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Invasive meningococcal disease in Norway in the two decades before the COVID-19 pandemic

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ABSTRACT

Objectives: Disease caused by the bacterium *Neisseria meningitidis* remains a worldwide public health challenge, despite the steadily decreasing incidence in Western countries. The objective of this study was to explore the epidemiology of invasive meningococcal disease in Norway over the last two decades.
Design: All isolates sent to the National Reference Laboratory from patients with invasive meningococcal disease between the years 2000 and 2019 were analyzed using whole genome sequencing (total: 625).
Results: A five-fold decrease in case numbers occurred over this period, and the situation has gone from being dominated by serogroup B to one where serogroups Y and W are more prevalent. Concurrently, the mean age at infection has increased from 18 to 33 years. Among the 350 serogroup B isolates, 87% were an exact match or cross-reactive with one or both the currently available serogroup B vaccines, but the proportion decreased in the past decade. Core genome analyses revealed a high variation in the number of allelic differences accumulated in epidemiologically linked isolates to the point that near-identical isolates were found several years apart.
Conclusion: Allelic distance is an imprecise metric for the degree of epidemiologic linkage between isolates in *N. meningitidis*.

Introduction

Despite the availability of vaccines, invasive meningococcal disease (IMD) is still a major public health concern worldwide. The clinical forms of disease caused by the gram-negative bacterium, *Neisseria meningitidis,* include principally meningitis and/or septicemia and, less frequently, pneumonia, arthritis, and gastrointestinal disorders [1]. With a case fatality rate of about 10% and severe neurological sequelae reported in approximately 10-20% of survivors, IMD is a severe burden both for the affected individuals and for the society [2,3].

The polysaccharide capsule of *N. meningitidis* is the main virulence factor, with nonencapsulated meningococci rarely causing disease in immunocompetent individuals. Of the 12 serogroups defined based on the immunochemical structure of the capsule, six (A, B, C, W, Y, and X) are responsible for nearly all IMD cases worldwide. The serogroup-specific conjugate meningococcal vaccines have been developed against A, C, W, and Y disease and are under development against serogroup X strains [4]. Because the serogroup B capsular polysaccharide is not a suitable vaccine antigen, vaccines against serogroup B disease are targeting proteins that have the inconvenience of being variable. Thus, serogroup B vaccines have necessitated the use of combinations of several proteins or several variants of the same protein to improve strain coverage [5].

In Norway, the incidence of IMD peaked in 1983, with 8.9 cases per 100,000, mostly resulting from a hyperendemic wave caused by a serogroup B strain [6] belonging to the clonal complex (CC) 32, as defined by multilocus sequence typing (MLST) [7]. Since then, the incidence has gradually decreased to 0.3 cases per 100,000 in 2019 and has continued to decline during the pandemic caused by SARS-CoV-2. Almost all IMD cases in Norway are isolated cases, with only 1-3% being part of a local cluster. The following measures are implemented around each case: look for co-primary cases, inform the population, eradicate carriage, and vaccinate the index case and close-contacts. In the event of a local cluster, the range of vac-

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Table 1
Isolates assumed to be linked based on occurrence in time (within 3 months) and geography (same region) before the whole genome sequencing analysis.

Cluster	Year of Isolation	No. of isolates	CC	ST	Geographic region	Time interval (in days)	No. of allele differences
1	2000	2	32	1332	Eastern	56	15
2	2000	2	41/44	1127	Northern	1	3
3	2001	4	32	32	Eastern	3	5-35
4	2001	2	11	11	Eastern	2	9
5	2001	2	11	11	Eastern	33	23
6	2002	2	32	34	Western	5	6
7	2002	2	11	11	Eastern	17	9
8	2002	2	11	11	Eastern	50	6
9	2003	2	UA	2884	Southern	7	11
10	2005	2	41/44	44	Western	53	12
11	2007	2	41/44	44	Western	81	21
12	2008	2	23	6973	Eastern	55	3
13	2008	2	11	11	Western	8	16
14	2009	2	23	23	Eastern	26	15
15	2009	2	41/44	41	Western	7	47
16	2009	2	11	11	Western	67	20
17	2009	2	23	4183	Northern	91	18
18	2009-10	2	11	11	Eastern	88	31
19	2010	2	32	32	Western	0	9
20	2010	3	11	11	Western	55-88	8-15
21	2010	2	23	23	Eastern	3	19
22	2011	2	23	23	Northern	63	42
23	2012	2	11	11	Western	9	35
24	2012	2	11	11	Eastern	5	27
25	2013	3	11	11	Middle	8-38	15-39
26	2013	4	23	23	Eastern	7-73	5-33
27	2013	2	11	11	Eastern	10	5
28	2016	2	11	11	Eastern	3	20
29	2017	2	23	1655	Eastern	57	18
30	2017	2	23	23	Eastern	40	25
31	2018	2	23	23	Eastern	1	6
32	2018	2	11	11[a]	Western	87	38

[a] One of the isolates was ST-14792, differing from ST-11 at the *pgm* locus by two nucleotides only.CC, clonal complex; ST, sequence type; UA, unassigned.

cinated contacts may be extended and a carriage study may be undertaken (https://www.fhi.no/nettpub/smittevernveilederen/sykdommer-a-a/meningokokksykdom-veileder-for-he/).

In the current study, we report the genetic changes in the meningococcal population in Norway associated with the decrease in incidence, using whole genome sequences from a collection of 625 IMD isolates collected by the National Reference Laboratory for meningococci at the Norwegian Institute of Public Health over the period 2000 to 2019. Using epidemiological data, we identified clusters of potentially related cases and analyzed the genomic changes that have occurred between the involved isolates. We showed that the number of allelic differences between isolates of *N. meningitidis* is an imprecise measure of their epidemiologic linkage.

Materials and methods

Clinical isolates

The Norwegian Institute of Public Health hosts the National Reference Laboratory for meningococci and receives all cultured cases from Norway. A total of 628 meningococcal isolates were available for analysis, representing 86% of all IMD cases reported to the Norwegian Surveillance System for Communicable Diseases (MSIS, http://www.msis.no/) during the period; the remaining cases being diagnosed mainly by polymerase chain reaction and, for a very few cases, by microscopy and/or clinical manifestation only. The isolates were serogrouped by slide agglutination immediately upon receipt using Remel polyvalent and monovalent antisera (Thermo Fischer, Norway).

Table 2
Days apart in recovery of isolates suggested to be linked based on whole genome sequencing using a 5-, 10-, or a 25-loci difference in the cg multilocus ST according to CC.

No. of loci	CC	No. of pairs	No. of days between cases
≤5	CC11	5	10, 11, 150, 307, 398
	CC23	5	55,73, 955, 1004, 1959
	CC32	1	1
	CC41/44	3	1, 71, 273
	CC174	1	5
	CC226	1	90
6-10	CC11	25	2, 4, 5, 7, 9, 9, 17, 50, 71, 99, 100, 153, 168, 184, 298, 302, 309, 315, 361, 407, 409, 425, 436, 468, 834
	CC23	8	1, 45, 326, 390, 532, 583, 606, 661
	CC32	5	0, 1, 2, 50, 140
	CC41/44	4	66, 332, 361,1032
11-25	CC11	86	3-1943
	CC23	62	1-3393
	CC32	19	8-1957
	CC41/44	10	53-1341

CC, clonal complex; ST, sequence type.

Epidemiological data

Patient sex, age, and county of residency were obtained from MSIS (Supplementary Table S1). This information was used to identify clusters of potentially related cases: isolates with the same serogroup recovered from patients in the same region of Norway within a period of 3 months or less were assigned to a cluster (Table 1).

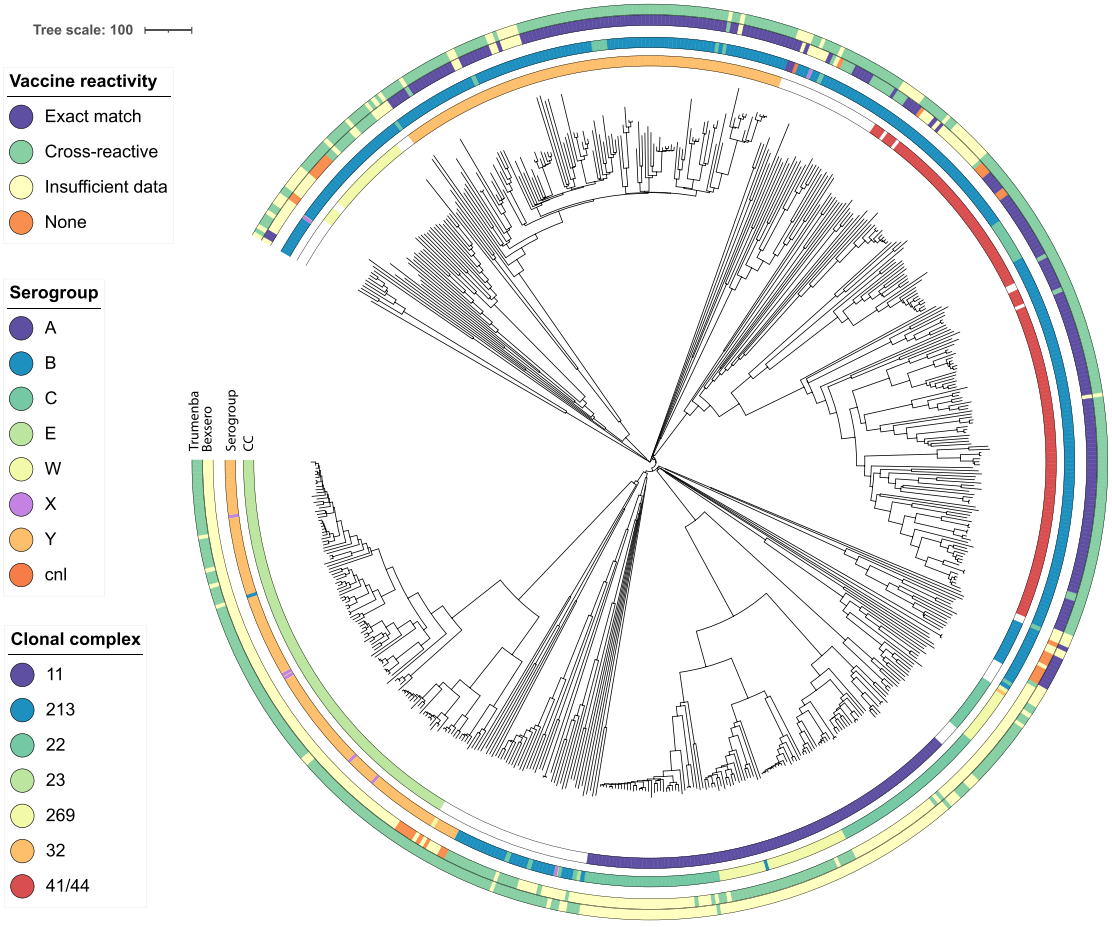


Figure 1. Phylogenetic tree based on cgMLST of all isolates included in this study. The rings are, from inner ro outer: CC - clonal complex, annotated only for clonal complexes with 10 or more isolates; serogroup; Bexsero reactivity MenDeVar index; Trumenba reactivity MenDeVar index.

Sequencing

The isolates were grown overnight at 37°C in an atmosphere of 5% CO_2 on chocolate blood agar plates before DNA extraction. DNA was extracted using MagNA Pure 96 (Roche Life Science) and DNA sequencing libraries were prepared from the extracted DNA using KAPA HyperPlus kits (Roche Life Science) with NEXTflex DNA barcodes (Bioo Scientific), following the manufacturer's instructions. The DNA libraries were sequenced on the MiSeq or NextSeq sequencing platform (Illumina) using the v2 500 cycles and the v3 600 cycles or the midoutput 300-cycle reagent kits (Illumina), respectively, following the manufacturer's instructions.

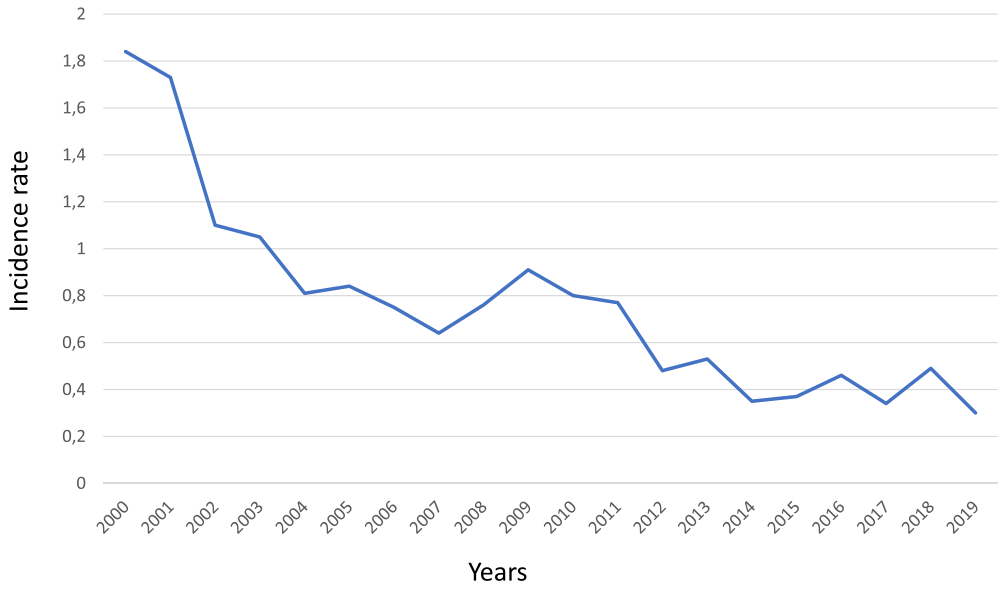
Genome analyses

Raw Illumina reads were quality controlled with FastQC v0.11.9, Kraken v1.1.1 (database version October 2017) and MultiQC v1.9 [8–10]. Three isolates were excluded due to low sequencing yield. Sequences of the remaining 625 isolates were trimmed using Trimmomatic v0.39 [11] and assembled *de novo* using SPAdes v3.14.1 [12] in «isolate» mode. The assemblies were further filtered to remove contigs with a kmer-coverage <3 and length <500 nucleotides.

All assemblies were uploaded to the *Neisseria* PubMLST database [13] (https://pubmlst.org/bigsdb?db=pubmlst_neisseria_isolates). The accession numbers can be found in Supplementary Table S1. We used the *N. meningitidis* cgMLST v1 scheme [14] to produce distance matrixes among all isolates, ignoring incomplete and paralogous loci, but counting the missing loci as true differences. We additionally validated that there was no relationship between data quality (measured as sequencing depth and base quality) and the number of missing loci because such a relationship would confound the analyses of relatedness. The BIONJ algorithm [15] was used to produce phylogenetic trees. Trees were rooted at midpoint. All statistical analyses were done in R v3.6.3, with the following additional packages: ggplot2 v3.3.5, ape v5.4.1, cowplot v1.1.0, forcats v0.5.0, dplyr v1.0.0, plyr v1.8.6, Hmisc v4.5-0, tidyverse v1.3.0, readr v1.3.1 [16–21]. Figure 1 was made using Interactive Tree of Life [22].

Results

During the period of the study, the IMD incidence decreased from 1.84 per 100,000 in 2000 to 0.30 in 2019 (Figure 2). A total of 625 isolates collected from patients with IMD from 2000 to 2019 were included in the analyses, representing 86% (625 of 729) of the total number of IMD cases reported to MSIS. Of the 625 isolates, 296 (47.4%) were from Eastern Norway, 169 (26.9%) from Western, 60 (9.6%) from northern, 58 (9.3%) from middle, and 35 (5.6%) from southern Norway (Supplementary Figure S1). One isolate came from the offshore, artic archipelago of Svalbard. For six isolates, information about the geographical origin was missing. Sexes were evenly distributed, with 322 cases among females and 303 among males. The number of cases per year ranged from 73 (year 2000) to 10 (year 2015). There was a clear downward trend over time in the number of cases per year (t-test, P <0.0001).



No. of analysed isolates	73	65	45	46	27	34	30	27	29	37	36	32	20	23	12	10	22	17	24	16
No. of registered cases	83	78	50	48	37	39	35	30	36	44	39	38	24	27	18	19	24	18	26	16

Figure 2. Incidence of invasive meningococcal disease in Norway, 2000-2019.

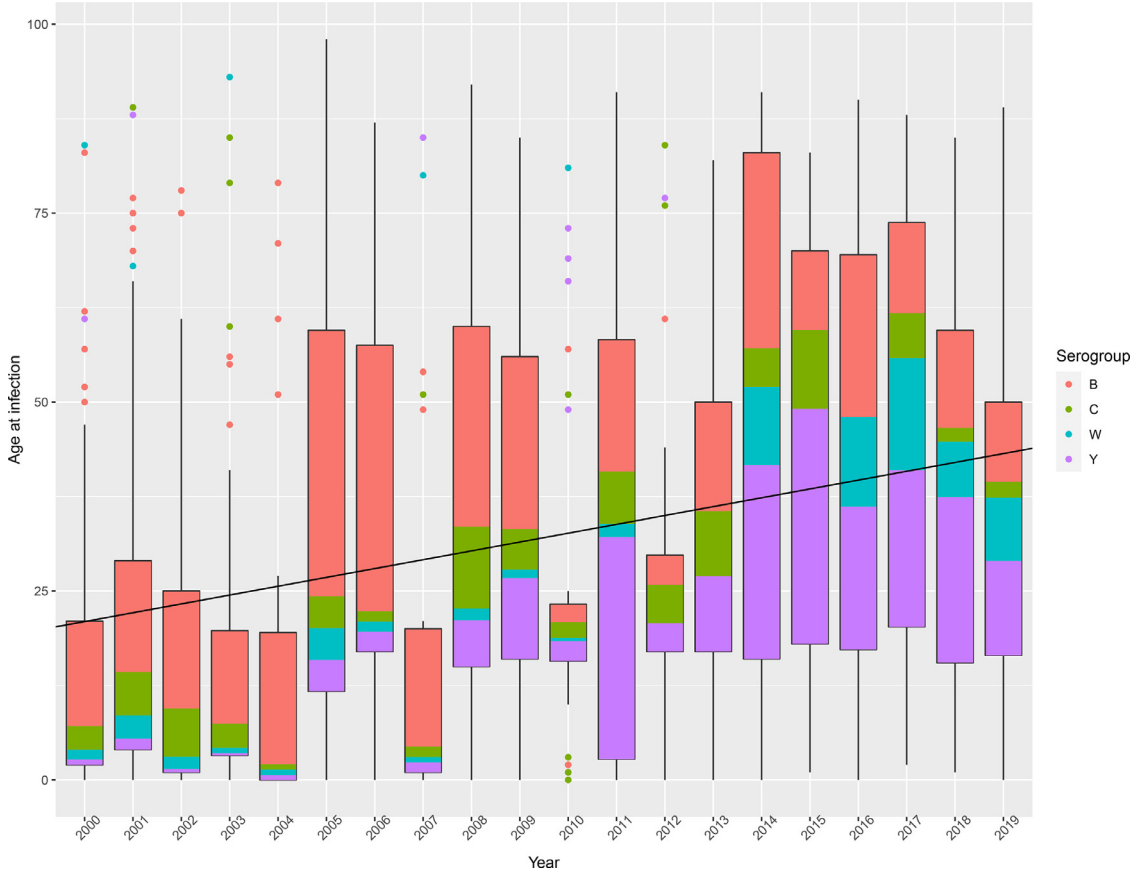


Figure 3. Boxplot of patient age of infection over the period 2000-2019. The line corresponds to the best-fitting linear regression model of (mean) age of infection. Additionally, the relative areas of the boxes correspond to the composition of the serogroups B, C, W and Y.

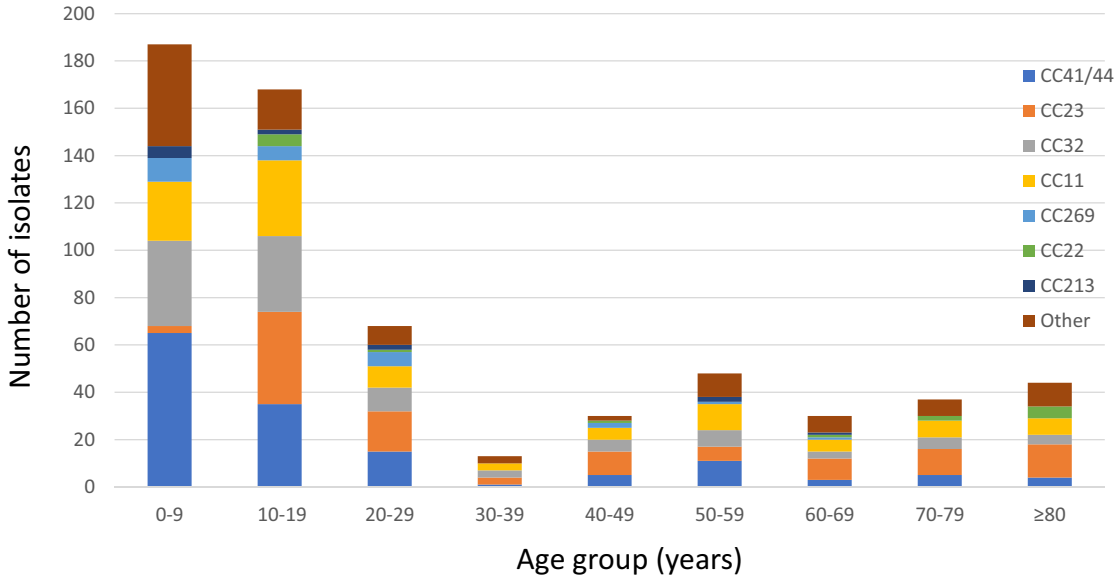


Figure 4. CCs of the 625 meningococcal isolates according to age of the patients.
CC, clonal complex.

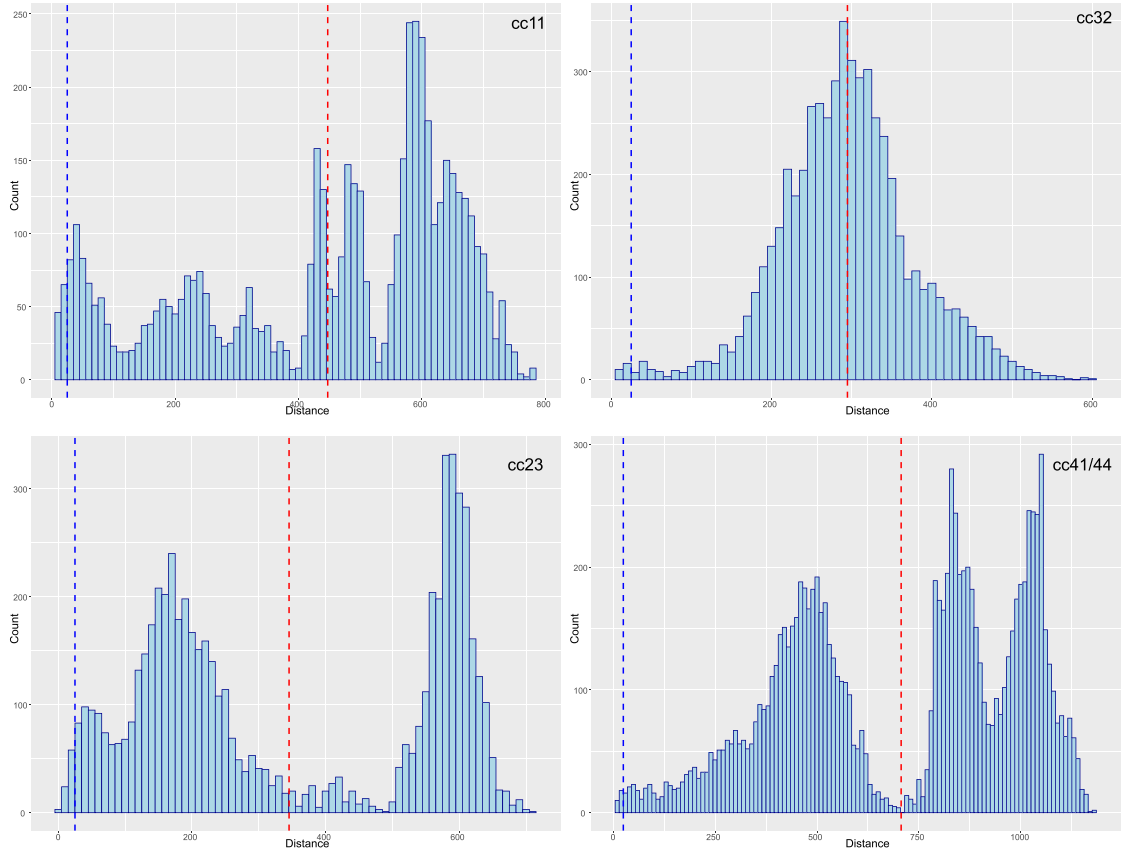


Figure 5. Distribution of pairwise distances, as measured through cgMLST, for the major CCs 11, 23, 32 and 41/44.
CC, clonal complex; MLST, multilocus sequence type..

There was also a clear shift in the mean age at infection over this period (*t*-test, $P < 0.0001$), with the mean age being 17.6 years in 2000 and 32.8 years in 2019 (Figure 3). Because the mean age can be severely influenced by outliers, we also ran a median regression: the median age at infection was 8 years in 2000 and 21.5 years in 2019, and the model fit indicated a median yearly increase in age at infection of 0.66 years (95% confidence interval 0.15-1.32).

The most common clonal complexes were, in decreasing order, CC41/44 (144; 23%), CC23 (112; 18%), CC32 (105; 17%), CC11 (104;

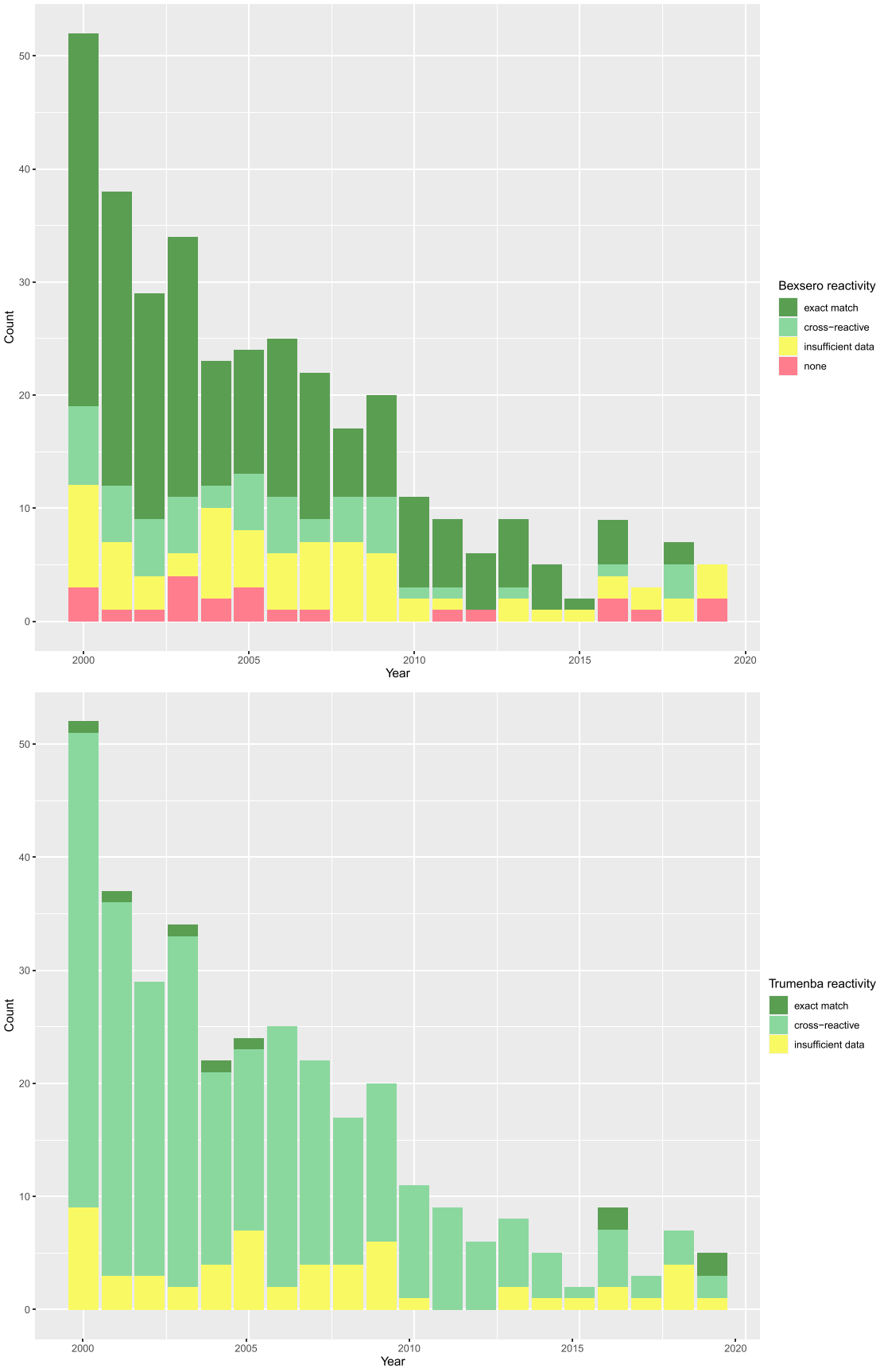


Figure 6. Bexsero (panel a) and Trumenba (panel b) MenDeVAR index over time toward serogroup B isolates specifically.

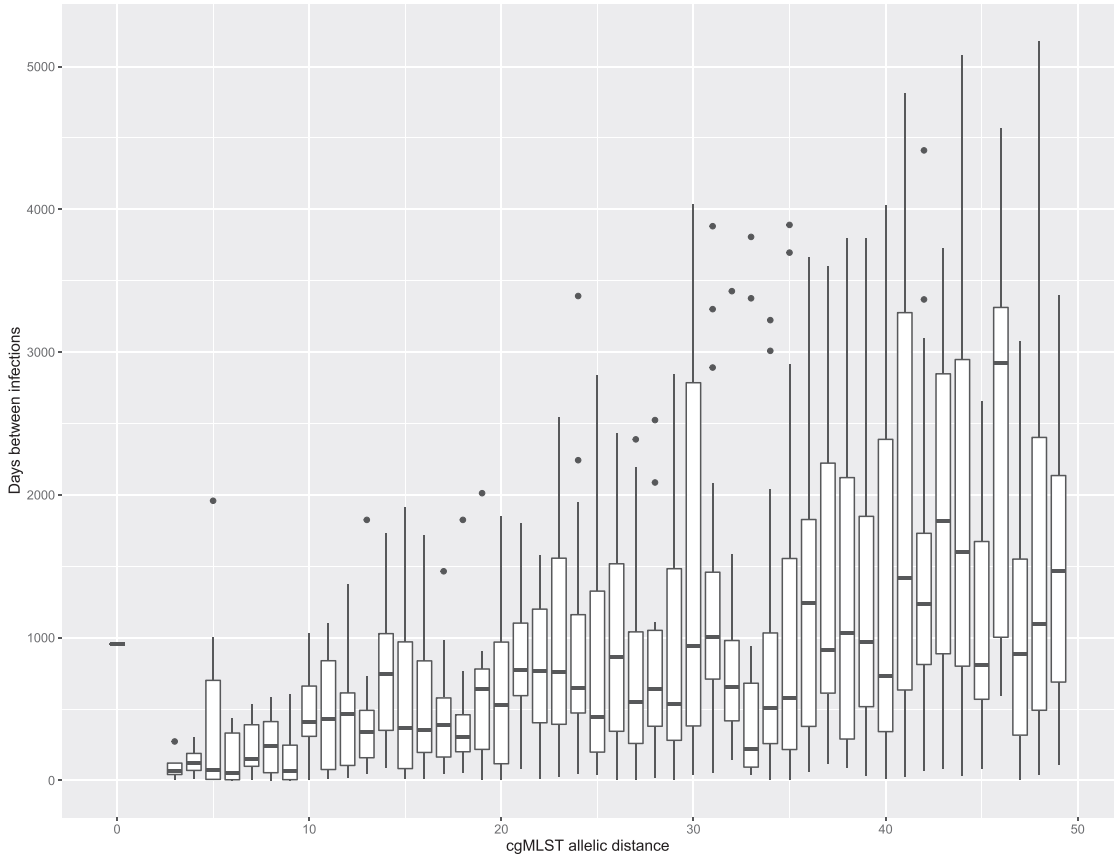


Figure 7. Pairwise allelic distances, as measured through cgMLST, plotted against the number of days between the time of infection for the pair constituents. MLST, multilocus sequence type.

17%), CC269 (26; 4%), CC22 (15; 2%), and CC213 (12; 2%). The remaining isolates belonged to CCs with 10 or fewer cases (51; 8%) or had a sequence type that did not belong in any defined CC (56; 9%) (Figure 1). CC41/44 and CC32 dominated among those aged 0-9 years, whereas the major clonal complexes were more evenly distributed in the other age groups (Figure 4). By examining the distribution of pairwise distances, it became clear that several of the major CCs were composed of several finer-scale clones (Figure 5). For example, CC41/44 clearly had three clusters of equidistant isolate pairs, corresponding to the three major branches on the phylogenetic tree in Figure 3. Likewise, CC23 consisted of two major clusters and CC11 of six to eight clones with small intracluster distances. Of the major clonal complexes in Norway, only CC32 had the structure of a largely homogenous cluster. In general, CC11 had the highest number of clustered isolate pairs, followed by CC23, CC32, and finally, CC41/44, with respectively 100, 77, 25, and 24 pairs with an allelic distance of less than 25. This means that among the 104 CC11 isolates collected over a 20-year period, 100 (1.87%) pairs differed by less than 25 of the 1605 cgMLST genes, whereas among the 144 CC41/44 isolates, only 24 (0.23%) pairs differed by less than 25 genes.

The genogroups (capsule groups) were determined *in silico:* 350 (56%) isolates were genogroup B, 113 (18%) were genogroup Y, 102 (16%) were genogroup C, and 45 (7%) were genogroup W. In addition, there were two genogroup A, two genogroup X, one genogroup E, and one capsule null (*cnl)* isolates, the last one from a patient with complement deficiency. The capsule type could not be defined *in silico* for nine isolates, but serogrouping with antisera revealed that of these, two were serogroup X and five were serogroup Y. The final two isolates were nonserogroupable. The capsule group distribution had a clear shift over time, with B being common in the early 2000s and a gradual increase in the proportion of serogroups W and Y toward the later 2010s (Figure 2).

We further explored the theoretical efficacy of the multicomponent recombinant vaccines, Bexsero [23] and Trumenba [24], both of which are designed primarily to prevent serogroup B meningococcal disease, using the deduced vaccine antigen reactivity (MenDeVAR) index [25]. For Bexsero, 222 of all isolates were deemed to have exact matches to vaccine components, 90 were cross-reactive (isolates containing ≥1 antigenic variant are deemed cross-reactive to vaccine variants through experimental studies), and there was no protection from this vaccine toward 32 of the isolates. There were insufficient data on cross-reactivity for 281 of the isolates. For Trumenba, nine were exact matches, 443 were cross-reactive, and there was insufficient data for 173 isolates. For serogroup B isolates only, equivalent numbers were similar: for Bexsero, 202 had exact matches, 52 were cross-reactive, there was insufficient data for 73 isolates, and no protection against 23 isolates; for Trumenba, nine were exact matches, 281 were cross-reactive, and there was insufficient data for 60 isolates. Of the 350 serogroup B isolates, 303 were exact matches or cross-reactive with either vaccine. In general, both vaccines could offer good protection against most variants within the major circulating CCs; although, there was insufficient evidence of reactivity toward some of the variants (Figure 6).

We used the data generated in this study to analyze the power of sequencing for epidemiological purposes relative to traditional outbreak tracking, exploring the definition of an epidemiological pair as two isolates with a cgMLST distance of a maximum of five, 10, and 25 alleles (0.3%, 0.6% and 1.5% of loci, respectively) as a

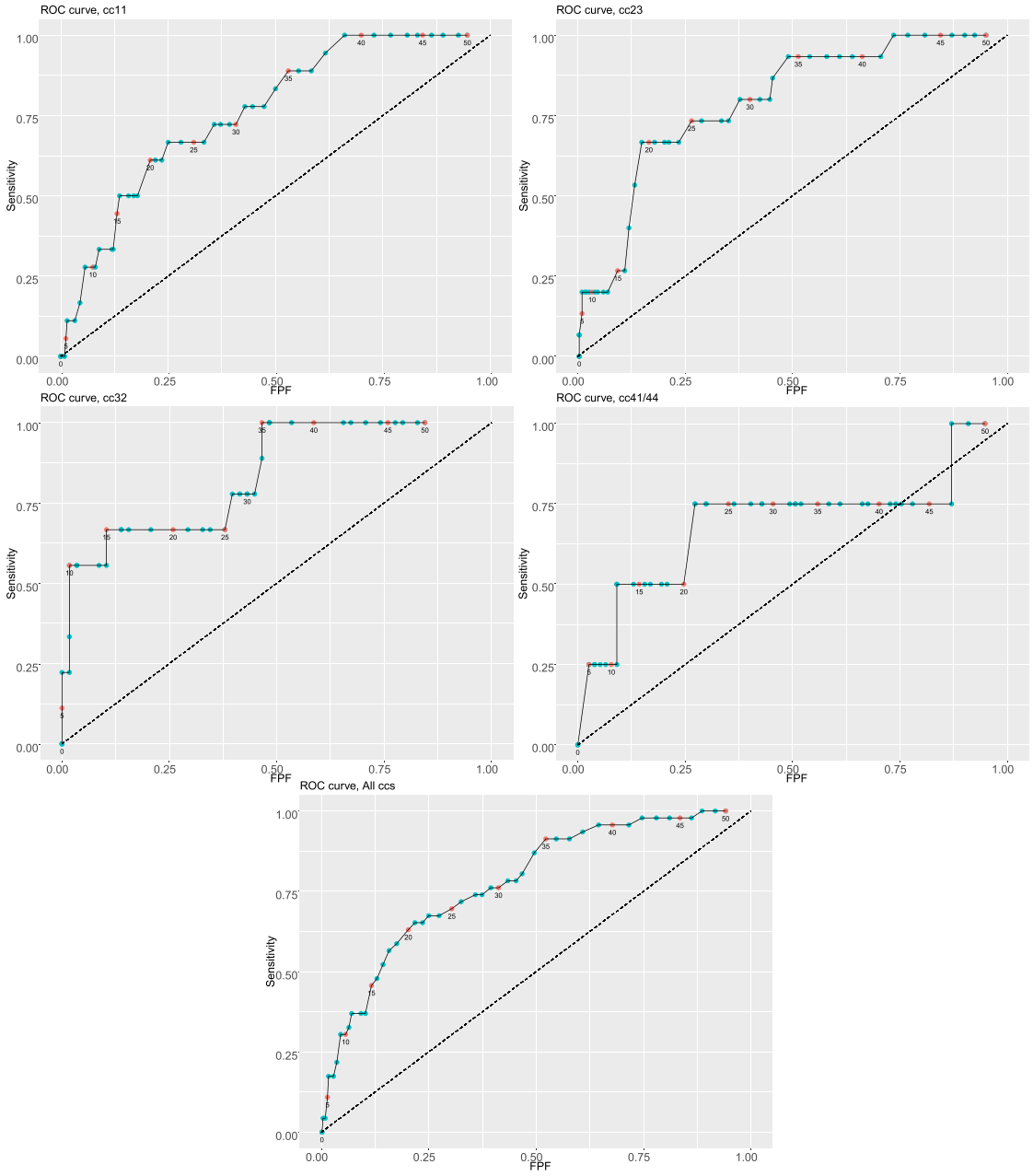


Figure 8. ROC curves showing sensitivity and FPF at various allelic distance thresholds for determining linkage. The top four panels are for CCs 11, 23, 32, and 41/44, whereas the bottom is for all isolates in the study.
CC, clonal complex; FPE, false-positive fraction; ROC, receiver operating characteristic.

cut-off. Using these definitions, we found a total of 16, 58, or 245 potentially linked isolate pairs (Table 2). However, we noticed that the number of days between infections within these isolate pairs varied immensely (Figure 7). In the most extreme example, two isolates had no allelic differences by cgMLST; yet, they were isolated 955 days apart. There were many similar pairs where the time between infections far exceeded what we would expect given the genomic similarity: one CC23 pair had five allelic differences and the time between infections was 1959 days; another CC23 pair of isolates with the same distance gave an invasive disease 1004 days apart. In neither of these cases could this be ascribed to poor sequencing quality, a high fraction of missing loci, or any obvious mix-up. Other pairs displayed the opposite phenomenon: a CC32 pair isolated a single day apart and with known epidemiological link had 35 allelic differences, and a CC41/44 pair isolated 7 days apart had an allelic distance of 47. In conclusion, allelic distance, as assessed by cgMLST, was too variable to be considered clock-like in *N. meningitidis*, and it seems that the strains can sometimes remain largely in evolutionarily stasis, displaying a near-identical allelic profile even after several years have passed. It is therefore paramount to use a holistic approach when determining whether two isolates are epidemiologically linked using meta-information, such as time between infections, geographical information, and additional patient metadata, where available.

Nevertheless, crude assessments of linkage might sometimes have to be done without available metadata, for example, when comparing isolates from different jurisdictions or when metadata are incomplete because they have been deemed sensitive. In such cases, genomic distance-based metrics could be the only information at hand. We plotted the receiver operating characteristic curve

for allelic distance-based cut-offs to explore how distance cut-off values impact sensitivity and false-positive fraction (FPF). To do this, we defined epidemiologically linked isolates in our dataset as any isolate pair having identical sequence type, being isolated within 90 days from each other, and coming from the same geographic area, which was defined as being within 2 hours of driving by car (Table 1). The curves revealed trade-offs between sensitivity and FPF at different allelic distance cut-offs (Figure 8). For example, to flag 75% of pairs that are likely epidemiologically linked, a cgMLST distance of 30 will suffice. This threshold also limits the fraction of false-positives to less than 50%. There were minor differences in optimal cut-off by CC, but 30 alleles seem to be an appropriate universal cut-off that keeps the sensitivity and FPF at suitable values. All assumed epidemiologically linked pairs of isolates in this study had an allelic distance of less than 50. However, defining all isolates with an allelic distance of 50 or less as linked would mean an unacceptably high rate of false-positives (>90%), demonstrating that cgMLST allelic distance alone has limited value for predictions about epidemiological linkage.

Discussion

The meningococcal landscape in Norway has shifted substantially in the two decades before the COVID-19 pandemic. The incidence has dropped five-fold, a continuation of a trend that started in the 1980s: in the period 1977-1987 Norway had 250-350 cases per year, whereas the period 1988-1998 saw 100-150 annual cases. Concurrently, there has been a steady shift in the age of patients. Traditionally affecting young people, with roughly one-third of patients being below age 5 years and another third teenagers, the landscape has changed: the infection is now most common in the middle-aged and elderly. This has been accompanied by a shift from predominantly serogroup B to a mixed occurrence of B, Y, W, and C.

The low disease incidence in recent years and especially in infants and young children has resulted in the recommendation of meningococcal vaccines only for high-risk groups, such as patient contacts, people with complement deficiencies or diseases of the spleen, laboratory personnel, men who have sex with men, teenagers participating in the “russ” celebration (a traditional celebration for Norwegian high school pupils in their final spring semester [https://en.wikipedia.org/wiki/Russefeiring]), or people traveling to high-risk regions of the world. Although the serogroup B-specific Bexsero vaccine has been introduced in the infant immunization program in the United Kingdom from 2015 and is now included in the national immunization program of eight additional European countries [26], Norway is only recommending vaccination of high-risk groups and vaccination for outbreak control (https://www.fhi.no/publ/2014/meningokokksykdom-i-norge-og-anbefa/). Trumenba is licensed for individuals from the age of 10 years. Our study showed that although these serogroup B-specific vaccines do potentially offer some cross-protection against disease caused by different capsule types, there are insufficient data for many of the clones in circulation to fully assess their possible impact. The decrease in serogroup B IMD observed in Norway is parallel to the overall 67% decrease in Europe from a total of 4255 cases reported to the European Center for Disease Prevention and Control in 2000 to 1422 cases in 2019 (https://atlas.ecdc.europa.eu/public/index.aspx?Dataset=27&HealthTopic=36).

We used genomic data for all the IMD isolates from Norway in a 20-year period in an attempt to identify epidemiologically related cases/clusters that may have been missed by traditional epidemiology. We showed that allelic distances based on cgMLST were not well suited to determine the epidemiological linkage between isolates because identical or nearly identical isolates were recovered years apart, whereas the clearly linked isolates (*e.g.*, co-primary cases) could differ at more than 20 alleles. Our finding supports the results of the study from Retchless *et al.* [27] in the United States, who identified very similar isolates collected several years apart and from different states. In the highly transformable species, *N. meningitidis*, the rate of recombination differs between CCs [28], a phenomenon that may explain the stochastic allelic differences between isolates within clusters.

As part of this study, we investigated the value of cgMLST version 1.0 allelic distance as a predictor of epidemiologic linkage. We have been made aware that an updated *N. meningitidis* cgMLST scheme, namely version 2.0, has been made available in PubMLST. This scheme has fewer loci (1422 vs 1605) than version 1.0; so, it is possible that sensitivity and FPF at different cut-offs in this scheme will be different from that presented in this paper. Version 2.0 of the scheme already has a higher number of unique profiles (at the time of writing, 31,539 vs 23,766), indicating a higher variability in the loci included in version 2.0. This would imply that cut-offs should be somewhat increased rather than decreased as one would expect from the reduced number of loci compared. In any case, as we have shown in this paper, the *N. meningitidis* molecular clock, as assessed through cgMLST, is so variable that any conclusion about epidemiological linkage should be very cautiously assessed.

Declaration of competing interest

The authors have no competing interests to declare.

Funding

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Ethical statement

The current work does not involve experimental work with humans.

Acknowledgments

The authors gratefully acknowledge the clinical microbiology laboratories for submitting meningococcal isolates to the National Reference Laboratory and the staff at the reference laboratory for sequencing the isolates.

Author contributions

Conceptualization: DAC. Computational analyses: OBB and KA. Original draft preparation: OBB. Writing, reviewing, and editing the manuscript: DAC, SVW, KA, and OBB.

Data summary

Accession numbers for all sequence reads included in the study are available as supplementary materials, including any metadata used. Norwegian genomic short read sequences are available under ENA study accession PRJEB56004. Epidemiological data were retrieved from the MSIS (http://msis.no/).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.005.

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