# Surveillance of invasive *Neisseria meningitidis* with a serogroup Y update, Sweden 2010 to 2012

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**An increase of invasive meningococcal disease caused by *Neisseria meningitidis* serogroup Y has been noted in Sweden since 2005, and to a lower extent throughout Europe. The present study describes the epidemiology of invasive *N. meningitidis* isolates in Sweden in the period between 2010 and 2012, with a focus on serogroup Y. We also aimed to find an optimal molecular typing scheme for both surveillance and outbreak investigations. All invasive *N. meningitidis* isolates in Sweden during the study period (n=208) were genetically characterised. Serogroup Y predominated with 22/57, 31/61 and 44/90 of all invasive isolates (incidence 0.23, 0.33 and 0.46 per 100,000 population) in 2010, 2011 and 2012 respectively. In each of these years, 15/22, 22/31 and 19/44 of serogroup Y isolates were genetically clonal (Y: P1.5–2,10–1,36–2: F4–1: ST-23(cc23), '*porB* allele 3–36, *fHbp* allele 25 and *penA* allele 22). Our findings further support those of others that currently recommended FetA typing could be replaced by FHbp. Moreover, in line with a previous study that we conducted, the current results indicate that highly variable multilocus variable-number tandem repeat analysis (HV-MLVA) can be used as a first-hand rapid method for small outbreak investigations.**

## Introduction

*Neisseria meningitidis* (the meningococcus) is a Gram-negative diplococcus carried asymptomatically in the pharynx by approximately 10% of the population [1]. It is also a potentially devastating pathogen causing meningitis and septicaemia. Invasive meningococcal disease (IMD) occurs mainly in s*pora*dic cases but also as outbreaks and epidemics. Meningococcal populations are genetically and antigenically highly diverse [2] and vary greatly globally and over time, but the majority of IMD is caused by a limited number of clonal complexes, known as hyper-virulent lineages [3]. Therefore, detailed characterisation of circulating meningococcal strains is important in terms of vaccination policy decisions, outbreak management, as well as monitoring antibiotic susceptibility and vaccine coverage.

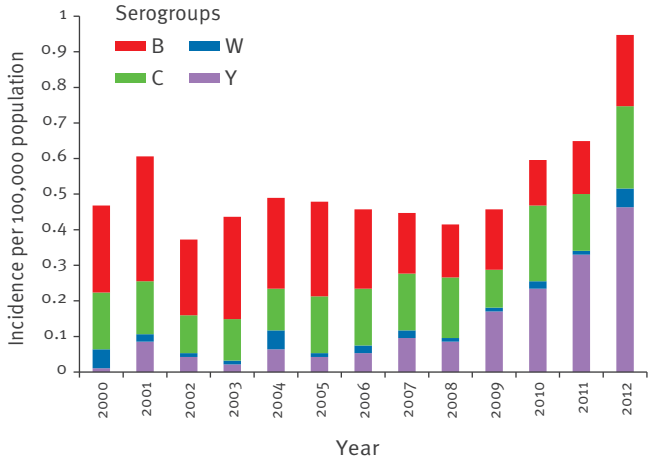
The polysaccharide capsule surrounding the bacterium defines the meningococcal serogroup. The capsule is an important virulence factor and IMD is mainly restricted to encapsulated meningococci belonging to serogroups A, B, C, W, X and Y. The capsule is also a polysaccharide vaccine component in available conjugate vaccines for serogroups A, C, W and Y [4]. The serogroup distribution is highly regional [5]. In Europe, the main circulating strains belong to serogroups B and C [6]. As previously described, serogroup Y has increased in Sweden from 0.04 per 100,000 population in 2005 to 0.23 per 100,000 population in 2010 [7]. An emergence of serogroup Y has also been noted in some other European countries, however, the highest relative proportions are found in Scandinavia [8,9].

In addition to serogroup designation, it is currently recommended by the European Meningococcal Disease Society (EMGM) that meningococcal strains are designated by variable regions (VR) in the Porin A (*PorA*) and the Ferric enterobactin transport protein A (FetA) proteins as well as multilocus sequence typing (MLST) sequence type (ST) and clonal complex (CC) [10]. *PorA* and FetA are two surface antigens, which are recommended for rapid investigation of disease outbreaks. MLST, based on seven housekeeping genes, is ideal for studying population biology and evolution of the organism on a national and international level. For enhanced resolution, genotyping of a third surface antigen, Porin B (PorB), may also be performed [11]. Finally, further characterisation can be achieved with the *penA* gene encoding the penicillin-binding protein 2 (used in surveillance of penicillin susceptibility) [12] and *fHbp* encoding the serogroup B vaccine component Factor H binding protein (*FHbp*) [13,14].

Another molecular method that has been proposed for an alternative typing paradigm, mainly suited for investigating localised outbreaks, is multilocus variable-number tandem repeat analysis (MLVA) [15-18]. MLVA is a polymerase chain reaction (PCR)-based technique, which uses the variability in the numbers of short tandem repeats to create DNA fingerprints used

**Figure 1**

Incidence of invasive meningococcal disease caused by *Neisseria meningitidis* serogroups B, C, W and Y in Sweden, 2000–2012 (n=642)




in epidemiological studies. The highly variable MLVA (HV-MLVA) developed by Schouls et al. [18] has shown high discriminatory capacity for serogroup C isolates and has been considered suitable for outbreak identification [19].

The aims of the present study were to describe the current epidemiology of invasive *N. meningitidis* isolates including the dominating serogroup Y in Sweden, and to find an optimal molecular typing scheme with appropriate resolution power for both surveillance and outbreak investigations.

# Methods

## Bacterial isolates and phenotypic characterisation

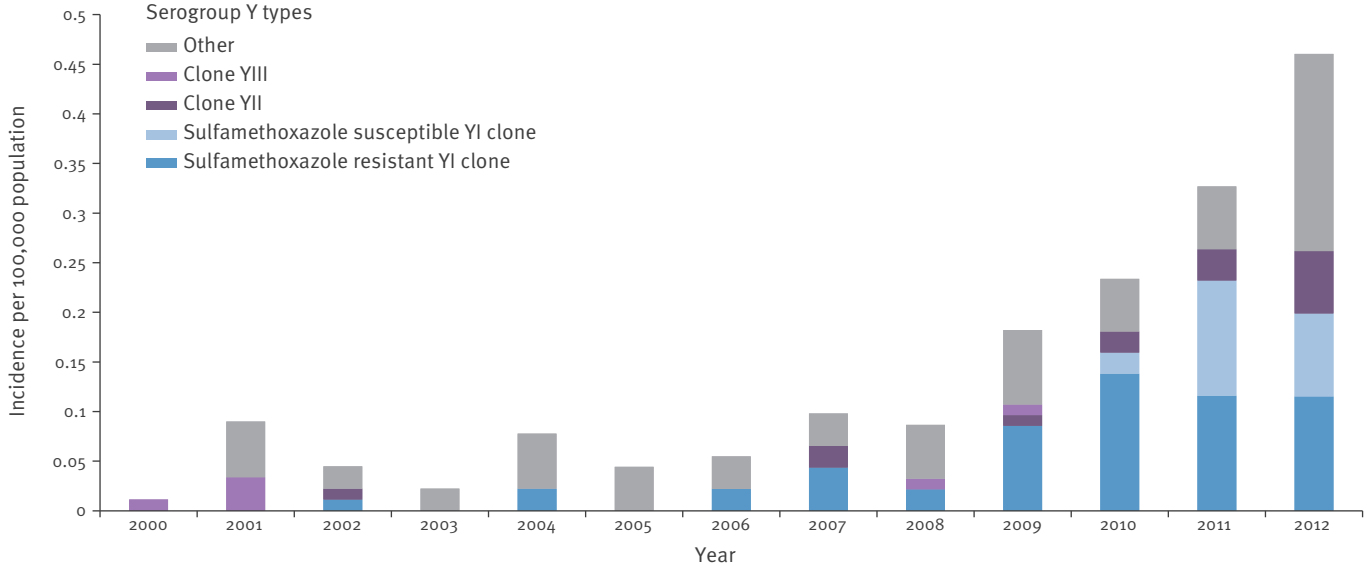
In Sweden, all invasive cases of meningococcal disease according to the European Union case definition are mandatorily reported by clinicians to the Swedish Institute for Infectious Disease Control (SMI) [20]. The corresponding isolates are sent to the Public Health Agency of Sweden, where they are routinely cultured on chocolate agar at 37°C with 5% $CO_2$ overnight and subsequently serogrouped by co-agglutination [21]. Further genosubtyping (*PorA* typing) is then conducted as previously described [22] and antibiotic susceptibility determined using the Epsilometer (E)test method (bioMérieux, Marcy l'Etoile, France). Basic epidemiological data (age, sex, area of residence, clinical site of isolation and date of sample collection) are gathered for all isolates from cases. This study included all invasive *N. meningitidis* isolates in Sweden between 2010 and 2012. The serogroup B strain MC58 [23] was included in all analyses as a reference.

## Nucleic acid extraction

The DNA used for sequence-based typing methods and MLVA was extracted with a NorDiag Bullet instrument (DiaSorin, Dublin, Ireland). For the automatic extraction, 20 colonies from each cultured organism were suspended in 2 ml NaCl (0.85%) and 100 µl of this solution were subsequently processed with the NorDiag Bullet with the Bullet BUGS'n BEADS kit according to the manufacturer's recommendation (DiaSorin). All DNA preparations were stored at 4°C prior to the PCR.

**Figure 2**

Distribution of the genetically defined predominant *Neisseria meningitidis* clone YI with different sulfamethoxazole susceptibilities, the second and third most common serogroup Y clones (YII and YIII) and all other invasive *N. meningitidis* serogroup Y isolates in Sweden, 2000–2012 (n=163)

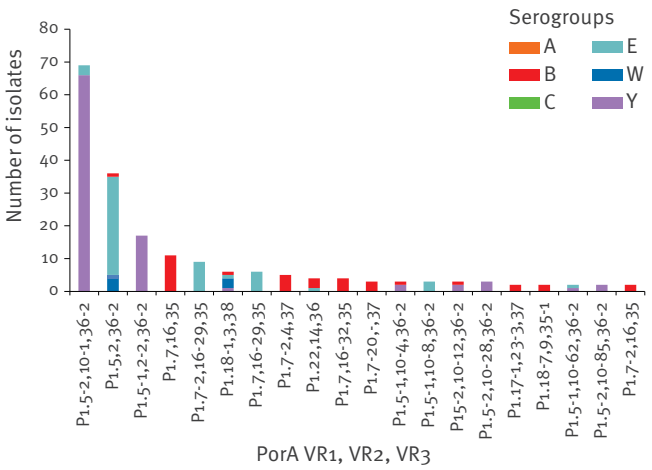



Data for this Figure originate both from this study and a previous one [7].

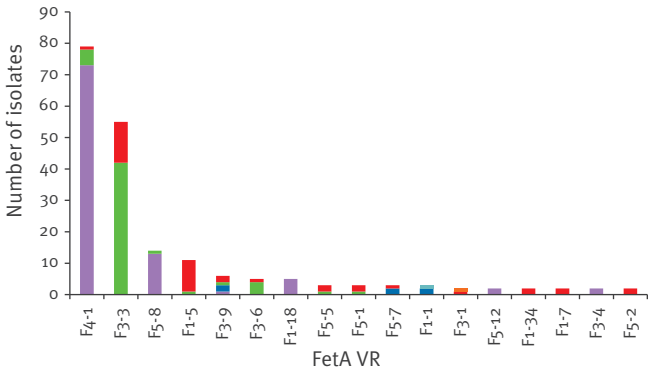
**Figure 3**

Serogroup distribution in the most frequent *PorA*, FetA and *fHbp* variable regions (VRs) or alleles of invasive *Neisseria meningitidis* isolates in Sweden, 2010–2012

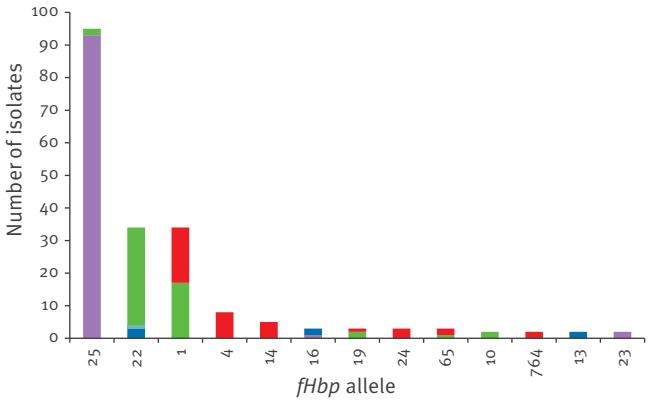
A. Serogroup distribution by *PorA* VR




B. Serogroup distribution by FetA VR




C. Serogroup distribution by *fHbp* allele




FetA: Ferric enterobactin transport protein A; *PorA*: Porin A.
Only the types represented by at least two isolates are displayed.

## Polymerase chain reaction and DNA sequencing

The MLST genes: *abcZ, adk, aroE, fumC, gdh, pdhC* and *pgm* together with *fetA, fHbp, porB* and *penA* were amplified and sequenced as previously described [7,19]. In short, the PCR was performed using a Rotor-Gene Q real-time PCR system (Qiagen, Hilden, Germany) and detected with SYBR green I. The nucleotide sequences were determined by capillary electrophoresis using an ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using BigDye Terminator v3.1 (Applied Biosystems, Warrington, UK). The sequence alignments for the MLST genes were assembled using the Bionumerics software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium, http://www.applied-maths.com/bionumerics) and all other genes were assembled using the ChromasPro software version 1.33 (Technelysium Pty Ltd, Brisbane, Australia, http://technelysium.com.au/). The different sequences were assigned allele numbers using the *N. meningitidis* sequence query database [24].

## Multilocus variable-number tandem repeat analysis

The HV-MLVA with four highly variable variable-number tandem repeat (VNTR) loci (VNTR4–4, VNTR9–2, VNTR4–2, and VNTR4–3) was performed as previously described by Törös et al. [19] with the primers from Schouls et al. [18]. In short, the PCR was performed on an Applied Biosystems 9700 or 2720 PCR machine and the fragments were separated on an ABI PRISM 3130*xl* Genetic Analyzer using the GeneScan LIZ 500 size standard and GeneScan module with filter set G5 (Applied Biosystems). The sizing of the fragments was performed with the GeneMapper software v4.0 (Applied Biosystems). All isolates were run in duplicates in separate runs.

## Data analysis

The ability of each method to discriminate between strains was evaluated on the basis of their discrimination index, using Simpson's index of diversity (ID) [25]. The ID determines the probability that two randomly picked strains are allocated to different types. Confidence intervals (CI) of 95% were calculated [26]. Cluster analysis of the MLST data was performed using a categorical coefficient and displayed in a minimum spanning tree (MST) created with the Bionumerics software version 7.1, with the priority rule of first linking types which have the highest number of single-locus variants. For the HV-MLVA, a dendrogram was generated using a categorical coefficient and unweighted pair group method with arithmetic average (UPGMA).

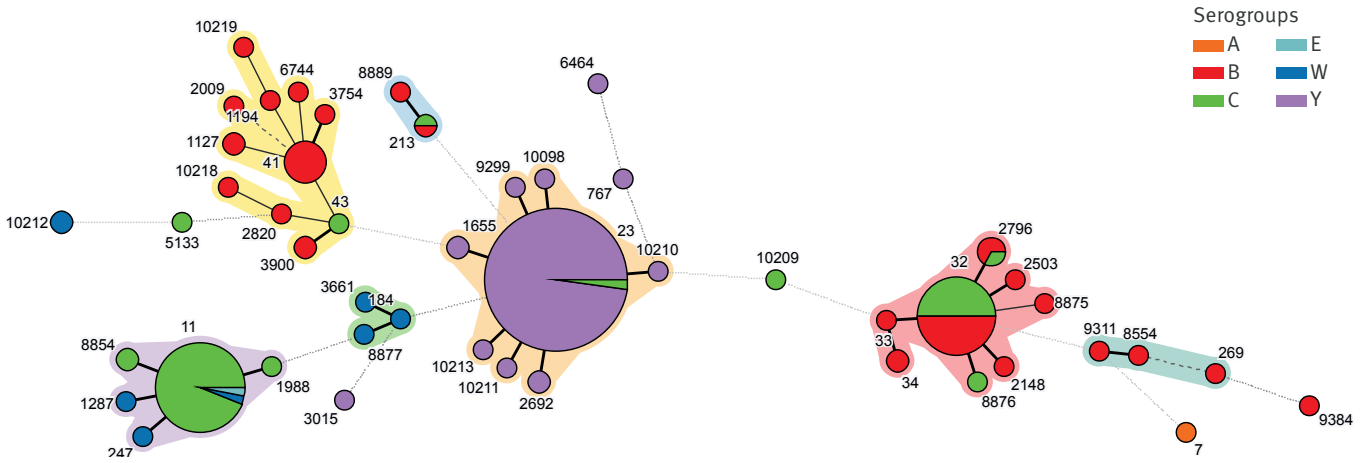
# Results

## Epidemiology

A total of 208 invasive *N. meningitidis* isolates were characterised during the study period, including 57 in 2010, 61 in 2011 and 90 in 2012. The isolates originated from clinical specimens of cerebrospinal fluid (n=44),

**Figure 4**

Minimum spanning tree from multilocus sequence typing profile data for *Neisseria meningitidis* isolates in Sweden, 2010–2012 (n=208)




Each circle represents a sequence type (ST), and the serogroup distribution in each ST is represented by the respective colour.

Lines connecting the STs that are thick and solid, thin and solid, dashed and dotted denote 1, 2, 3 and 4 or more loci differences, respectively. Halos surrounding the circles denote different clonal complexes. Purple: clonal complex ST-11; yellow: clonal complex ST-41/44; green: clonal complex ST-22; blue: clonal complex ST-213; peach: clonal complex ST-23; pink: clonal complex ST-32; turquoise: clonal complex ST-269.

tissue (n=1), joint fluid (n=4) and blood (n=159). Among all isolates, 97 belonged to serogroup Y, 57 to serogroup C, 44 to serogroup B, eight to serogroup W, one to serogroup A and one to serogroup E. The annual incidences of all meningococcal serogroups in Sweden from 2000 to 2012 are described in Figure 1. The figure also shows that the total incidence of IMD in Sweden has increased prominently in 2012, from approximately 0.6 per 100,000 population in 2010 and 2011 to 0.95 per 100,000 population in 2012. The predominant serogroup in the period between 2010 and 2012 was serogroup Y, which accounted for 22/57 (39%, incidence 0.23 per 100,000 population), 31/61 (51%, incidence 0.33 per 100,000 population) and 44/90 (49%, incidence 0.46 per 100,000 population) of all invasive isolates in Sweden in 2010, 2011 and 2012, respectively.

The clonal pattern of the invasive serogroup Y isolates is presented in Figure 2. Of all serogroup Y isolates, 15/22 (68%) in 2010, 22/31 (71%) in 2011 and 19/44 (43%) in 2012 were genetically identical to clone YI as previously described by Thulin Hedberg et al. [7] (Y: P1.5–2,10–1,36–2: F4–1: ST-23(cc23), '*porB* allele 3–36, *fHbp* allele 25 and *penA* allele 22). However, a sulfamethoxazole susceptible variant of the clone seems to have appeared in 2010, which in 2011 corresponded to 11/22 (50%) of the predominant clone, and 8/19 (42%) in 2012. As seen in Figure 2, the previously described clone YII [7] (sulfamethoxazole susceptible, with type Y: P.5–1,2–2,36–2, F5-8, ST 23(cc23), '*porB* allele 2–55, *fHbp* allele 25 and *penA* allele 22) is still the second most frequent serogroup Y clone corresponding to 3/22 (14%) in 2010, 3/31 (10%) in 2011 and 6/44 (14%) in 2012.

The current clonal pattern among serogroup Y isolates is further outlined in Figure 3 and 4 where the *PorA* types P1.5–2,10–1,36–2 and P1.5–1,2–2,36–2, FetA VR F4–1 and 5–8, *fHbp* allele 25 and ST-23 are the most frequent. Furthermore, 29/57 (51%) of all serogroup C isolates have *PorA* type P1.5,2,36–2, FetA VR F3–3, *fHbp* allele 22 and ST-11 (Figures 3 and 4).

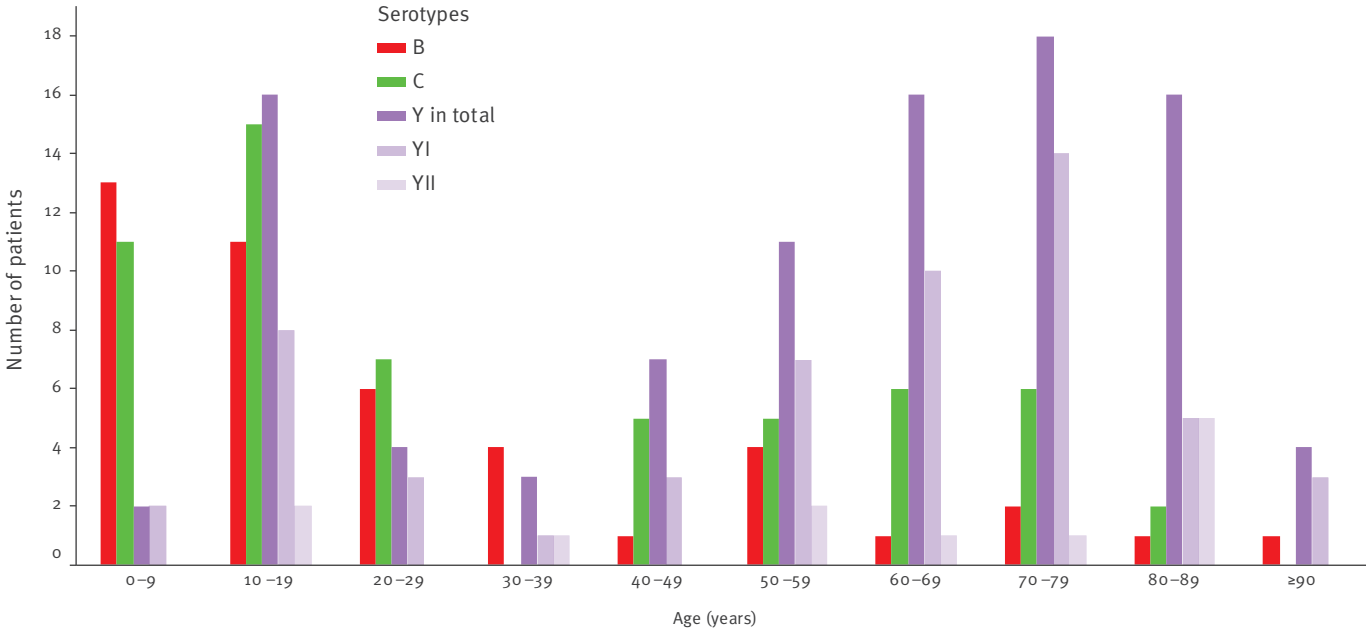
The age distribution of patients with IMD during 2010 to 2012 is shown in Figure 5. Serogroup B was most common among children (median age: 19 years; inter quartile range (IQR): 7–33), serogroup C among young adults (median age: 20 years; IQR: 17–59) and serogroup Y among older adults (median age: 64 years; IQR: 37–77). The figure shows that among IMD caused by serogroup Y during this time period, the genetically defined predominant clone YI and clone YII are mainly common among an older age group compared to the other serogroup Y isolates. The mortality rate among patients with IMD caused by serogroup Y from 2010 to 2012 was 6/97 (6%; 95% CI: 1.4–11%) compared to the total mortality rate due to all serogroups combined during the same time period which was 18/208 (9%; 95% CI: 5–12%]). The IMD mortality rate in Sweden during 2000 to 2009 was 67/569 (12%; 95% CI: 9–14%).

## Resolving power of molecular typing methods used for surveillance

The discriminative ability of a MLST and the *porA*, *porB*, *fetA*, *fHbp* and *penA* genes shows that as single targets PorA VR 1, 2, and 3 had the highest Simpson's ID (0.849; 95% CI: 0.811–0.886) and serogroup had the lowest (0.664; 95% CI: 0.628–0.700]). Among combinations of four, on the basis of serogroup, *porA* and MLST, this combination including *fetA* had the highest

**Figure 5**

Age distribution of patients with invasive meningococcal disease caused by *Neisseria meningitidis* serogroups B, C, Y, the genetically defined predominant serogroup Y clone YI and the second most common serogroup Y clone (YII) in Sweden, 2010–2012 (n=208)




ID (0.956; 95% CI: 0.940–0.972) and serogroup, *porA* and MLST including *fHbp* had the lowest (0.950; 95% CI: 0.933–0.967).

## Outbreak investigations

A spatiotemporal association was defined as isolates of the same serogroup, collected in the same central county within a timeframe of one month. Of the 208 *N. meningitidis* invasive isolates from 2010 to 2012, 35 isolates were spatiotemporally associated in 16 different clusters and represented potential outbreaks. There were 13 clusters of two isolates and three clusters of three isolates. Eight of the spatiotemporal clusters (cluster nr 1–8 in Figure 6) did not share a common *PorA* type within the clusters and the cases had not been in direct contact with each other and therefore there were no further outbreak investigations. In spatiotemporal clusters nr 10–13, the isolates were identical within each cluster regarding serogroup and all 12 target genes (Figure 6), but no connection between cases was found and the isolates were separated by HV-MLVA. Within each of the three spatiotemporal serogroup B clusters 14–16 (Figure 6) the isolates were identical regarding all 12 sequenced genes (the isolates in cluster 14 all had one insertion in *aroE* allele 9 but were still regarded as belonging to ST-41). In addition, the isolates were clustered together in the HV-MLVA when MLVA types did not differ in more than one VNTR locus (single-locus variant, SLV). However, spatiotemporal cluster 14 and 16 were the only spatiotemporal clusters which had confirmed connections between cases. The HV-MLVA results from all 208 isolates showed another seven HV-MLVA clusters comprising fifteen isolates in total (if SLVs were allowed), of which four did have identical genetic profiles, but none of them shared a spatiotemporal link and were therefore not included in Figure 6.

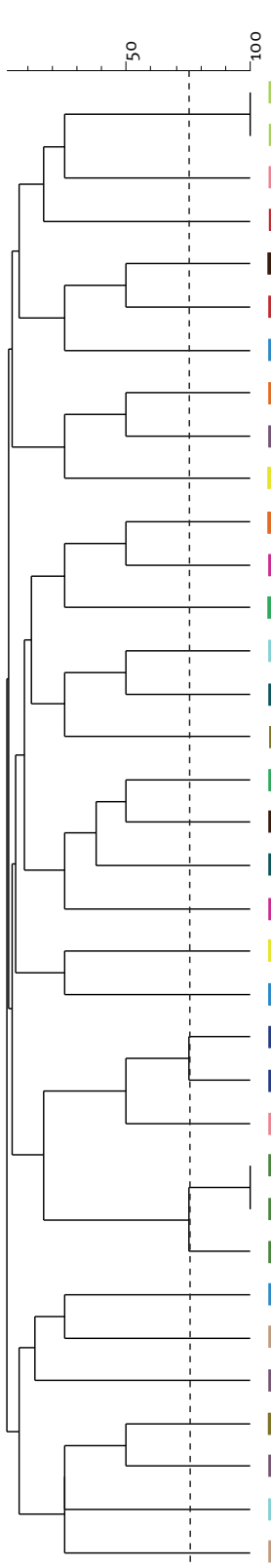
## Discussion

This study aimed to describe the epidemiology of invasive *N. meningitidis* isolates in Sweden between 2010 and 2012, specifically the dominating serogroup Y, and to identify an optimal molecular typing scheme with appropriate resolution power for both surveillance and outbreak investigations in low-endemic areas. Although whole genome sequencing is becoming more cost beneficial than traditional Sanger sequencing per isolate, optimal molecular typing schemes will still be important when fast results are needed, and for smaller laboratories that lack the need of a next generation sequencer. All isolates causing IMD in Sweden during 2010, 2011 and 2012 were characterised by capsular group, MLST, sequencing of the *porA*, *fetA*, *porB*, *penA*, *fHbp* genes and a MLVA using four highly variable loci.

The epidemiology in Sweden has changed most notably with an increase of IMD between 2010 and 2012. IMD caused by serogroup C has declined slightly from 2010 to 2011 and 2012, and serogroup B somewhat increased (Figure 1). The genetic characterisation of circulating *N. meningitidis* causing IMD shows that serogroup Y was the most prevalent in Sweden, and the previously genetically described predominant clone YI [7] was still dominating among serogroup Y strains. However, the overall IMD incidence is still low and although a vaccine against serogroup Y is available, it is probably not

## Figure 6

Dendrogram of invasive *Neisseria meningitidis* isolates generated from a highly variable multilocus variable-number tandem repeat analysis, Sweden, 2010–2012 (n=35 isolates)




| Spatiotemporal cluster | SG | PorA VR1, VR2, VR3 | FetA VR | ST | *'porB* | *fHbp* | *penA* |
|---|---|---|---|---|---|---|---|
| 16 | B | P1.17-1,23-3,37 | F1-34 | 1127 | 3-37 | 14 | 391 |
| 16 | B | P1.17-1,23-3,37 | F1-34 | 1127 | 3-37 | 14 | 391 |
| 10 | B | P1.7,16,35 | F3-3 | 32 | 3-24 | 1 | 3 |
| 6 | Y | P1.5-1,2-19,36-2 | F5-8 | 10211 | 3-36 | 25 | 1 |
| 2 | Y | P1.5-1,2-2,36-2 | F5-8 | 23 | 2-55 | 25 | 22 |
| 6 | Y | P1.5-1,2-2,36-2 | F1-18 | 10210 | 2-55 | 25 | 22 |
| 7 | C | P1.7,16-29,35 | F3-3 | 32 | 3-24 | 1 | 3 |
| 12 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 11 | Y | P1.5-1,10-1,36-2 | F4-1 | 1655 | 3-117 | 25 | 22 |
| 3 | B | P1.7,16-66,35 | F5-69 | 2796 | 3-24 | 1 | 15 |
| 12 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 8 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 1 | C | P1.7-2,16-29,35 | F3-3 | 8876 | 3-24 | 1 | 3 |
| 13 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 9 | Y | P1.5-2,10-1,36-2 | F4-1 | 10098 | 3-455 | 25 | 22 |
| 5 | C | P1.7-2,16-29,35 | F1-49 | 11 | 3-24 | 1 | 3 |
| 1 | C | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 2 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 9 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-35 | 25 | 392 |
| 8 | Y | P1.5-2,10-12,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 3 | B | P1.5-2,10-12,36-2 | F3-3 | 32 | 3-1 | 1 | 3 |
| 7 | C | P1.5-1,10-8,36-2 | F3-6 | 11 | 2-223 | 669 | 2 |
| 15 | B | P1.7,16-32,35 | F3-3 | 32 | 3-82 | 1 | 3 |
| 15 | B | P1.7,16-32,35 | F3-3 | 32 | 3-82 | 1 | 3 |
| 10 | B | P1.7,16,35 | F3-3 | 32 | 3-24 | 1 | 3 |
| 14 | B | P1.7-20,-,37 | F1-5 | 41 | 3-64 | 4 | 368 |
| 14 | B | P1.7-20,-,37 | F1-5 | 41 | 3-64 | 4 | 368 |
| 14 | B | P1.7-20,-,37 | F1-5 | 41 | 3-64 | 4 | 368 |
| 7 | C | P1.5,2,36-2 | F3-3 | 11 | 2-2 | 22 | 1 |
| 4 | C | P1.7,16-29,35 | F3-3 | 32 | 3-24 | 1 | 3 |
| 11 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 5 | C | P1.5-2,10-1,36-2 | F5-8 | 10209 | 2-224 | 380 | 1 |
| 11 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 13 | Y | P1.5-2,10-1,36-2 | F4-1 | 23 | 3-36 | 25 | 22 |
| 4 | C | P1.5,2,36-2 | F3-3 | 11 | 2-2 | 22 | 1 |

FetA: Ferric enterobactin transport protein A; *PorA*: Porin A; SG: serogroup; ST: sequence type; VR: variable region.

A similarity line has been drawn at 75% (maximum one single-locus variant). Only spatiotemporally associated isolates (Sweden, 2010–2012) are included and have been designated a spatiotemporal cluster number 1–16. Additional information about serogroup and allele or VR from sequencing the *porA, fetA, 'porB* (partial fragment), *fHbp* and *penA* genes and the ST from the multilocus sequence typing is provided for each strain.

justifiable to change the vaccine policy in Sweden which currently does not include any vaccines against IMD in the general vaccination programme. Moreover, a shift of sulfamethoxazole susceptibility has occurred sometime around 2010 where isolates otherwise identical to the predominant serogroup Y clone instead are susceptible to sulfamethoxazole. Sequencing of the *folP* gene in a representative collection of the serogroup Y isolates in this study has not shown any of the mutations previously associated with sulphonamide resistance (data not shown) [27,28]. Investigating larger parts of the genome, including differences in expression, can possibly elucidate the mechanisms responsible for this sulfamethoxazole susceptibility shift. Like serogroup Y, serogroup C also presented a fairly clonal pattern whereas serogroup B isolates were rather genetically heterogeneous (Figure 3 and 4).

The age distribution of patients with IMD in Sweden during 2010 to 2012 regarding serogroups B and C is similar to the age incidence pattern during the period from 1995 to 2009 where serogroup B is most common among patients under 10 years of age and serogroup C is most common in the age group including 10 to 19 year-olds (data not shown). The mean age among patients with IMD caused by serogroup Y in 2010 to 2012, 58.9 years, has somewhat increased compared to the mean age of 54.5 years for this serogroup in 2000 to 2009 (data not shown). A considerably lower average age of serogroup Y patients in 2011 has been reported in Denmark (26 years), France (20 years), Italy (26.9 years), Portugal (15.5 years) and Spain (31 years) [9]. Further studies need to be performed to give a clearer picture as to whether the virulence or transmissibility is increased in the predominant serogroup Y clone. Moreover, the mortality rate for IMD has somewhat decreased during 2010 to 2012 compared to the mortality rate during 2000 to 2009, however this was not statistically significant. The mortality rate among serogroup Y cases has also decreased slightly between the periods 2000 to 2010 and 2010 to 2012, however the difference was again not statistically significant. The decrease may be partly due to the recent small decrease in median age among patients with IMD caused by meningococci belonging to this serogroup.

The resolving power of a molecular typing method is recommended to have a discrimination level of at least 0.90 [25]. Serogroup:*porA*:*fetA* typing has previously shown a value of 0.963 [29] which is similar to the discrimination index achieved in this study (0.952; 95% CI: 0.935–0.969) with the same typing targets (data not shown). Although serogroup:*porA*:*fetA*:ST had the highest index of diversity achieved in this study, our results show that replacing the *fetA* gene with *fHbp* only reduces the discrimination ability by 0.006 (no statistical significant difference). Concurrently, Lucidarme et al. [30] investigated the comparability between *fetA* and *fHbp* in terms of diversity, after it had been recommended in England and Wales to additionally incorporate *fHbp* for routine genotypic surveillance. Their study (on 613 invasive isolates) actually showed that *fHbp* had significantly (non-overlapping 95% CIs) better resolving power than *fetA*. These findings indicate that the level of discrimination gained from *fHbp* is partially complementary to that of *fetA*. This could strengthen the argument that considering labour and cost, and with regard of the new serogroup B vaccines, it would be beneficial to substitute the current routine marker *fetA* with *fHbp*.

In terms of outbreak investigations, HV-MLVA detected three small clusters with spatiotemporal connections and identical genetic profiles (spatiotemporal clusters 14–16 in Figure 6), which further supports that these strains were truly involved in small outbreaks. Although no connection could be confirmed between cases in spatiotemporal cluster 15, the cases were both of similar age and from the same county and thus the cases could still have been related.

Fifteen isolates in the present study were clustered in seven HV-MLVA clusters without having a spatiotemporal connection (data not shown). However, without a spatiotemporal connection, these would normally never have been subjected to a HV-MLVA analysis. Moreover, the results of the HV-MLVA suggest that, after identifying the capsular group and receiving clinical data, it could have been sufficient to perform only the HV-MLVA to get indications of potential outbreaks. In an outbreak situation where time is of high importance, not having to await the results of PorA typing or whole genome sequencing for the subsequent HV-MLVA analysis would be beneficial.

We have previously compared HV-MLVA to rep-PCR with the DiversiLab system and DNA sequencing on invasive serogroup C isolates which showed that HV-MLVA helped strengthen all of the spatiotemporal linkages [19]. The use of MLVA to trace transmission has been deemed questionable due to the low stability of VNTRs. Transmission-dependent variation of tandem repeats in meningococci has been investigated by Elias et al. [31] in a study using four highly variable VNTR loci together with another eight standard MLVA loci. The observed overall variation was considerably smaller than predicted and the method was considered most useful for outbreaks containing few transmissions. Considering this, HV-MLVA could be valuable in low-endemic areas such as Sweden where outbreaks are fairly rare and connected cases usually only consists of no more than three contacts, as shown by our results. A fast detailed typing of spatiotemporally linked cases which can separate outbreaks from sporadic cases is important to inform public health measures to control IMD, such as deciding whether or not to offer prophylaxis in the form of antibiotics or vaccines.

In summary, serogroup Y was found to be the most prevalent serogroup in Sweden, and the previously genetically described predominant clone YI [7] (Y: P1.5–2,10–1,36–2: F4–1: ST-23(cc23), '*porB* allele 3–36,

*fHbp* allele 25 and *penA* allele 22) is still dominating. However, a sulfamethoxazole susceptibility subvariant of the clone appears to have emerged in 2010, and in 2011 represented 11/22 (50%) of the genetically defined predominant clone YI isolates. Our study supports previous studies that suggests that the FetA typing could be replaced by FHbp in the recommended designation (serogroup:*porA*:*fetA*:ST(CC)), which may be more suitable in the current vaccine era. Furthermore, this study including the additional serogroups A, B, E, W and Y, strengthens previous results on serogroup C isolates [19] suggesting that HV-MLVA is a first-hand rapid method for investigating outbreaks with few transmission events, where isolates have an identical serogroup and a common spatiotemporal connection.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## Conflict of interest

None declared.

## Author's contributions

Bianca Törös, Sara Thulin Hedberg and Paula Mölling were mainly responsible for the design and supervision of the study. Bianca Törös and Susanne Jacobsson performed the laboratory work. Bianca Törös analysed the results and all authors were involved in the discussion of the results. Bianca Törös drafted the paper and Sara Thulin Hedberg, Susanne Jacobsson, Hans Fredlund, Per Olcén and Paula Mölling revised the paper.

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