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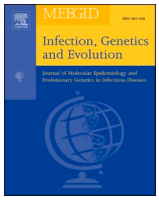

Phylodynamic analysis of a prolonged meningococcal epidemic reveals multiple introductions and pre-epidemic expansion

Zuyu Yang [a], Heather Davies [a], Jane Clapham [a], Liza Lopez [a], Holly B. Bratcher [c], Audrey Tiong [a], Xavier Didelot [b], Martin C.J. Maiden [c], Philip E. Carter [a], Xiaoyun Ren [a,*]

[a] Institute of Environmental Science and Research, Porirua, New Zealand
[b] School of Life Sciences and Department of Statistics, University of Warwick, Coventry CV4 7AL, United Kingdom
[c] Department of Biology, University of Oxford, Oxford, United Kingdom

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ABSTRACT

Neisseria meningitidis is the causative agent of invasive meningococcal disease (IMD), a form of bacterial meningitis and septicaemia, leading to isolated cases, outbreaks, and epidemics worldwide. Between 1991 and 2008, Aotearoa/New Zealand (NZ) experienced a prolonged hyperendemic group B IMD outbreak caused by the NZMenB epidemic strain, belonging to clonal-complex 41/44 (cc41/44) and identified by the PorA variant P1.7–2,4 (B:4:P1.7–2,4:cc41/44). NZMenB continues to account for approximately one-quarter of group B meningococcal disease cases in NZ. To understand NZMenB origin and initiation we used phylodynamic tools to analyse approximately 97 % of all NZMenB isolates submitted to the NZ Meningococcal Reference Laboratory from 1990 to 2019. We found NZMenB can be divided into three major clades: clade41, clade154, and clade42, each with distinct origins and expansion patterns. Our evidence from molecular dating and clonal expansion analysis suggests that NZMenB was circulating and had expanded before the epidemic. Comparison with international data showed multiple importations and re-introductions of NZMenB into NZ, while not suggesting close relationships with international variants. The recent COVID-19 health emergency and differing governmental responses have brought societal and environmental contributions to epidemics and pandemics into focus. We propose the NZMenB epidemic may have been triggered by increasing societal inequality and household crowding resulting from government policies at the time.

1. Introduction

Infectious disease epidemics are complex events influenced by a multitude of societal, human, and pathogen evolutionary factors. Genomic epidemiological modelling can be used to dissect these complex interactions. We applied phylodynamic tools (Didelot et al., 2018; Helekal et al., 2022) to investigate the origin and spread of a prolonged invasive meningococcal disease (IMD) epidemic in Aotearoa/New Zealand (NZ). Phylodynamic analysis integrates phylogenetics, epidemiology, and population models to investigate the spatial and temporal dynamics of pathogens and help understand their spread and evolution.

Invasive meningococcal disease (IMD) caused by the human specific bacterium *Neisseria meningitidis* (the meningococcus) remains a global concern. Meningococci reside as commensals in the nasopharynx of 5 to 30 % of the population (Yazdankhah and Caugant, 2004; Peterson et al., 2018) but, rarely, they invade causing meningitis and/or septicaemia. Variations in the capsular polysaccharide classify meningococci into 12 capsule-groups, of which six (A, B, C, W, X, and Y) are responsible for most IMD (Pardo de Santayana et al., 2023). Meningococci are further classified by clonal-complex (cc) based on multilocus sequence type (MLST, ST) (Maiden et al., 1998) and molecular typing of major antigens such as PorA (Jolley et al., 2007). Different variants show different prevalence and distribution around the world (Pardo de Santayana et al., 2023) influenced by factors not fully understood. It is often postulated that outbreaks and epidemics occur when particular variants are introduced into a population. Understanding how biological and environmental processes influence the population dynamics of meningococcus remains crucial to reducing the public health burden related to

* Corresponding author.
E-mail addresses: zoe.yang@esr.cri.nz (Z. Yang), heather.davies@esr.cri.nz (H. Davies), jane.clapham@esr.cri.nz (J. Clapham), liza.lopez@esr.cri.nz (L. Lopez), holly.bratcher@mpls.ox.ac.uk (H.B. Bratcher), audrey.tiong@esr.crin (A. Tiong), xavier.didelot@warwick.ac.uk (X. Didelot), martin.maiden@biology.ox.ac.uk (M.C.J. Maiden), philip.carter@esr.cri.nz (P.E. Carter), Una.Ren@esr.cri.nz (X. Ren).

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IMD.

Hyperendemic group B meningococcal disease was first identified during the second half of the 20th century. Hyperendemic disease, or often described as an 'epidemic', describes an elevated level of disease caused by a particular variant over years or decades. Cuba, Norway, and NZ all experienced such group B epidemics during this time (Racloz and Luiz, 2010). The NZ group B epidemic (1991 to 2008) strain B:4:P1.7–2,4:cc41/44 (NZMenB), also known as B:4:P1.4, was first identified in the Netherlands in 1980 (Martin et al., 1998; Caugant et al., 1990). NZMenB, first detected in 1990 with five cases, rose to 650 cases at the peak of the epidemic in 2001, with a population incidence rate of 17.4 per 100,000 (*The epidemiology of meningococcal disease in New Zealand in* 2001, 2001). Throughout the epidemic, the disease rate was higher in Māori and Pacific people populations, compared to the European population. Māori and Pacific people are more likely to experience socioeconomic hardship in NZ. MeNZB™, an outer membrane vesicle (OMV) vaccine developed against NZMenB, was first introduced from July 2004 to June 2006 to people under 20 years old (Sexton et al., 2004), then stopped in 2008. Though the MeNZB™ is no longer available, the NZMenB OMV is part of the 4CMenB vaccine (O'Ryan et al., 2014). The NZMenB strain is still circulating in NZ, causing about a quarter of all group B meningococcal cases (Sexton et al., 2004).

Much is known about the epidemiology and impact of the epidemic; however, many questions remain regarding the emergence of the NZMenB strain. This strain mainly comprises three STs: ST-41, ST-154, and ST-42 (*Invasive Meningococcal Disease Report January–December* 2023, 2023), but previous molecular typing could not resolve the relationship within or among these STs, nor distinguish how NZMenB is related to B:4:P1.7–2,4 from the rest of world, including the Netherlands. In 1990, three ST-42 isolates, and one ST-41 isolate were detected in Auckland (upper North Island), while one ST-154 isolate was detected in Nelson (upper South Island) (Dyet and Martin, 2006). Early detection of all three STs raises questions as to whether they resulted from multiple introductions or evolved from a single introduction, and when these introductions occurred.

Here we analysed the genomes for 97 % of all available NZMenB isolates (76 % of all cases, 2371 isolates in total) collected from 1990 to 2019 in NZ to describe the meningococcal population structure and spatiotemporal dynamics during the epidemic and post-epidemic period. To our knowledge, this study is the largest and most comprehensive analysis of meningococcal epidemic-associated isolates to date. The historically recent appearance of this hyperinvasive strain, its continued circulation, and associations with IMD, and the complete dataset of NZMenB epidemic present a unique and important opportunity to explore meningococcal evolution and population structure in an epidemic setting.

2. Methods

2.1. Prevalence analysis and sample selection

IMD is notifiable in NZ with all cases referred to the Meningococcal Reference Laboratory at the Institute of Environmental Science and Research (ESR). Disease incidence was extracted from the national notifiable disease surveillance database, EpiSurv. Annual population denominators were from Stats NZ. From 1990 to 2019, 2737 NZMenB isolates were sent to ESR for molecular typing: 2607 represented unique cases, of these 232 were lost, not viable, or contaminated. Sequencing was attempted on all available isolates associated with unique cases.

We used R version 4.0.3 (R Core Team, 2021) (https://www.r-project.org) to perform all statistical tests. The inflection point of the time series was identified by breakpoint analysis using the R package strucchange (Zeileis et al., 2020) and tested by the Chow test, using yearly disease rates (cases/100,000) during 1990–2019. We adopt Principal component analysis (PCA) for population structure analysis PCA were performed using FactoMineR (FactoMineR, n.d.) and interpreted by factoextra (*Factoextra: Extract and Visualize the Results of Multivariate Data Analyses*, n.d.).

2.2. Whole genome sequencing and processing

Isolates were cultured on Sheep blood agar plates. Genomic DNA was extracted using the Gentra Puregene Yeast/Bact. Kit (QIAGEN) or High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's protocols. For non-culturable isolates, DNA was extracted from glycerol stocks. Paired-end short-read sequencing was performed by ESR or Oxford Genomics using Illumina chemistry. Long-read sequencing was performed on the Oxford Nanopore system using Rapid Barcoding Sequencing (SQK-RBK004) and R9.4.1 MinION flow-cells. Sequencing data is available from the NCBI SRA (PRJNA592848 and PRJEB28859) and PubMLST.

Illumina reads were quality trimmed using fastp_v.0.20.1 (Chen et al., 2018). Samples with contamination or depth $< 40\times$ were excluded. Short-reads were assembled with SPAdes_v.3.13.0 (Bankevich et al., 2012). Long-reads were assembled with flye_v.2.8–1 (Freire et al., 2022) and polished using short-reads with Unicycler_v.0.4.8 (Wick et al., 2017). in silico MLST, capsule grouping, 4CMenB antigen typing including fHbp, NHBA, NadA, PorA-VR1 and PorA-VR2, and their corresponding Bexsero® Antigen Sequence Type (BAST) (Brehony et al., 2016) were derived from assemblies using Meningotype_v.0.82-beta (Kwong et al., n.d.). The statistics for the sequencing data for each sample are listed in Table S1.

Please see supplementary material for detail method for DNA extraction, initial sequencing quality control.

2.3. Identification of Single-nucleotide polymorphisms (SNPs)

We used FreeBayes_v. 1.3.4 to detect sequence variations among isolates (Garrison and Marth, 2012). Core variants were filtered using vcffilter from vcflib and vcftools_v.0.1.12b with QUAL >30 and $10\times$ minimum depth (Garrison et al., 2022; Danecek et al., 2011) Vcfallelicprimitives from vcflib was used to decomplex multiple nucleotide polymorphisms (MNPs) and complex variations, retaining biallelic SNPs with a minimum variant allele frequency of 70 %. Sites located in the tandem repeat regions were removed using the intersect function from BEDTools_v.2.23.0 to obtain the final core SNPs (Quinlan and Hall, 2010).

2.4. Phylogenetics and recombination correction

Maximum likelihood (ML) phylogenies were constructed from core SNP alignment using the best-fit model with IQ-TREE_v.2.0.6 (Minh et al., 2020) with 4000 ultra-fast bootstrap (UFB) replicates. ClonalFrameML_v.1.25 (Didelot and Wilson, 2015) was used to construct recombination-corrected phylogeny based on core genome alignments (Parsnp_v.1.2) (Treangen et al., 2014) and core SNP tree topology.

2.5. Molecular dating

We used BactDating_v1.1, a Bayesian framework, to estimate the divergence times of the nodes in phylogenies (Didelot et al., 2018). ClonalFrameML outputs with recombination correction were used as the input for molecular dating analysis. To test for the presence of a temporal signal, we performed a linear regression analysis between report dates and root-to-tip distances using the root-to-tip function in BactDating_v1.1. We employed the default additive relaxed clock model for all datasets. Markov Chain Monte Carlo (MCMC) chains in BactDating were run for 10,000,000 iterations, removed the first half as burn-in and the remainder sampled every 5000 iterations to compute the credible intervals. We performed molecular dating for clade41_SC1, clade154 and clade42.

2.6. Clonal expansions in dated phylogenies of clade154 and clade42

We employed CaveDive, a Bayesian framework, for the detection clonal expansion events in time-scaled phylogenies of clade154 and clade42 (Helekal et al., 2022). We used the default priors for both inferences. Markov Chain Monte Carlo (MCMC chains in CaveDive were run for 10,000,000 iterations, removing the first 40 % as burn-in and the remainder was sampled every 1000 iterations to compute the posterior probability of clonal expansion.

2.7. Effective population size, growth rate of clade154 and clade42

We applied Skygrowth_v 0.3.1, using Bayesian Gibbs-within-Metropolis MCMC and fast maximum a posteriori algorithm, to estimate the effective population size through time and the growth rate of effective population size (Volz and Didelot, 2018). Manual inspection of the trees revealed some outliers before the expansion, which were removed for the Skygrowth analysis (Fig. S5). We used a smoothing parameter, tau0 = 10, to control the smoothness of the estimated trajectories, since we lacked a priori information about volatility of these trajectories. MCMC chains in Skygrowth were run for 10,000,000 iterations, with the first half removed as burn-in, and the remainder sampled every 5000 iterations to compute the credible intervals. The final results were produced by averaging across 8000 time trees estimated for each dataset. Phylogenies were annotated using iTOL (Letunic and Bork, 2016) and Microreact (Argimon et al., 2016).

2.8. NZMenB in the context of global cc41/44

To determine how NZMenB isolates were related to international cc41/44 isolates, we downloaded all group B cc41/44 genomes from PubMLST (14th May 2021). After removing the isolates with MASH distance larger than 0.004, 4161 genomes were retained for phylogenetic analysis (Table S2). A reference-free and assembly-based analysis approach was used with kSNP3 (Gardner et al., 2015). ML phylogeny was constructed from SNPs present in at least 80 % of the isolates under the best-fit model using IQ-TREE_v.2.0.6 with 2000 UFB replicates.

3. Results

3.1. Prevalence of NZMenB in NZ

From 1990 to 2019, 7348 IMD cases were notified in NZ and NZMenB was responsible for 58 % of the confirmed cases (5625, Fig. 1, Table S3–4). We divided the 1990 to 2019 period into four stages: EE, early epidemic (1990 to 1994); EP, epidemic (1995 to 2003); VA, vaccination (2004 to 2008); and PE, post epidemic (2009 to 2019). The disease rates (cases/100,000) increased during EE (coefficient = 0.9582, R^2 = 0.9182, p = 0.0102), remained high during EP (coefficient = 0.4051, R^2 = 0.1641, p = 0.2794), then declined over VA (coefficient = 0.9194, R^2 = 0.8453, p = 0.0271), and stayed low in PE (coefficient = −0.0999, R^2 = 0.0100, p = 0.7701) (Fig. 1A, Table S3). 63 % of all IMD cases during the study period were reported in EP. To confirm the stage divisions assigned were appropriate for the different points of the epidemic, we used two-proportion Z-test to compare the proportion of NZMenB cases among the stages: 29 % (65/225) in EE, 72 % (2202/3046) in EP, 55 % (455/821) in VA, and 24 % (234/974) in PE (Fig. 1B). Paired comparisons showed a significant difference for all combinations.

3.2. NZMenB consists of three distinct lineages with high and increasing diversity in the early epidemic period

We analysed the genomes of 2371 NZMenB isolates collected from 1990 to 2019 (Table S5), representing approximately 97 % of all available isolates and 76 % of confirmed NZMenB cases (Fig.S1, Table S5). For EE (early epidemic), we sequenced 97.2 % (209/215) of confirmed NZMenB cases. For EP, VA, and PE, 77 % (1697/2202), 69.5 % (316/455), and 63.7 % (149/234) of NZMenB confirmed cases,

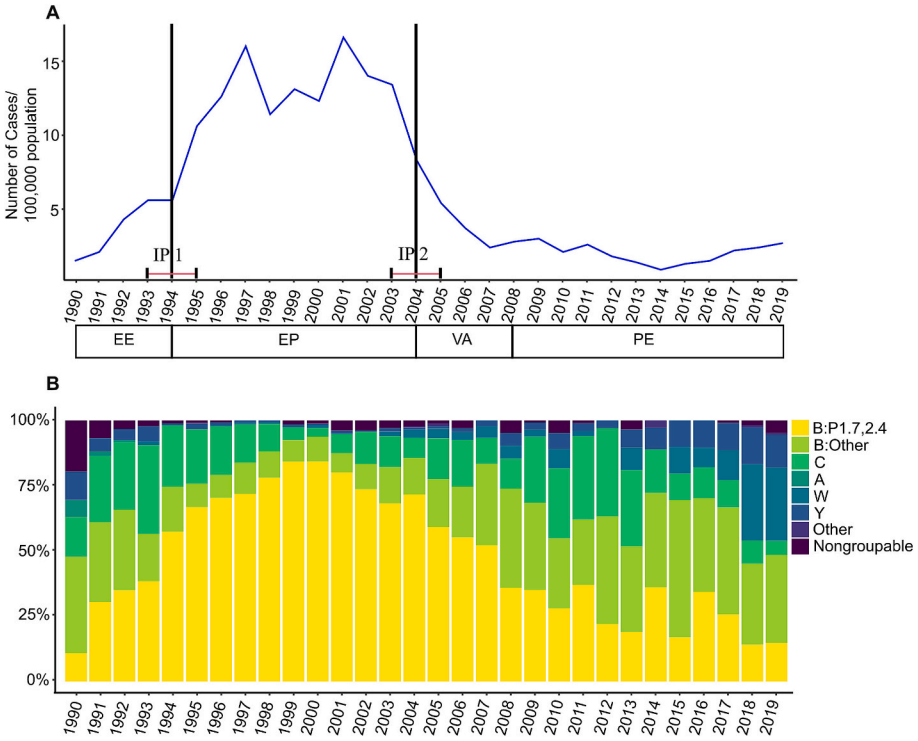

Fig. 1. Meningococcal disease in New Zealand, 1990–2019. A) Two inflection points were identified based on the yearly disease rates (cases/100,000): one in 1994 and the other in 2004. The 1990 to 2019 period of IMD in NZ were divided into four stages: EE, early epidemic (1990 to 1994); EP, epidemic (1995 to 2003); VA, vaccination (2004 to 2008); and PE, post epidemic (2009 to 2019). B) Proportion of meningococcal disease by group.

respectively, were sequenced. Due to prophylactic, antibiotic use for all suspected IMD cases, isolates are not available for all cases.

We found 105 STs among the 2371 isolates, confirming the three major STs previously identified (Table S5, Fig.S2 A) (Dyet and Martin, 2006). We also confirmed the isolates from 1990 belonged to three different STs: ST-42; ST-154; and ST-41 (Dyet and Martin, 2006). Phylogenetic and principal component analysis showed all sequenced isolates could be divided into three major clades, named clade41, clade42 and clade154 (Fig. 2A-C, Fig.S2B). The majority of isolates belonged to two highly supported (100 % UBF) monophyletic clades: clade154 and clade42. Clade154 contained 913 isolates with 46 known STs, while clade42 included 1366 isolates with 55 STs and three unassigned isolates. The remaining 92 isolates, with 10 different STs, did not form a monophyletic clade and were either ST-41 or grouped with ST-41. We named this group clade41. Stages EE through PE were represented by 209, 1697, 316, and 149 isolates, with 9, 87, 29, and 18 STs, respectively.

Despite having fewer isolates, clade154 was more diverse than clade42, with an average nucleotide diversity (π) of 0.0202, compared to 0.0175 for clade42. Removing a post-epidemic clade154 sub-lineage reduced π to 0.0184 but remained higher than that of clade42. The overall π increased over time: 0.0181 for EE, 0.0235 for EP, 0.0289 for VA, and 0.0346 for PE. Among the 209 EE isolates sequenced, nine different STs were identified. The top five STs in this early stage, ST-42, ST-154, ST-41, ST-10989, and ST-6118, were also the top five STs in the full dataset. Early epidemic isolates were distributed across the phylogeny, occupying relatively basal positions (Fig. 2D). These results suggest that the NZ epidemic strain was genetically diverse from the outset.

3.3. Molecular dating analysis supports early emergence and diversification

To refine the phylogenetic relationships within each clade, we constructed clade-specific ML core-SNP phylogenies using ST-specific references: NMI01191 for clade41, NMI97348 for clade154, and NZ-05/33 for clade42. Phylogenies were corrected for recombination using ClonalFrameML (Didelot and Wilson, 2015). For each of the clade-specific phylogenies, major clades containing over 40 isolates with UFB >95 % were defined as subclades (unless specified). One subclade was defined for clade41 (C41_SC1), five for clade154 (C154_SC1–5), and three for clade42 (C42_SC1–3). Members of each subclade were refined to align with the recombination-corrected phylogeny (Table S6 and Fig. S3).

To investigate when these subclades arose, we performed molecular dating analysis using BactDating (Didelot et al., 2018). The root-to-tip genetic divergence against sampling dates showed a weak but significant temporal signal (Fig.S4). The emergence of clade154 and clade42 was dated to 1972.2 (1967.1 to 1977) and 1972 (1965.7 to 1976.4), respectively (Fig. 3A-B). The estimated times for subclades and major nodes of clade154 and clade42 are found in Table 1 and S5. Within C154_SC4, the root date for the lineage of isolates from 2013 to 2019 was estimated to be 2007.4 (2005.1 to 2009.3). All major subclades apart from C154_SC2 were predicted to have arisen before the epidemic. These dates support the high genomic diversity observed in the early epidemic period.

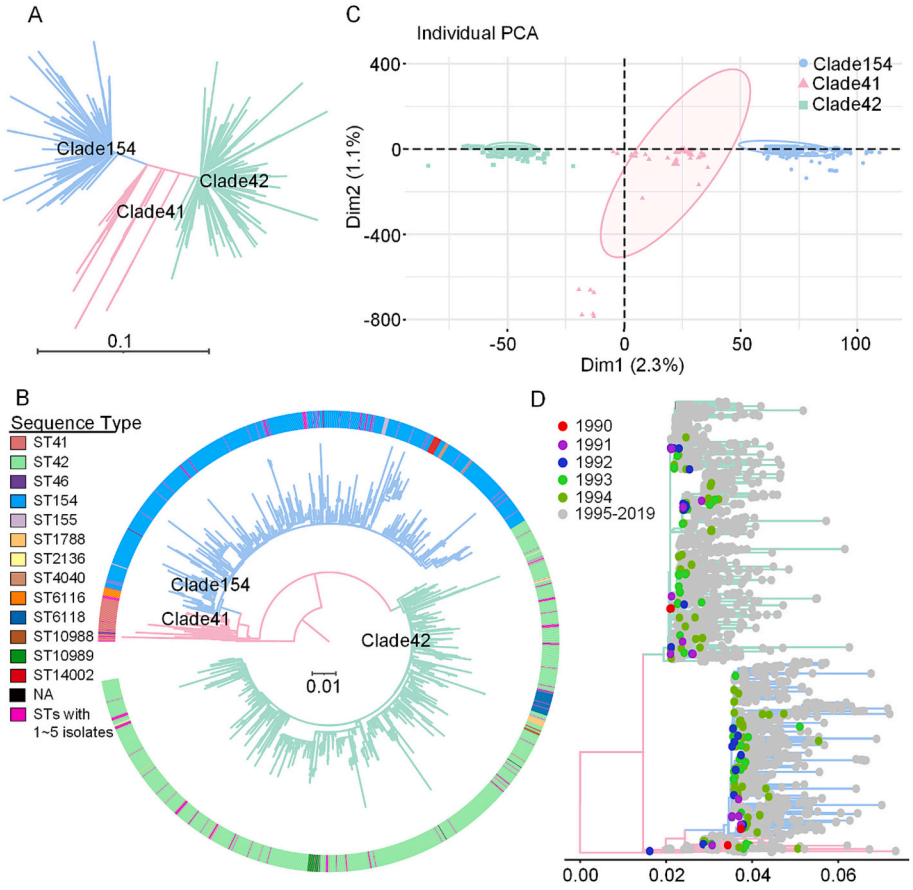

Fig. 2. Phylogenetic analysis of the NZMenB 2371 dataset. A) An unrooted maximum likelihood phylogeny was constructed using generalised time reversible substitution model using core SNP alignments with IQ-TREE. Three major group were identified: clade41, clade154, and clade42. B) The core SNP phylogeny of NZMenB was rooted with a sublineage within Clade41. The ring indicates the multi-locus sequence types identified. C) Principal component analysis supports three major groups within the NZMenB population. D) Isolates from stage S1, the early stage of the epidemics (from 1990 to 1994), occupies the basal positions on the phylogeny.

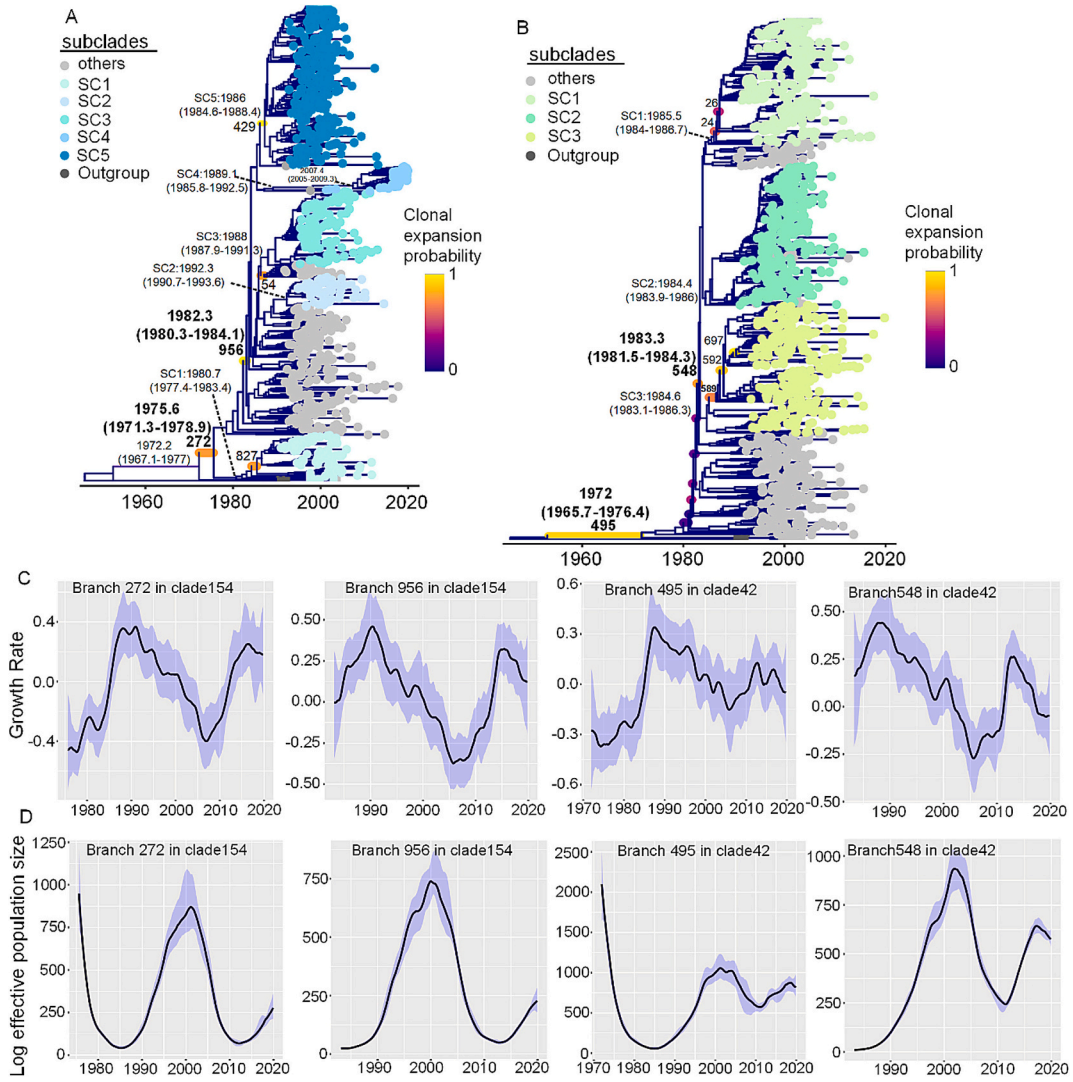

Fig. 3. Time based phylogeny for clade154 and clade42 and their population dynamics. A) Five clonal expansion events in clade154 were identified by CaveDive, marked colors purple (probability) to yellow (high probability). B) Seven clonal expansion events in clade42 were identified by CaveDive, marked colors purple (probability) to yellow (high probability). C) Growth rate dynamic changes over time for clonal expansion events of branch 272, 956 in clade154, and branches 495 and 548 in clade42, were estimated by Skygrowth. D) Effective population size dynamics over time for clonal expansion events of branch 272, 956 in clade154, and branches in 495 and 548 in clade42, were estimated by Skygrowth. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Estimated root date of defined subclades of NZMenB.

Subclade	Estimated date
C41_SC1	1987 (1983.7 to 1989.45)
C154_SC1	1980.7 (1977.4 to 1983.4)
C154_SC2	1992.3 (1990.7 to 1993.6)
C154_SC3	1988 (1987.9 to 1991.3)
C154_SC4	1989.1 (1985.8 to 1992.5)
C154_SC5	1987.2 (1986 to 1988.4)
C42_SC1	1985.5 (1984 to 1986.7)
C42_SC2	1984.4 (1983.9 to 1986)
C42_SC3	1984.6 (1983.1 to 1986.3)

3.4. Clonal expansions within clade154 and clade42 were responsible for the majority cases in the epidemic

To understand the origins of the subclades and their contributions to the epidemic, we performed clonal expansion analysis using CaveDive (Helekal et al., 2022). CaveDive estimates the posterior probability for varying numbers of clonal expansion events within a population.

For clade154, five expansion events were inferred to have the highest posterior probability, while seven expansion events were inferred for clade42. Within clade154, the first expansion occurred at branch 272, which gave rise to two expansions at branches 827 and 956. Branch 956 further led to two expansions at branch 54 and 429 (Fig. 3A). Branch 272 was a basal branch from which almost all clade154 isolates originated. Branch 827 gave rise to C154_SC1, which was the sister clade to all other subclades (Fig. 3A). Branch 956 gave rise to all other subclades. A separate event at branch 429 led to C154_SC5. Within clade42, the first expansion was predicted at the basal branch 495. The next expansion at branch 548 led to two nested expansions: one containing branches 589, 592, and 697, and the other containing branches 24 and 26 (Fig. 3B). The expansion event on branch 548 gave rise to 1143 isolates of clade42, including all three subclades. The expansion event at 589 resulted in the majority of isolates in C42_SC3, while the expansion events at 24 and 26 produced the majority of isolates in C42_SC1.

To estimate the growth rates and effective population size over time, for both clade154 and clade42, we applied a nonparametric model,

Skygrowth (Volz and Didelot, 2018). The input for the Skygrowth model estimations was based on the basal clonal expansions at branch 272 of clade154 and branch 495 of clade42, and the second clonal expansion at branch 956 of clade154 and branch 548 of clade42. The inferred growth rates for both clades showed similar patterns: increasing from the early to middle 1970s to the late 1980s and early 1990s, reaching their peaks, and then progressively declining to a trough around 2006. After this, the growth rate increased again, reaching second peaks around 2016–2017 for clade154, and around 2013–2014 for clade42, followed by subsequent declines (Fig. 3C). The lowest growth rate for both clades was around 2006, following the national rollout (Caugant et al., 1990) of MeNZB™. The effective population size for each clade based on the basal clonal expansions indicated an early decline before the NZMenB epidemic, likely due to the basal lineages that took a long time to coalesce with the rest of the clades (Fig. S5C, Fig. S5D, Fig. 3D). This suggests that the dynamics of these basal lineages differed from the clonal expansions that occurred before and during the NZ epidemic. After removing those basal lineages, the estimated growth rate of the effective population for samples from branch 272 of clade154 and 495 of clade42 showed consistent dynamics with those estimated from branch 956 of clade154 and branch 548 of clade42, respectively (Fig. 3C, and Fig.S5). These effective population dynamics also aligned with the incidence curve for the NZMenB strain (Fig. 1A and 3D) except for a second peak in clade42. This peak was also likely due to the long coalescence time, attributable to the small number of samples from diverse lineages.

3.5. NZMenB in the international cc41/44 context

To put NZMenB in the context of international cc41/44 variants and estimate the number of introductions into NZ, we compared NZMenB to an international set of cc41/44 genomes. This dataset consisted of seven meningococcal isolates from the Netherlands (gifted by The Netherlands Reference Laboratory for Bacterial Meningitis) and four from Norway (gifted by Norwegian Institute of Public Health) in the ESR collection, supplemented by 197 Netherland genomes from Kremer et al., 2020 (Kremer et al., 2020), and all available cc41/44 assemblies from PubMLST (as of 14/05/2021). After excluding distantly related isolates, the final dataset included 4161 genomes - 2372 from NZ and 1789 international. The international dataset, predominantly from the UK and other European countries, had ST-41 as the most common sequence type (570/1789). Within the international dataset there were 17 genomes from pre-1990 and 22 from the early epidemic period (1990–1994). As expected, the phylogenetic analysis revealed two highly supported clades: clade154 (1244 isolates) and clade42 (1559 isolates), with the majority of isolates (73 % of clade154 and 88 % of clade42) from NZ (Fig. 4A and Table S6). ST-41 isolates were polyphyletic in the context of

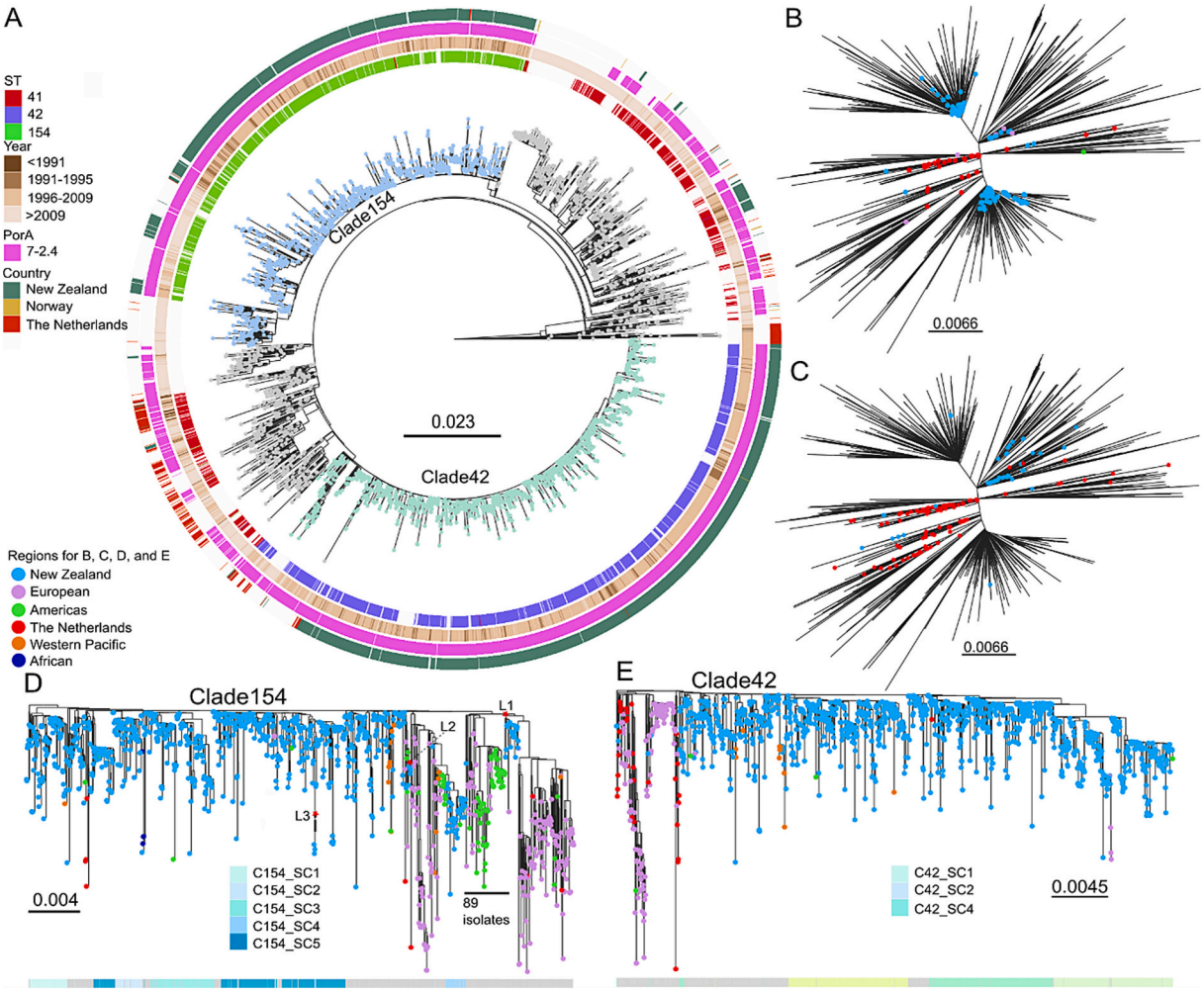

Fig. 4. NZMenB in the international cc41/44 context. A) A maximum-likelihood phylogeny of 4161 cc41/44 genomes, constructed based on SNPs found in at least 80 % of all genomes using a reference-free k-mer approach. B) Early epidemic NZMenB isolates. These isolates are scattered across different lineages and do not closely cluster with any international isolates from before or during the early epidemic period (1990–1994). C) ST-41 isolates are dispersed across various lineages in the international cc41/44 context. D) Clade 154 in the international cc41/44 context: subclade isolates of clade154 remained largely intact, with the exception of C154_SC4 and two small clusters (L1 and L2). E) Clade 42 in the international cc41/44 context. Isolates of NZ clade42 remain clustered in one well-supported clade.

cc41/44, 67 out of 637 isolates were from NZ. Early epidemic NZMenB isolates did not cluster closely with any international isolates from before or during the early epidemic period (Fig. 4B).

NZMenB was likely the result of multiple importations. NZMenB ST-41 isolates were scattered across different lineages (Fig. 4C). C41_SC1 remained largely intact. Its sister clade contained 41 isolates from Europe, with the earliest isolate from 1994. Clade154 remained mostly intact, except for C154_SC4 and two small clusters (L1 and L2), indicating a major importation event with minor subsequent introductions (Fig. 4D). The sister clade to recent C154_SC4 isolates contained isolates from North America, Europe, and Australia, with the earliest isolate from Australia (2011), suggesting a separate importation event post-epidemic. For clade42, NZ isolates remained clustered in one well-supported clade (Fig. 4E), along with a sister clade consisting mostly of isolates from the Netherlands (2001 to 2018). The oldest isolates in this sister clade were from 2001. This likely suggests one importation event for clade42.

An interactive phylogeny with metadata is available in Microreact project: https://microreact.org/project/nzmenb-international with detailed views for each clade.

4. Discussion

We present here, to our knowledge, the largest and most comprehensive analysis of a national IMD epidemic to-date using phylodynamic tools. We provide evidence that NZMenB was circulating before the epidemic. We found the three main clades of NZMenB had different expansion patterns explaining their contributions to the epidemic. By comparing NZMenB with international cc41/44 genomes, we identified multiple importations events; however, we did not find close linkages with international genomes. We contend that NZMenB was circulating in carriage before the onset of the epidemic, which may have been triggered by societal changes around the time of the epidemic.

B:4:P1.7–2,4:cc41/44 was first identified in the Netherlands around 1980 and became the most prevalent variant by 1990 (Caugant et al., 1990). It was thought that NZMenB was most likely introduced from the Netherlands, given the close timing of the outbreaks in both countries (Dyet and Martin, 2006). However, we consider it unlikely that direct importation from the Netherlands caused the NZMenB epidemic. ST-41 isolates were rare in the NZMenB epidemic but were the most common ST among international genomes. NZMenB ST-41were not closely related to the ST-41 isolates from the Netherlands (Fig. 4C). International comparison showed NZ ST-41 and clade154 isolates resulted from multiple importations. While NZ clade42 remained largely intact within the international phylogeny, suggesting a single importation event into NZ. The cohesiveness of the NZ clade154 and clade42 isolates within the international phylogeny suggests separate introductions for clade154 and clade42. The probability that the three NZMenB lineages resulted from diversification within NZ from a single introduction is unlikely, given the similar dating predictions (Fig. 3 and Table 1). The estimated clonal expansion events also occurred within similar time frames (Fig. 3). It is more probable that clade154 and clade42 were introduced around the same time. The most basal positions of both clades were occupied by isolates from European countries (Fig. 4), indicating introductions from Europe. However, due to limited international data available from the early epidemic period and regions outside of Europe, the origin of NZMenB lineages remains uncertain. A European origin would not be unlikely, considering the strong links between NZ and European countries.

Early diversification and expansion of NZMenB suggest circulation prior to the epidemic. Dating analysis showed NZMenB was circulating in NZ before the epidemic. Two predicted clonal expansion events (branch 956 for clade154; branch 548 for clade42), from which most of the isolates were derived, dated back to 1980 to 1984 (Fig. 3A and B), approximately seven to ten years before the first cases were detected. Their effective population sizes (Fig. 3D) were also congruent with the NZMenB incidence curve (Fig. 1), suggesting that these clonal expansion events were the main contributors to the epidemic. The more derived subclades and clonal expansion events dated closer to when the first cases were detected in 1990. For clade154, the clonal expansion event detected at branch 429, at the root of the largest subclade clade154_SC5, is predicted to have risen between 1984 and 1988. For clade42, events predicted at branches 589, 592, 697, 24 and 26 estimated to have risen between 1984 and 1991 (Table S6). Meningococci can be carried asymptomatically without causing disease, and transmission often occur among carriers. Time may be required for a newly introduced variant to establish itself in sufficient density for disease to be detected. The relatively high diversity of NZMenB observed in the early epidemic period (Fig. 2D) supports diversification before 1990. Knowing the extend of the "epidemic strain" presence and transmission within NZ before the cases were detected at the beginning of the epidemic may have accelerated the decision to make the be-spoke vaccine, MeNZB™, for this strain. In the modern era, with two protein-based vaccines available for group B meningococcal disease, 4CMenB (O'Ryan et al., 2014) and MenB-FHbp Trumenba, 2018, knowing the genomic makeup of an outbreak strain could help with deciding which vaccine to use as well as the extend of the vaccination campaign to reduce disease (Rodrigues et al., 2020).

An increase in carriage, exposure, or transmission likely played a role in initiating the NZMenB epidemic. B:4:P1.7–2,4:cc41/44 had caused an elevated level of disease in several countries, but only NZ experienced a large, prolonged epidemic ('hyperendemic outbreak') associated with this variant. It is particularly intriguing that Australia, despite its close travel connections with NZ, did not experience a large-scale NZMenB outbreak. We provided evidence that this strain likely circulated in carriage before the epidemic, suggesting that differing environmental factors may have triggered the epidemic in NZ. The early NZMenB epidemic period coincided with an era of economic policy changes and rising inequalities in NZ. In 1991, the NZ government removed income-related rents for state housing, leading to market-rate rents. Although average house crowding has remained largely unchanged since 1991, crowding in rental housing, particularly in social housing, rose from 1991 to 2006. Multi-family households also increased from 1991 to 2006 (*Analysis of Household Crowding Based on Census* 2013 data, 2013; *Living in a crowded house: Exploring the ethnicity and well-being of people in crowded households*, 2018; *The Social Report* 2016 – Te pūrongo oranga tangata, 2016). While no single policy could have solely initiated the epidemic, increasing inequalities may have exacerbated it.

Baker et al. (2000) demonstrated that household crowding, especially the number of individuals older than 10 years in a household, was a major risk factor for developing epidemic meningococcal disease in Auckland children (Baker et al., 2000). Transmission of meningococcus occurs through respiratory secretions passed between individuals in close proximity (Glover, 1918). It is known that meningococcal carriage is higher in high-density settings. It has also been suggested that high-density living facilitate strain persistence (Pardo de Santayana et al., 2023; O'Connor et al., 2015). NZMenB carriage rate among the household contacts of IMD patients (Simmons et al., 2001) during the epidemic (1996–1998) was estimated to be 11.3 %, similar to the rate (13 %) identified among Irish traveller community during B:4:P1.7–2,4: ST-6697(cc41/44) (KA, Bray J, Bennett D, Maiden MC, Cunney R., 2016) outbreak. Parallels exist between the NZMenB and the traveller community outbreak where people most affected were also those more likely to experience societal inequalities and housing-density played a role in exacerbating and possibly prolonging the outbreak.

Our study revealed that the NZMenB epidemic likely resulted from multiple introductions of B:4:P1.7–2,4 lineages into NZ, with these lineages circulating in carriage before the epidemic. Socioeconomic factors unique to NZ at the time may have aided the spread of the epidemic. A more comprehensive analysis of the subclades, integrating detailed epidemiological data such as movement, age, housing, and school attendance, is needed to understand factors that aided the transmission

of these lineages. This study highlights the importance of meningococcal carriage studies, which could reveal introductions of new virulent strains before disease detection and help us to better understand meningococcal transmission and emergence. Near real-time phylodynamic analysis has been applied to predicting and controlling viral outbreaks, we believe that these techniques could also be valuable to the analysis of bacterial outbreaks. For vaccine preventable infectious diseases, such as IMD, understanding the genomic diversity and estimating effective population size early on in an outbreak, could lead to better decision making around vaccine purchases and extend of the vaccine campaign.

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Contribution

ZY carried out the study and wrote the first draft of the manuscript. XR, PEC and MCJM conceptualized and led the study and contributed to the writing of the manuscript. HD and LL extracted laboratory and epidemiological data. JC assisted in laboratory work. XD assisted in data analysis and writing of the manuscript. HCB oversaw the genomic sequencing and data management at PubMLST. AT oversaw the management of laboratory.

Data statement

Sequencing data is available from the NCBI SRA (PRJNA592848 and PRJEB28859) and PubMLST. Report date of the cases is omitted from the supplementary information to ensure patient privacy. This data can be requested from corresponding author una.ren@esr.cri.nz and need to be approved. Analysis scripts will be made available at GitHub repository: https://github.com/ZoeYang2020/NZMenB_MS_analysis. Genomic assemblies used in this manuscript are available at https://doi.org/10.26091/ESRNZ.28377044.v1.

Ethical Statement

Ethical approval was not required for the present study because no identifiable information, clinical or patient data were used or requested.

CRediT authorship contribution statement

Zuyu Yang: Writing – original draft, Formal analysis. **Heather Davies:** Writing – review & editing, Data curation. **Jane Clapham:** Methodology. **Liza Lopez:** Data curation. **Holly B. Bratcher:** Data curation. **Audrey Tiong:** Supervision. **Xavier Didelot:** Writing – review & editing, Methodology. **Martin C.J. Maiden:** Writing – review & editing, Funding acquisition, Conceptualization. **Philip E. Carter:** Writing – review & editing, Funding acquisition, Conceptualization. **Xiaoyun Ren:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

Authors of this article has no conflict of interest to disclose.

Data availability

Sequencing data is available from the NCBI SRA (PRJNA592848 and PRJEB28859) and PubMLST. Report date of the cases is omitted from the supplementary information to ensure patient privacy. This data can be requested from corresponding author una.ren@esr.cri.nz and need to be approved. Analysis scripts will be made available at GitHub repository: https://github.com/ZoeYang2020/NZMenB_MS_analysis. Genomic assemblies used in this manuscript are available at https://doi.org/10.26091/ESRNZ.28377044.v1.

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Appendix A. Supplementary data

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References

Analysis of Household Crowding Based on Census 2013 data, 2013.
Argimon, S., Abudahab, K., Goater, R.J.E., et al., 2016. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. Microb Genom 2, e000093.
Baker, M., McNicholas, A., Garrett, N., et al., 2000. Household crowding a major risk factor for epidemic meningococcal disease in Auckland children. Pediatr. Infect. Dis. J. 19, 983–990.
Bankevich, A., Nurk, S., Antipov, D., et al., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.
Brehony, C., Rodrigues, C.M.C., Borrow, R., Smith, A., Cunney, R., Moxon, E.R., Maiden, M.C.J., 2016. Distribution of Bexsero® antigen sequence types (BASTs) in invasive meningococcal disease isolates: implications for immunisation. Vaccine 34 (39), 4690–4697.
Caugant, D.A., Bol, P., Hoiby, E.A., Zanen, H.C., Froholm, L.O., 1990. Clones of serogroup B *Neisseria meningitidis* causing systemic disease in the Netherlands, 1958-1986. J. Infect. Dis. 162, 867–874.
Chen, S.F., Zhou, Y.Q., Chen, Y.R., Gu, J., 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, 884–890.
Danecek, P., Auton, A., Abecasis, G., et al., 2011. The variant call format and VCFtools. Bioinform 27, 2156–2158.
Didelot, X., Wilson, D.J., 2015. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. PLoS Comput. Biol. 11 (2), e1004041.
Didelot, X., Croucher, N.J., Bentley, S.D., Harris, S.R., Wilson, D.J., 2018. Bayesian inference of ancestral dates on bacterial phylogenetic trees. Nucleic Acids Res. 46, e134.
Dyet, K.H., Martin, D.R., 2006. Clonal analysis of the serogroup B meningococci causing New Zealand's epidemic. Epidemiol. Infect. 134, 377–383.
Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. https://cran.r-project.org/web/packages/factoextra/index.html.
FactoMineR. http://factominer.free.fr/.
Freire, B., Ladra, S., Parama, J.R., 2022. Memory-efficient assembly using Flye. IEEE/ACM Trans. Comput. Biol. Bioinform. 19, 3564–3577.
Gardner, S.N., Slezak, T., Hall, B.G., 2015. kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. Bioinformatics 31, 2877–2878.
Garrison, E., Marth, G., 2012. Haplotype-based variant detection from short-read sequencing. In: arXiv preprint arXiv:1207.3907 [q-bio.GN].
Garrison, E., Kronenberg, Z.N., Dawson, E.T., Pedersen, B.S., Prins, P., 2022. A spectrum of free software tools for processing the VCF variant call format: vcflib, bio-VCF, cyvcf2, hts-nim and slivar. PLoS Comput. Biol. 18 (5), e1009123.
Glover, J.A., 1918. "Spacing-out" in the prevention of military epidemics of cerebro-spinal fever. Br. Med. J. 2 (3019), 509–512.
Helekal, D., Ledda, A., Volz, E., Wyllie, D., Didelot, X., 2022. Bayesian inference of clonal expansions in a dated phylogeny. Syst. Biol. 71, 1073–1087.
Invasive Meningococcal Disease Report January–December 2023, 2023.
Jolley, K.A., Brehony, C., Maiden, M.C., 2007. Molecular typing of meningococci: recommendations for target choice and nomenclature. FEMS Microbiol. Rev. 31, 89–96.

KA, Bray J, Bennett D, Maiden MC, Cunney R., 2016. Resolution of a protracted serogroup B meningococcal outbreak with whole-genome sequencing shows interspecies genetic transfer. J. Clin. Microbiol. 54 (12), 2891–2899.
Kremer, P.H.C., Lees, J.A., Ferwerda, B., et al., 2020. Genetic variation in *Neisseria meningitidis* does not influence disease severity in meningococcal meningitis. Front Med (Lausanne) 7, 594769.
Kwong, J.C., da SA, Gonçalves, Stinear, T.P., Howden, B.P., Seemann, T., 2025. Meningotype: in silico typing for *Neisseria meningitidis*. GitHub. https://github.com/MDU-PHL/meningotype.
Letunic, I., Bork, P., 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44, W242–W245.
Living in a crowded house: Exploring the ethnicity and well-being of people in crowded households, 2018.
Maiden, M.C., Bygraves, J.A., Feil, E., et al., 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. 95, 3140–3145.
Martin, D.R., Walker, S.J., Baker, M.G., Lennon, D.R., 1998. New Zealand epidemic of meningococcal disease identified by a strain with phenotype B:4:P1.4. J. Infect. Dis. 177, 497–500.
Minh, B.Q., Schmidt, H.A., Chernomor, O., et al., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534.
O'Connor, L., Ward, M., Bennett, D., et al., 2015. A prolonged outbreak of invasive meningococcal disease in an extended Irish Traveller family across three health service executive (HSE) areas in Ireland, 2010 to 2013. Euro Surveill. 20 (21), 21139.
O'Ryan, M., Stoddard, J., Toneatto, D., Wassil, J., Dull, P.M., 2014 Jan. A multi-component meningococcal serogroup B vaccine (4CMenB): the clinical development program. Drugs 74 (1), 15–30.
Pardo de Santayana, C., Tin Tin Htar, M., Findlow, J., Balmer, P., 2023. Epidemiology of invasive meningococcal disease worldwide from 2010-2019: a literature review. Epidemiol. Infect. 151, e57.
Peterson, M.E., Mile, R., Li, Y., Nair, H., Kyaw, M.H., 2018. Meningococcal carriage in high-risk settings: a systematic review. Int. J. Infect. Dis. 109–117.
Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinform 26, 841–842.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Racloz, V.N., Luiz, S.J., 2010. The elusive meningococcal meningitis serogroup: a systematic review of serogroup B epidemiology. BMC Infect. Dis. 10, 175.
Rodrigues, C.M.C., Jolley, K.A., Smith, A., Cameron, J.C., Feavers, I.M., Maiden, M.C.J., 2020. Meningococcal deduced vaccine antigen reactivity (MenDeVAR) index: a rapid and accessible tool that exploits genomic data in public health and clinical microbiology applications. J. Clin. Microbiol. 59 (1) e02161–20.
Sexton, K., Lennon, D., Oster, P., et al., 2004. The New Zealand meningococcal vaccine strategy: a tailor-made vaccine to combat a devastating epidemic. N. Z. Med. J. 117, U1015.
Simmons, G., Martin, D., Stewart, J., Jones, N., Calder, L., Bremner, D., 2001. Carriage of among household contacts of patients with meningococcal disease in New Zealand. Eur. J. Clin. Microbiol. Infect. Dis. 20, 237–242.
The epidemiology of meningococcal disease in New Zealand in 2001. In: Pubic Health Surveillance Report, 2001.
The Social Report 2016 – Te pūrongo oranga tangata, 2016.
Treangen, T.J., Ondov, B.D., Koren, S., Phillippy, A.M., 2014. The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol. 15, 524.
Trumenba® (meningococcal group B vaccine) . Full Prescribing Information, 2018. Wyeth Pharmaceuticals Inc (a subsidiary of Pfizer Inc), Philadelphia, PA.
Volz, E.M., Didelot, X., 2018. Modeling the growth and decline of pathogen effective population size provides insight into epidemic dynamics and drivers of antimicrobial resistance. Syst. Biol. 67, 719–728.
Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595.
Yazdankhah, S.P., Caugant, D.A., 2004. *Neisseria meningitidis*: an overview of the carriage state. J. Med. Microbiol. 53, 821–832.
Zeileis, A.L.F., Hornik, K., Kleiber, C., Hansen, B., Zeileis, M.A., 2020. Package 'Strucchange' [Cited 2020 Nov 3].