster A4 | 0 (0·0) | 6 (6·4) | 6 (2·0) |
| ST-41/44 complex = Lineage 3 | 0 (0·0) | 5 (5·3) | 5 (1·7) |
| ST-23 complex = Cluster A3 | 0 (0·0) | 1 (1·1) | 1 (0·3) |
| ST-334 complex | 1 (1·1) | 0 (0·0) | 1 (0·3) |
| UA: unassigned to a clone complex (not a hypervirulent strain) | 6 (3·0) | 20 (21·3) | 26 (8·9) |
| No isolate/not genotyped | 156 (78·4) | 7 (7·4) | 163 (55·6) |

* B:15:P1.7,16 includes B:15:P1.7 and B:15:P1.16; Other B:15 strains: P1.2, P1.5, P1.7, P1.12, P1.13(a), P1.13a, P1.15, NST; B:4 strains: P1.4, P1.5, P1.7, P1.12, P1.14, P1.15, P1.16 and NST; Other B strains: B:8, B:16, B:17, B:19.
† NT, Non-serotypable; NST, non-serosubtypable.
‡ C:2a strains: P1.5, P1.2, P1.14,22, NST. Other C strains: C:2b, C:21.

phenotyped strains not characterized by MLEE or MLST to specific clonal complexes [13]. For the statistical analyses, the phenotyped and/or genotyped strains were divided into five phenotype/clonal complex groups (Table 2).

### Statistical methods

All univariate analyses were performed using exact $\chi^2$ tests for categorical variables (Table 2). The joint effect of phenotypes/clonal complexes and time period on fatality could not be analysed due to the small numbers, except for the Norwegian epidemic strain B:15:P1.7,16/ST-32 complex. 95% confidence intervals (CIs) were calculated for the CFRs of the phenotypes/clonal complexes. SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used to process the data. A value of $P \leqslant 0{\cdot}05$ was considered significant.

## RESULTS

### Fatality

The association between fatality and phenotypes/clonal complexes was statistically significant ($P = 0{\cdot}02$) (Table 2). The C:15:P1.7,16/ST-32 and the C:2a/ST-11 complex strains had the highest CFRs of 21·1% and 18·2% respectively. The lowest CFRs were found in patients with strains of other phenotypes/clonal complexes (CFR 2·2%), no isolates/non-characterized strains (CFR 4·3%), and other B-strains of the ST-32 complex (CFR 5·1%).

The Norwegian epidemic strain B:15:P1.7,16/ST-32 complex had an overall CFR of 12·0%, but it varied from 7·4% during 1985–1993 to 23·8% during 1994–2002, although the difference did not prove significant ($P = 0{\cdot}11$). B:15:P1.7,16/ST-32 complex strains were the most common strains among the 24 fatal cases ($n = 9$), followed by C:15:P1.7,16/ST-32

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Table 2. *Phenotypes/clonal complexes of patient strains related to case-fatality rates (CFR) and time period*

| Strain characterization | Fatal cases (*n*/total) | CFR (%) | 95% CI of CFR* | *P* Fatality | *P* Time period† |
|---|---|---|---|---|---|
| Phenotype/clonal complex | | | | | |
| B:15:P1.7,16/ST-32 complex[a] | 9/75 | 12·0 | 6·2–20·9 | 0·02 | <0·001 |
| Other B strains/ST-32 complex[b] | 2/39 | 5·1 | 0·9–17·0 | | |
| C:15:P1.7,16/ST-32 complex[a] | 4/19 | 21·1 | 7·5–43·4 | | |
| C:2a strains/ST-11 complex[c] | 4/22 | 18·2 | 6·5–38·9 | | |
| Other phenotypes/complexes‡[d] | 1/46 | 2·2 | 0·1–11·2 | | |
| No. isolates/not characterized strains | 4/92 | 4·3 | 1·5–10·2 | | |

* Blyth–Still–Casella 95% CI (StatXact, Cytel Corp., Cambridge, MA, USA).
† Comparison of phenotypes/clonal complexes by time period.
‡ Other: A-4/ST-8, Lineage 3/ST-41/44, Cluster A3/ST-23, UA = unassigned to clone complex.
[a] Strains that react with only one of the two serosubtype mAbs, P1.7 and P1.16.
[b] Merely B:15 strains, except for two B:4:P1.12 strains genotyped ST-32.
[c] C:2a strains of the ST-11 (ET-37) complex.
[d] Strains with other phenotypes and/or belonging to other clonal complexes.

and C:2a/ST-11 complex strains (both $n=4$) (Table 2). Of the remaining seven fatal cases, three had other serogroup B strains, one had a non-characterized serogroup B strain and from three no isolates were made.

### Patients with no isolates or non-characterized strains

Altogether 92 patients, 83 of them hospitalized during 1985–1993, had no isolates made ($n=52$), serogrouped isolates that were not available for further characterization ($n=34$) or phenotyped isolates that could not be categorized to any of the five phenotype/clonal complex groups ($n=6$). The number of patients with no isolates/non-characterized strains decreased significantly from 41% during 1985–1993 to 11% during 1994–2002 ($P<0·001$). The proportion of patients with no isolates/non-characterized strains did not differ between age groups ($P=0·2$) or between genders ($P=0·7$), but was higher in disease categories I (meningitis) and III (meningitis and severe septicaemia) than disease categories II (severe septicaemia) and IV (bacteraemia/septicaemia) ($P<0·001$).

### Patients with characterized strains

Of the 241 patients with bacteriologically confirmed McD, 182 patients had serogroup B disease, 54 serogroup C disease, one serogroup Y disease, one W-135 disease and three had non-groupable (NG) strains (Table 1). (The strains have not been tested with mAbs against the X, Z, 29E polysaccharide antigens.) The most frequent phenotypes were B:15:P1.7,16 ($n=53$), B:15:P1.16 ($n=18$) and C:15:P1.7,16 ($n=16$). The distribution of phenotypes/clonal complexes varied significantly between the two 9-year periods 1985–1993 and 1994–2002 ($P<0·001$), with a substantial decrease in B:15/ST-32 complex strains and a marked increase in both C:15:P1.7,16/ST-32 complex strains and strains with other phenotypes/clonal complexes.

Altogether 130 isolates were genotyped, most of which belonged to the ST-32 and ST-11 complexes (Table 1). Among the patients with genotyped strains, there were no significant changes in the distribution of clonal complexes between the two time periods ($P=0·07$). The proportion of genotyped strains was substantially higher during 1994–2002 and strains belonging to three clonal complexes [ST-8 complex (cluster A4), ST-41/44 complex (lineage 3) and ST-23 complex (cluster A3)] were identified only in this period.

### Age, gender, and disease category

Of the 293 McD patients, 144 were males and 149 females aged 0–98 years. The two age groups, $\leqslant 4$ years old and teenagers, accounted for 71% of the patients. Among the patients with characterized strains, the distribution of phenotypes/clonal complexes varied significantly between age groups ($P<0·001$), genders ($P=0·03$), and disease categories ($P=0·03$). The B:15:P1.7,16/ST-32 complex strains were most frequently found in children aged 0–4 years

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and in teenagers, whereas the C:15:P1.7,16 5/ST-32 complex strains were mainly isolated from teenagers. The C:2a/ST-11 complex strains were mainly found in patients >50 years, teenagers, and children aged 0–4 years. Strains with other phenotypes/clonal complexes were most common in children aged 0–4 years. The B:15:P1.7,16/ST-32 complex and C:15:P1.7,16 5/ST-32 complex strains were more common in males than females, whereas the opposite was the case for serogroup B strains of the ST-32 complex harbouring different serotype/serosubtype and for the C:2a/ST-11 complex strains. These C:2a/ST-11 complex strains were frequently isolated from patients in disease categories II (severe septicaemia) and IV (bacteraemia). The B:15:P1.7,16/ST-32 complex, the C:15:P1.7,16 5/ST-32 complex strains and the strains of other phenotypes/clonal complexes were mainly isolated from patients in disease category IV (bacteraemia).

## DISCUSSION

In this clinical study on McD patients from Western Norway, we found that fatality was significantly associated with the phenotype and genotype of the patients' strains, and the highest fatality was caused by serogroup C strains characterized as C:15:P1.7,16/ST-32 complex and C:2a/ST-11 complex. These results are consistent with studies showing that certain strains are more virulent and cause more deaths [5, 14–16].

The C:2a/ST-11 complex strains were identified in patients throughout the 18-year study period. They caused sporadic cases but no outbreaks, which is in accordance with previously published data from Norway [17]. In contrast, the introduction of a new genetic variant of serogroup C strains of the ST-11 complex in several countries in the 1990s has been associated with both increased incidence rates and outbreaks [18, 19]. The prevalence of C:2a strains was low in our study population, but caused disease in six of the 13 patients >50 years old, and two of the six patients died [4]. C:2a strains were often found in patients categorized as disease category II (severe septicaemia) on admission, which was the disease category with the highest CFR. These results are in line with those of other studies showing that C:2a strains are associated with a high CFR [20–23]. The C:2a strains of the ST-11 complex have been present in Western Norway for more than two decades. This in combination with the low prevalence and high CFR of these strains indicates that C:2a/ST-11 complex strains have a low transmissibility but a high virulence [24].

The C:15:P1.7,16/ST-32 complex strain had only been identified in four patients from Western Norway before it was responsible for a local outbreak in Nordhordland, just outside Bergen during 1995–1997 [25]. Only two cases of McD had been registered in this area in the 5 years preceding the outbreak, but most cases during the outbreak were caused by this new C:15:P1.7,16 strain. The outbreak had a CFR of 20% and the incidence rate in Nordhordland increased to 22/100 000 during the 12 months prior to the implementation of interventions. This is consistent with the results of many studies finding increased incidence rates by the emergence of new meningococcal strains in a population [1, 26–28]. However, whether it was the virulence of the outbreak strain or the susceptibility of the population that was mainly responsible for the high CFR, remains unclear.

It is well-known that the meningococcal strains causing invasive disease worldwide belong to few clonal complexes, and molecular typing methods revealed that the outbreak strain C:15:P1.7,16 and the Norwegian epidemic strain B:15:P1.7,16 were genetically related [17, 29]. Meningococci possess a large genetic diversity, have a high recombination rate, and through horizontal genetic exchange, new virulent strains evolve constantly [30]. The surface structures of the meningococci in particular are under immunological selection pressure [17]. When a clone has been prevalent in a population for some time, e.g. the Norwegian epidemic strain, the surface structures may change, leading to heterogeneity of phenotypes and genotypes within a clone complex [31, 32]. The Norwegian serogroup B vaccination programme could not provide a selection pressure on B:15:P1.7,16 favouring the C:15:P1.7,16 phenotype, since the Norwegian OMV vaccine does not contain the serogroup B polysaccharide. The outbreak strain C:15:P1.7,16 and the Norwegian epidemic strain B:15:P1.7,16 both belonged to the ST-32 (ET-5) clonal complex and were phenotypically very similar, only differing by serogroup, which could imply that a capsule switch had occurred. The ability to switch capsule enables meningococci to escape anti-polysaccharide antibodies, which are important for the host defence against McD [33, 34]. Such capsule switches have occurred many times during the

Norwegian serogroup B epidemic, but this was the only one that resulted in an outbreak.

Our study suffered from three weaknesses. First, the small size of the patient sample did not allow for analyses of the joint influence of different variables on fatality. Second, the proportion of genotyped patient strains was low especially during 1985–1993, but since each clonal complex exhibits typical phenotypes, phenotyped strains could be assigned to specific ST complexes accordingly [13]. Third, there were many patients without isolates/non-characterized strains, and with a low CFR. One explanation may be that around 50% of these patients were children <12 years old; many of them were categorized to the meningitis group and many had not had a lumbar puncture. Despite these limitations, our results highlight some important issues regarding disease causing meningococcal strains.

Strains of the same clonal complex may perform differently when associated with different phenotypes. The Norwegian epidemic strain (B:15:P1.7,16/ST-32 complex) caused sporadic cases with a CFR of 12% whereas the C:15:P1.7,16/ST-32 complex strain caused an outbreak with a CFR of around 20%. Acquisition of the serogroup C polysaccharide capsule appears to result in increased virulence and higher CFR of the strain. However, it is noteworthy that the CFR for the B:15:P1.7,16/ST-32 complex in the period 1994–2002 was even higher (23·8%) than that of the C:15:P1.7,16/ST-32 complex strain. Thus, it is very important to have large number of patients and corresponding characterized strains to be able to control for various parameters. Phenotypically identical strains of the same clonal complex may perform diversely in different populations; e.g. the C:2a/ST-11 complex strains caused only sporadic cases in Western Norway while they were responsible for outbreaks in many other countries. The strains in the 'other phenotypes/clonal complexes' group had a very low CFR. All these findings involve the interaction between strain and host, and it is still unclear which elements are of importance as to whether or not a host develops invasive and/or fatal disease [35].

In conclusion, our results indicate that C:15:P1.7,16/ST-32 and C:2a/ST-11 complex strains were highly virulent strains contributing to the high CFR found in McD patients from Western Norway during 1985–2002, confirming previous findings that certain strains cause more severe disease and more deaths [17]. The future identification of such highly virulent strains therefore calls for early and specific measures including:

(1) Information to the public about the early symptoms of McD including meningococcal skin rash.
(2) Early admission to hospital of patients with suspected McD for prompt and adequate treatment [36, 37].
(3) Vaccination of populations at increased risk of disease, especially close contacts [38].
(4) Carriage eradication should be considered when clustering of patients occurs [25].

Finally, the continued effort to develop more efficient meningococcal vaccines, including serogroup B vaccines, remains essential to further reduce the disease burden and fatality of McD.

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## DECLARATION OF INTEREST

None.

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