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RESEARCH ARTICLE

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4CMenB vaccine coverage of invasive serogroup B meningococci collected in Belgium between 2016 and 2022

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ABSTRACT

Neisseria meningitidis infections can cause life-threatening meningitis and septicemia. In Europe, serogroup B (MenB) is the leading cause of invasive meningococcal disease (IMD), particularly in young children. Genomic surveillance of circulating MenB strains through whole genome sequencing (WGS) provides a powerful tool to assess the potential impact of vaccination strategies, including the 4CMenB vaccine, which is available for infants from 2 months of age. Here, we present a retrospective WGS-based analysis of clinical MenB IMD cases (n = 311) recovered in Belgium from 2016 to 2022 by the Belgian National Reference Center. High-quality WGS data were obtained for 281 of these strains, demonstrating high genetic diversity of the antigen targets included in the 4-component meningococcal serogroup B vaccine 4CMenB (fHbp, PorA, NHBA and NadA) and at the 4CMenB Antigen Sequence Types (BAST) level. Novel antigen combinations, not yet assigned a BAST ID, were detected in 23.5% of isolates. Vaccine coverage was predicted using the Genetic Meningococcal Antigen Typing System (gMATS) and the Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) index. Of the 281 strains, 79.5% (lower limit–upper limit: 68.0–91.5%) were predicted to be covered by the vaccine by gMATS, and 80.7% (lower limit–upper limit: 66.5–95.4%) by MenDeVAR. No evidence of variation in vaccine coverage was found throughout the study period nor between different age groups, demonstrating the broad applicability of 4CMenB. This study highlights the benefits of a pathogen surveillance program and the need for experimental characterization of continuously evolving antigenic subvariants of *Neisseria meningitidis*.

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Introduction

Neisseria meningitidis is a Gram-negative bacterium that asymptomatically colonizes the upper respiratory tract of ~10% of people.[1] Invasive meningococcal disease (IMD) occurs when the bacterium occasionally crosses the mucosal epithelium and disseminates and causes an invasive infection of the meninges. The most common clinical manifestations of IMD are meningitis and/or septicemia. IMD is a life-threatening condition with a high mortality rate, affecting mainly children and young adults.[2]

N. meningitidis can be classified into serogroups based on the structure of its capsular polysaccharides. Currently, 12 serogroups have been described, of which six (A, B, C, W, X and Y) account for almost all cases of IMD worldwide.[3] In Europe, serogroup B (MenB) is the most common, accounting for up to 51% of all documented cases, and is particularly prevalent in infants and young children.[2] Polysaccharide-based vaccines are available for serogroups A, C, W, X and Y, while MenB vaccines are based on protein antigens due to safety and efficacy concerns. Two vaccines against MenB, 4CMenB (Bexsero, MenB-4C; GSK Vaccines Srl, Sovicille, Italy) and MenB-FHbp (Trumenba, bivalent rLP2086; Pfizer Inc, New York, NY, USA ipv Philadelphia), were licensed in 2013 and 2017 by the European Medicines Agency, respectively.[4,5] 4CMenB is available from 2 months of age in most countries worldwide, helping to protect children and infants, while Trumenba has been approved by the European Medicines Agency for use in children from 10

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years of age. 4CMenB is a multicomponent vaccine containing three recombinant protein antigens: neisserial adhesin A (NadA), neisserial heparin-binding antigen (NHBA) and factor H-binding protein (fHbp), combined with the outer membrane vesicle (OMV) of the New Zealand vaccine strain containing the porin A protein (PorA) as main antigen.[5] Furthermore, two additional protein antigens present in the OMV, OpcA and PorB, have been shown recently to contribute to the immune responses in infant sera vaccinated with 4CMenB.[6]

Assessing the efficacy of protein-based vaccines is complicated by the sequence diversity and variable expression levels of the *N. meningitidis* surface antigens. The Meningococcal Antigen Typing System (MATS) assay was developed to predict whether MenB strains are likely to be covered by the 4CMenB vaccine.[7] MATS provides conservative vaccine coverage predictions by determining the cross-reactivity of antigenic variants with vaccine-induced antibodies. As this experimental approach is very time and resource intensive, MATS has been largely replaced in recent years by methods based on genotype and whole genome sequencing (WGS). Several techniques have been developed to predict 4CMenB vaccine coverage based on the combination of phenotypic and genomic data. The Bexsero Antigen Sequence Types (BASTs) scheme was the first reproducible method developed to predict vaccine coverage based on the genetic characterization of vaccine antigens.[8] Within BAST, a unique identification number is assigned to each combination of the four 4CMenB antigen variants, analogous to conventional multi-locus sequence typing (MLST).[9] This approach facilitates the interpretation of the combination of allelic variants of the antigens contained in the 4CMenB vaccine, including cases where one or more loci targeted by the vaccine are missing.[8]

Two additional methods to infer potential vaccine coverage of clinical isolates have been proposed in recent years. Similar to BAST, the Genetic Meningococcal Antigen Typing System (gMATS)[10] and Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR)[11] schemes use experimental data on vaccine antigens targeted by the MATS assay. Both methods rely on genotyping data to predict vaccine coverage, using partially overlapping datasets and slightly different algorithms.[10,11] The gMATS assay classifies isolates as ‘covered‘, ‘not covered‘ or ‘unpredictable‘. An isolate is considered as ‘covered‘ by the vaccine if at least one antigen is classified as ‘covered’ according to the gMATS antigen typing system. Strains are classified as ‘not covered‘ if fHbp, NHBA, NadA and PorA-VR2 are all predicted as 'not covered' by gMATS. The remaining isolates, which contain novel or rare variants that require additional phenotypic testing, are classified as 'unpredictable'.[10] The MenDeVAR reactivity index classifies isolates into four categories: (i) 'exact match' for samples containing at least one of the peptide variants used in the 4CMenB vaccine; (ii) 'cross-reactive' if at least one antigenic variant is predicted to be susceptible to a vaccine-induced immune response by applying specific thresholds to MATS experimental data; (iii) 'none' if all antigenic variants are predicted not to be cross-reactive when applying the same thresholds and (iv) 'insufficient data' if there are insufficient experimental data for the detected antigenic variants.[11]

This study used whole-genome sequencing to retrospectively characterize all serogroup B *N. meningitidis* strains received by the Belgian National Reference Centre (NRC) between 2016 and 2022. Clinical information on these cases is presented, and the predicted 4CMenB vaccine coverage over the surveillance period was estimated using gMATS and MenDeVAR. The differences between the two prediction methods were also analyzed, along with circulating antigenic variants, to better assess the potential impact of vaccination.

Material and methods

Clinical information and phenotypic characterization

The *N. meningitidis* isolates were obtained from patient blood or cerebrospinal fluid samples and collected by the Belgian National Reference Centre between 2016 and 2022. While the dataset includes all isolates collected by the NRC and reporting is mandatory in Belgium, sending samples to the NRC is on voluntary basis. Therefore, not all IMD cases may be represented due to underreporting or diagnostic limitations, which could impact generalizability. Serogroup determination was performed using slide agglutination with commercially available antisera (Becton Dickinson, Sparks, USA), confirming 311 isolates as MenB. Anonymized clinical and epidemiological data were available for each isolate including the isolation date, sampling source (*i.e.* 'blood', 'cerebrospinal fluid' or 'other'), patient’s sex, zip code, age, clinical outcome of

the infections (*i.e.* 'favourable', 'unfavourable' or 'unknown'), clinical manifestation (*i.e.* 'meningitis', 'sepsis', 'other/unknown') and hospitalization (*i.e.* 'yes', 'no' or 'unknown'). Patient age at the time of infection was categorized as follows: <1 year, 1–4 years, 5–9 years, 10–14 years, 15–19 years, 20–24 years, 25–44 years, 45–64 years, and >65 years.

Whole genome sequencing and genomic analysis

The bacterial isolates from cerebrospinal fluid or blood were grown at 37°C on Columbia Sheep Agar (Oxoid, Dilbeek, Belgium) in a candle air-exhaustion chamber. Genomic DNA from pure bacterial cultures was obtained using the MagCore Genomic DNA Bacterial Kit (RBC Bioscience, New Taipei City, Taiwan) according to the manufacturer's instructions. NexteraXT sequencing libraries were prepared and sequenced on an Illumina MiSeq (Illumina Inc., San Diego, California, US) using MiSeq Reagent Kit v3 (Illumina Inc.), generating 250 or 300 bp paired-end reads, depending on the run.

The *Neisseria* pipeline (v1.3) (Figure S1) was used for *de novo* assembly and typing of the whole genome sequencing (WGS) data as described elsewhere.[12,13] A schematic overview of the bioinformatics workflow is provided in the Supplementary Material. Blastn (v2.14.0)[14] was used for classical MLST and typing of the nucleotide variants of the vaccine antigens, using the MLST (retrieved on 2024–08–18) and BAST (retrieved on 2024–08–18) schemes obtained from PubMLST.[15,16] For peptide loci, Blastx (v2.14.0)[14] was used instead, with the 'seg' parameter set to 'no' and the 'comp_based_stats' parameter set to 0. Imperfect matches for of the *nadA* and *nhba* loci were manually queried for 'frameshift' or 'stop-codon' flags (which may indicate that a detected variant is nonfunctional) on the PubMLST website. For some samples, no exact matches could be detected but multi-hits (*i.e.* multiple imperfect matches with identical % coverage and % sequence identity) were returned instead for the NadA (n = 13) and NHBA peptides (n = 3). Novel alleles and BAST profiles were submitted to the PubMLST database.

MenDeVAR-based vaccine coverage predictions

Vaccine coverage was predicted using the MenDeVAR index on the detected alleles for the fHbp, NadA, NHBA and PorA-VR2 vaccine antigens[11] (Table S1). 'Exact match' refers to strains with an exact allele match for at least one of the four target antigens. fHbp, NadA and NHBA antigen peptides are defined as 'cross-reactive' or 'not cross-reactive' by applying two rules. The first rule takes only peptides present in at least five strains into account, while the second rule considers as 'cross-reactive' peptides that are covered in at least 75% of the strains and as 'not cross-reactive' peptides (*i.e.* the 'none' MenDeVAR index) that are covered at maximum in 25% of the strains. All the other peptides are assigned to the 'insufficient data' category. Isolates were considered to be covered by the vaccine if at least one vaccine antigen was classified as an 'exact-match' or 'cross-reactive'. MenDeVAR vaccine coverage predictions were estimated by adding half of the 'insufficient data' strains to the covered isolates. The lower limit of the estimate corresponds to the proportion of covered isolates, while the upper limit corresponds to the proportion of covered and 'insufficient data' strains.

gMATS-based vaccine coverage predictions

The gMATS scheme was used to predict whether isolates were 'covered', 'not covered' or 'unpredictable' by the 4CMenB vaccine[10] (Table S1). fHbp and NHBA antigen peptides are defined as 'covered' or 'not covered' on the basis of a statistical test on proportions rejecting 50% as null hypothesis (requiring at least 6 strains with the same prediction to be significant) and a threshold on the percentage of covered strains of 60% and 40% for 'covered' and 'not covered', respectively. All the other peptides fall into the 'unpredictable' category. For NadA, the prediction is always 'not covered' to reflect the results of the *in vitro* MATS assay, where the *nadA* gene is known to be artificially under-expressed.[17,18] Finally, PorA-VR2 was defined as 'covered' in case of a match with peptide 4 and was defined as 'not covered' otherwise. Analogous to the MenDeVAR-based predictions, the total panel coverage was estimated by adding half of the unpredictable strains to the covered isolates as done by Muzzi et al.[10] The lower limit of the estimate corresponds to the proportion of covered isolates, while the upper limit corresponds to the proportion of covered and unpredictable strains.[10]

Statistical analysis

The chi-squared test, or Fisher's exact test, in case of small sample sizes, was performed where appropriate using the stats 'chisq.test()' function in R. One patient with unknown sex and seven isolates with unknown or other sampling sites than blood or cerebrospinal fluid were excluded from the analysis. MenB incidence was estimated using the number of isolates collected by the Belgian NRC and the population sizes available on the Statbel portal (https://statbel.fgov.be/en) for the 2016–2022 period. Maps were plotted using the 'Belgium – Provinces' GIS dataset (last update: 2014/06/18) from the Esri BeLux Online public data (https://esribeluxdata.maps.arcgis.com/home/index.html). gMATS and MenDeVAR vaccine coverage predictions were stratified by year of strain collection, patient age group and region (*i.e.* Brussels, Flanders or Wallonia). For statistically significant differences, Pearson residuals below −2 and above 2 were considered departures from the null model. A 5% significance level was used for statistical inference. Data were analyzed and plotted using R (v4.3.2),[19] the tidyverse (v2.0.0),[20] ggpubr (v0.6.0),[21] ggupset (https://cran.r-project.org/web/packages/ggupset/index.html), sf (v1.0–14)[22] and mapsf (v0.8.0)[23] packages.

Results

Clinical manifestation of serogroup B IMD

Over the 2016–2022 period, the Belgian NRC received 311 MenB isolates from patients aged less than 1 year to 94 years at the time of infection. Around a third of the cases (35.7%) occurred in infants and children younger than 5 years. Infection peaks later in life occurred in young adults (15–19 years) and people between 45 and 64 years (Table 1). Until 2019, a similar annual trend in the number of cases stratified by age groups was observed. The highest number of cases was registered for infants and children younger than 5 years and adults between 45 and 64 years old (Table 1 and Figure 1). The number of cases in these age groups then decreased from 2019 on and remained low until 2022, with the exception of young adults between 15 and 19 years for which the number of cases increased already in 2021 and people between aged 25 and 44 and those 65 years old and above, for which a high increase in IMD cases was observed in 2022 (Figure 1).

Most IMD cases (n = 244) presented as either meningitis (n = 82), sepsis (n = 82) or both (n = 80) (Table 1). These clinical manifestations were independent from patients' age groups (chi-squared = 23.618, p-value = .092). In addition, two patients aged 50 and 68 years had pneumonia. The clinical manifestation for the remaining cases was unknown. MenB samples mostly originated from blood (n = 173) or cerebrospinal fluid (CSF) (n = 110) and rarely from clinical cases where isolates were obtained from both (n = 21). Specimen types such as 'blood' and 'cerebrospinal fluid' were also independent from the patient's age groups (chi-squared = 41.121, p-value = .130) (Table 1). There was no statistically significant difference in the number of infections between sexes (chi-squared = 8.774, p-value = .362) (Table 1).

MenB incidence in Belgium between 2016 and 2022

The annual incidence of MenB, based on strains received by the NRC, ranged from 0.13 cases per 100,000 population in 2021 to 0.61 cases per 100,000 population in 2016 (Figure 2) and can be split into two periods. Higher incidences occurred at the beginning of the study period between 2016 and 2019 and then dropped during two consequent years (2020–2021) before slightly increasing in 2022. A similar trend was observed at the level of the Belgian provinces where the highest incidence occurred in Namur in 2016 (1.43 cases per 100,000 inhabitants), followed by Liège and the Walloon Brabant in 2019 and 2017 with 1.26 and 1.25 cases per 100,000 inhabitants, respectively (Figure 2).

BAST analysis

Of the 311 sequenced MenB isolates, 281 passed the quality checks for the WGS data (Figure S2 and Table S2). Analysis of their genomes showed a high genetic diversity reflected at the level of BAST profiles (Figure 3). A

Table 1. Clinical information stratified by patient age group for MenB IMD cases for which isolates were collected by the Belgian NRC between 2016 and 2022.

	<1y	1–4y	5–9y	10–14y	15–19y	20–24y	25 – 44y	45-64y	>65y
Total*	46 (14.8%)	65 (20.9%)	29 (9.3%)	17 (5.5%)	41 (13.2%)	23 (7.4%)	25 (8%)	40 (12.9%)	25 (8%)
Patient sex, n									
Female, 156	23	26	17	10	27	11	12	17	13
Male, 154	23	38	12	7	14	12	13	23	12
Unknown, 1	–	1	–	–	–	–	–	–	–
Clinical manifestation, n									
Meningitis, 82	15	17	10	6	13	6	4	10	1
Meningitis and sepsis, 80	12	14	7	5	10	9	10	9	4
Sepsis, 82	14	23	6	2	10	3	2	13	9
Other/Unknown, 67	5	11	6	4	8	5	9	8	11
Sample isolation, n									
Blood, 173	24	43	18	6	22	10	11	19	20
CSF, 110	18	21	8	10	15	11	10	15	2
CSF and blood, 21	3	1	2	1	3	2	4	4	1
Other/Unknown, 7	1	–	1	–	1	–	–	2	2
Hospitalization, n									
No, 1	–	–	–	–	–	–	–	–	1
Unknown, 254	39	51	26	15	32	19	20	36	16
Yes, 56	7	14	3	2	9	4	5	4	8
Outcome, n									
Favourable, 162	28	30	15	11	29	10	11	16	12
Unfavourable, 17	2	3	1	1	2	1	2	3	2
Unknown, 132	16	32	13	5	10	12	12	21	11

(*) Values indicate the number of isolates in each age group and values inside parentheses the proportion of patients (%). CSF: cerebrospinal fluid.

total of 117 different profiles were identified in the successfully sequenced isolates, 89 of which were detected only once. Furthermore, 23.5% (66/281) of isolates presented 59 novel antigen combinations and were assigned a new BAST ID.

The most frequently observed BAST IDs were 238 (n = 23), 1077 (n = 15), 223 (n = 13), 5 (n = 12), 145 (n = 11) and 267 (n = 11), accounting for 30.2% (85/281) of the MenB isolates. With the exception of BAST 267, these frequent profiles were reported to be 'exact matches' or 'cross-reactive' to the 4CMenB vaccine. Only three of the BAST profiles observed in the complete Belgian MenB strain collection (*i.e.* BAST 267 (n = 11), BAST 556 (n = 1) and BAST 4794 (n = 1)) showed no cross-reactivity with the 4CMenB vaccine.

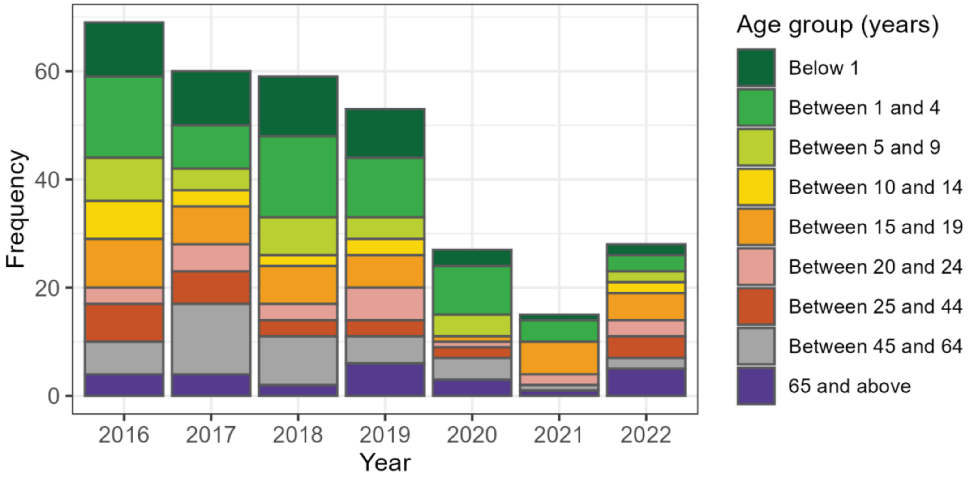

Figure 1. Annual MenB IMD cases by age group for clinical isolates collected by the Belgian NRC between 2016 and 2022 (n = 311). The x-axis shows the collection year, the y-axis the number of cases. The bars are colored by age group as indicated in the legend.

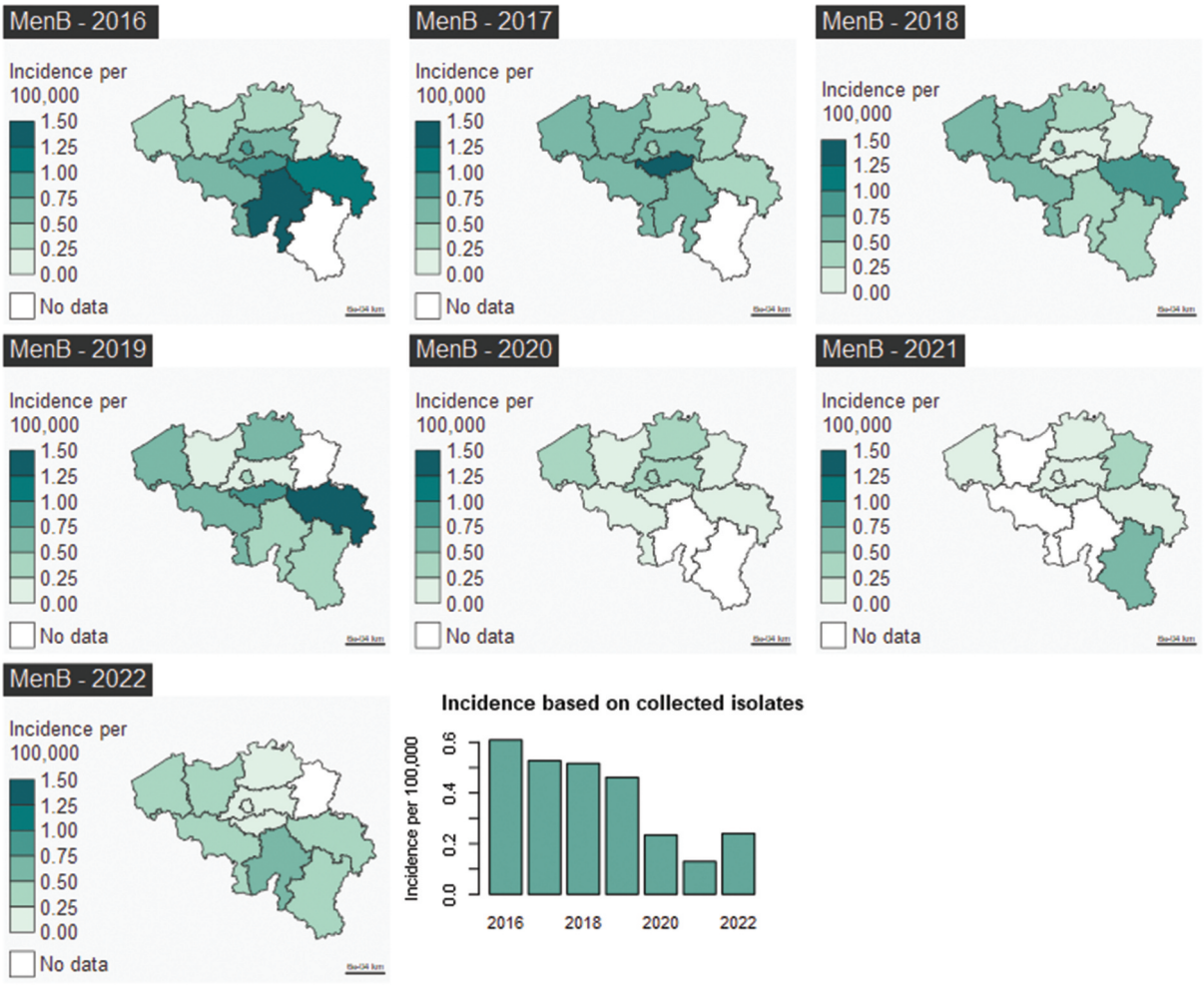

Figure 2. MenB incidence by province based on clinical isolates received by the Belgian NRC during the 2016–2022 period. Sources: ArcGIS hub (Esri BeLux online public data ' Belgium – Provinces' GIS dataset), Statbel (population size).

MenDeVAR-based predicted 4CMenB coverage

Of the 281 MenB isolates with high quality WGS data, 87 (31.0%) were reported by MenDeVAR as an 'exact match' to the 4CMenB vaccine and 100 (35.6%) were predicted to be 'cross-reactive'. For 81 (28.8%) isolates, 'insufficient data' was available and 13 (4.7%) were predicted to have no cross-reactivity with the vaccine (Table 2). This resulted in an estimated coverage of the Belgian panel of 80.7% (lower limit – upper limit: 66.5–95.4%). No statistically significant variations of vaccine coverage were observed over the period 2016–2022 (chi-squared = 12.15, p-value = .832), nor between age groups (chi-squared = 29.64, p-value = .205). While there is no evidence that the 4CMenB coverage varies by patients age or over the years, the number of strains with 'insufficient data' was unexpectedly high in the region of Brussels (chi-squared = 15.22, p-value = .021) and for novel BAST profiles (chi-squared = 25.52, p-value < .001). Of the samples with novel BAST profiles, 39.4% were categorized as 'insufficient data', compared to 12.2% of the other BAST profiles (Table S3). The predicted annual coverage by 4CMenB ranged from 78.2% (lower limit – upper limit: 62.2–94.3%) in 2017 to 83.4% (lower limit – upper limit: 68.7–98.1%) in 2019.

gMATS-based predicted 4CMenB coverage

Of the 281 MenB strains tested, gMATS classified 191 isolates (68.0%) as 'covered', 24 (8.5%) as 'not covered' and the remaining 66 (23.5%) as 'unpredictable' (Table 3). The overall predicted gMATS coverage was 79.5% (lower limit – upper limit: 68.0–91.5) for the period 2016–2022. Annual gMATS coverage predictions

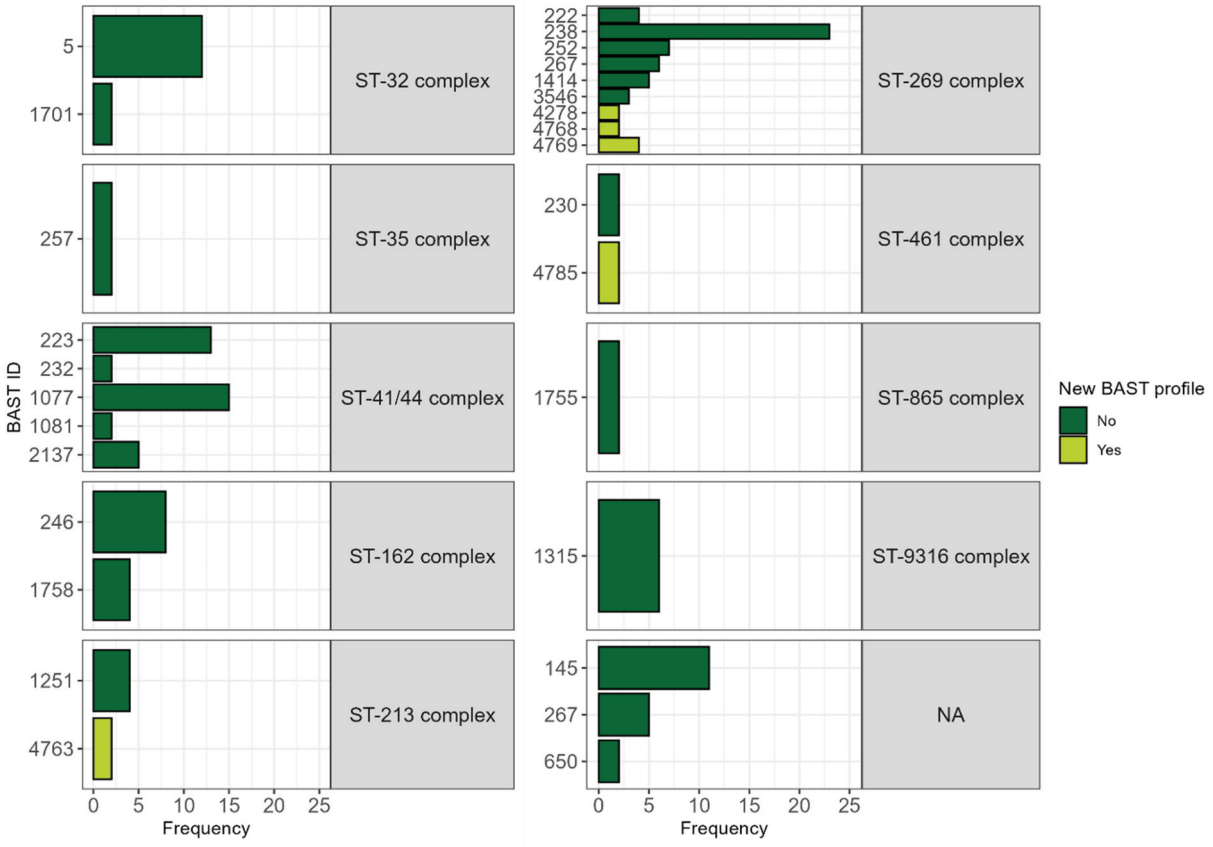

Figure 3. BAST frequencies stratified by clonal complex in sequenced MenB isolates collected by the Belgian NRC between 2016 and 2022. BAST profiles are grouped by clonal complex and shown for samples which passed the QC checks for WGS, excluding profiles observed only once (n = 89) and unknown BAST profiles due to missing peptides (n = 35).

varied between 74.9% (lower limit – upper limit: 53.8–96.1) and 86.0% (lower limit – upper limit: 76.0–96.0) but did not vary significantly over the 2016–2022 period (chi-squared = 10.37, *p*-value = .594). There were also no significant differences in predicted vaccine coverage between patient age groups (chi-squared = 15.74, *p*-value = .470). Significant differences were observed between regions (chi-squared = 10.74, *p*-value = .032) and for novel BAST profiles (chi-squared = 9.15, *p*-value = .010) (Table S4). As for the MenDeVAR index, a higher proportion of novel BAST profiles (25.8%) was 'unpredictable' compared to isolates with known BAST profiles (10.6%).

MenDeVAR – gMATS comparison

MenDeVAR and gMATS predictions differed slightly over the years with a tendency for MenDeVAR to be slightly more conservative than gMATS (*i.e.* predicting fewer strains as covered by 4CMenB). MenDeVAR assigned more samples to the 'insufficient data' category every year except in 2020 (Figures 4 and 5).

At the level of the Belgian panel, 244 out of the 281 isolates had the same vaccine coverage predicted by both schemes with 177, 55 and 12 MenB samples considered as 'exact match' or 'cross-reactive'/'covered', 'insufficient data'/'unpredictable' and 'none'/'not covered' respectively. Of the remaining 37 isolates, 24 isolates were predicted to be 'covered' by only one of the schemes. Of these, 14 isolates were predicted as 'covered' by gMATS and ' insufficient data ' by MenDeVAR and 10 isolates were in the opposite situation. These differences were due to NHBA alleles 3, 20 and 21 that were considered as having 'insufficient data' by MenDeVAR while gMATS considers these to be covered by the 4CMenB vaccine. The reverse cases were due to the NadA peptide 3 that is 'cross-reactive' in the MenDeVAR scheme while NadA is always considered as 'not covered' by gMATS. Additionally, the fHbp peptides 12 and 215 were considered as 'cross-reactive' by MenDeVAR but as 'unpredictable' by gMATS. Twelve of the 37 isolates with different

Table 2. MenDeVAR reactivity indexes for MenB isolates collected during the 2016–2022 period.

	MenDeVAR reactivity index			
	Exact match	Cross-reactive	None	Insufficient data
Total*	87 (31.0%)	100 (35.6%)	13 (4.6%)	81 (28.8%)
Year (No. of isolates)				
2016 (n = 59)	24	18	3	14
2017 (n = 53)	13	20	3	17
2018 (n = 53)	12	23	3	15
2019 (n = 51)	16	19	1	15
2020 (n = 26)	6	10	1	9
2021 (n = 14)	4	5	1	4
2022 (n = 25)	12	5	1	7
Age groups (No. of isolates)				
< 1 (n = 45)	16	15	3	11
1–4 (n = 55)	19	18	0	18
5–9 (n = 27)	8	10	0	9
10–14 (n = 16)	5	8	1	2
15–19 (n = 35)	10	17	1	7
20–24 (n = 23)	7	10	0	6
25–44 (n = 20)	9	3	3	5
45–64 (n = 37)	11	10	3	13
≥65 (n = 23)	2	9	2	10
Region (No. of isolates)				
Brussels (n = 29)	9	4	1	15
Flanders (n = 139)	38	61	7	33
Wallonia (n = 113)	40	35	5	33

(*) Values indicate the number of isolates in each category and values in parentheses indicate the proportion of isolates (%).

vaccine coverage predictions were labeled as 'unpredictable' by MenDeVAR but 'not covered' by gMATS due to their NadA peptides, NHBA allele 24 and fHbp variant 2 and 3 peptides. Finally, the last discrepancy occurring in these isolates was due to NHBA allele 197 that was not covered in the MenDeVAR scheme and fell into the 'unpredictable' gMATS category.

Table 3. gMATS predictions for MenB isolates collected during the 2016–2022 period.

	gMATS coverage predictions		
	Covered	Not covered	Unpredictable
Total*	191 (68.0%)	24 (8.5%)	66 (23.5%)
Year (No. of isolates)			
2016 (n = 59)	42	6	11
2017 (n = 53)	36	6	11
2018 (n = 53)	35	6	12
2019 (n = 51)	36	2	13
2020 (n = 26)	14	1	11
2021 (n = 14)	9	2	3
2022 (n = 25)	19	1	5
Age groups (No. of isolates)			
< 1 (n = 45)	31	4	10
1–4 (n = 55)	35	5	15
5–9 (n = 27)	19	1	7
10–14 (n = 16)	13	1	2
15–19 (n = 35)	27	1	7
20–24 (n = 23)	18	0	5
25–44 (n = 20)	13	4	3
45–64 (n = 37)	24	4	9
≥65 (n = 23)	11	4	8
Regions (No. of isolates)			
Brussels (n = 29)	16	4	9
Flanders (n = 139)	104	13	22
Wallonia (n = 113)	71	7	35

(*) Values indicate the number of isolates in each category and values inside parentheses the proportion of isolates (%).

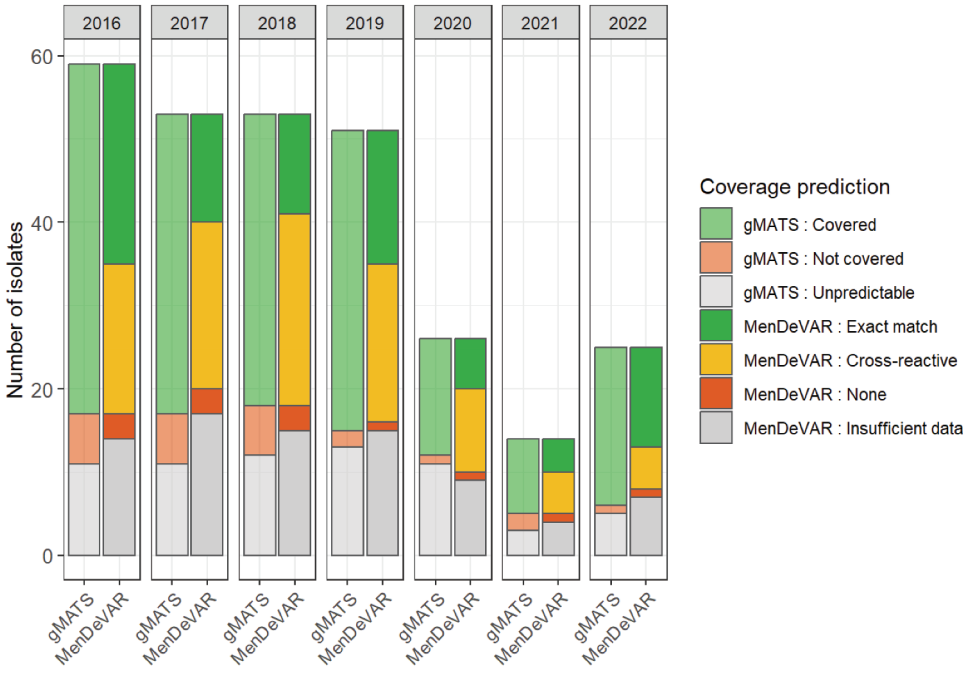

Figure 4. Comparison of the MenDeVAR and gMATS coverage predictions by year for isolates collected in Belgium between 2016 and 2022.

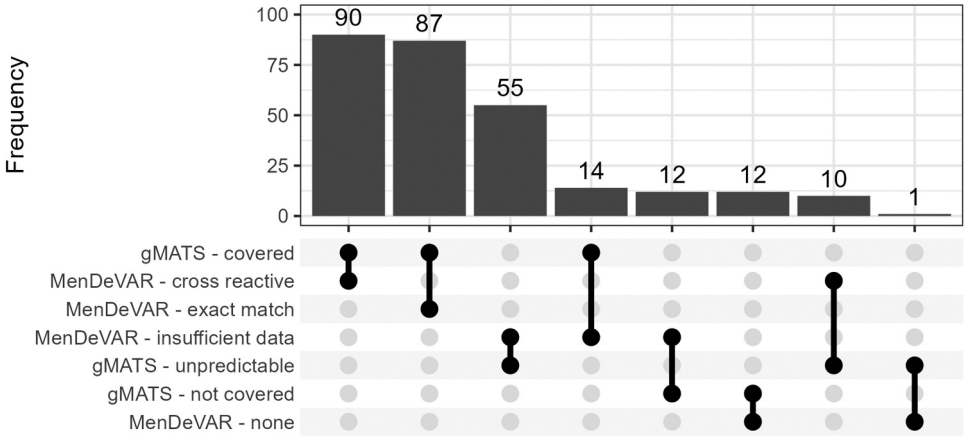

Figure 5. Upset plot of the MenDeVAR and gMATS coverage predictions for isolates collected in Belgium between 2016 and 2022. The barplot shows the size of the overlap between MenDeVAR and gMATS coverage predictions, as indicated by the black points and connecting lines below the plot. For example, the leftmost bar shows the number of isolates for which the gMATS prediction was 'covered' and the MenDeVAR prediction was 'cross-reactive'.

The fHbp and NHBA antigenic variants were predicted as 'covered' by gMATS in a high number of isolates (149 and 145, respectively) (Figure S3). fHbp antigenic subvariants were also the main factor in MenDeVAR predicted vaccine coverage (n = 153) (Figure S4). A high proportion of isolates encoded 'cross-reactive' fHbp subvariants (n = 140) such as allele 14 and 15 and some were 'exact matches' with fHbp subvariant 1 present (n = 13) (Figure S5). In contrast to the gMATS scheme, only 44 NHBA peptides contributed to the MenDeVAR predicted 4CMenB coverage (Figure S3–S4), while the bulk of isolates, including those with alleles 3 (n = 15), 20 (n = 25) and 21 (n = 61), were labeled as 'insufficient data' by MenDeVAR (Figure S6). NadA subvariants contributed minimally to the vaccine coverage predictions, with only nine isolates encoding subvariant three being covered in the MenDeVAR scheme (Figure S7). Additionally, the *nadA* gene was missing in 197 isolates and was never considered as covered in the

gMATS scheme. Finally, PorA-VR2 peptide 4 was detected in 55 isolates and predicted to be covered by 4CMenB by both schemes (Figure S8). Overall, the predictions were very similar between the two vaccine coverage prediction schemes, with gMATS assigning more isolates to the 'not covered' group, while MenDeVAR assigned more samples to the 'insufficient data' category.

Discussion

The Belgian NRC collected 311 *N. meningitidis* serogroup B strains between 2016 and 2022. Similar to other high-income regions, IMD is endemic in Belgium with a low incidence and occasional localized outbreaks.[24] The highest incidence of MenB was observed in the first year of this study, with 0.61 cases per 100,000 inhabitants in 2016, but this was lower than the pre-vaccine estimate of 0.79 per 100,000 inhabitants in 2012.[25] The MenB isolates from this collection were mostly isolated from infants, children and young adults from the age ranges 0–5 and 15–19 years. Adults between 45 and 64 years were the fourth most affected age group. A similar trend, with MenB shifting slightly toward older age groups over the 2011–2022 period, was observed in a study in France over 48 years.[26] Finally, IMD following MenB infection was most common in patients with septicemia and/or meningitis. Clinical manifestations and specimen types were independent of patient age groups.

As vaccine antigens may be subject to geographical and temporal variation, it is important to catalog circulating variants. The vaccine coverage predictions for both MenDeVAR and gMATS did not vary significantly of the years of the strain isolation as well as the patient's age, suggesting broad applicability of the 4CMenB vaccine. However, significant differences were observed between regions, with a higher proportion of 'unpredictable' strains collected from patients in Brussels. This could potentially be a consequence of the increased international mobility and greater population diversity in Brussels compared to other parts of Belgium. This could lead to a broader range of antigenic profiles that are underrepresented in the reference databases currently used by gMATS and MenDeVAR. However, the available metadata was insufficient to test this hypothesis. Similar to other recent studies,[27–29] the Belgian MenB isolates showed a high genetic diversity, which was reflected in the high number of antigenic subvariants detected for fHbp, NHBA and PorA-VR2. The different combinations of these vaccine antigens also accounted for a large number of BAST profiles observed (n = 117), 89 of which were present only once. Furthermore, 59 novel BAST profiles were submitted to PubMLST, for which coverage predictions were significantly more often classified as 'unpredictable'.

Despite this variation, the predicted 4CMenB vaccine coverage from both the MenDeVAR and gMATS schemes was high at 80.7% (lower limit – upper limit: 66.5–95.4%) and 79.5% (lower limit – upper limit: 68.0–91.5), respectively. Overall, there was a high consensus between the gMATS and MenDeVAR vaccine coverage predictions. Differences in vaccine coverage predictions between the two schemes were often caused by a small number of subvariants that are considered unpredictable by one of the schemes. These differences were most apparent for the NHBA subvariants for which some common alleles were considered as 'covered' by gMATS and as 'insufficient data' by MenDeVAR. For instance, the NHBA alleles 3, 20 and 21 are considered as 'covered' by gMATS but fall into the 'insufficient data' category in the MenDeVAR scheme. Conversely, the NHBA allele 24 is considered 'unpredictable' by MenDeVAR but 'not covered' by gMATS. The two prediction schemes use different thresholds for the percentage of MATS-covered isolates required for a subvariant to be considered as 'covered'/'not covered' by the 4CMenB vaccine. The gMATS method also applies a test of proportions to predict allele 4CMenB coverage, whereas MenDeVAR does not.[10,11] These differences lead to minor discrepancies between the MenDeVAR and gMATS coverage predictions. Other discrepancies could be attributed to the different datasets used to implement the two methods.[10,11] More specifically, MenDeVAR is based on MATS data derived from publications, while gMATS considers additional unpublished data. Both gMATS and MenDeVAR also inherit the limitations of the conservative estimates by design of MATS which does not take cooperative effects among antigens into account,[10,30–32] as well as the underestimation of the NadA contribution due to its limited *in vitro* expression[17,18] or the less well-known impact of minor OMV components.[6] These examples illustrate the limitations of using *in silico* methods to predict coverage, as scheme-specific differences can impact the results.

Strains not predicted to be covered by both schemes were predominantly classified as 'unpredictable' due to a lack of experimental data. The increase in this category reported in recent years is likely to reflect a shift in the circulating variants.[27–29] This highlights the need for continued experimental testing of the reactivity of novel peptide subvariants to ensure accurate predictions of vaccine coverage. In this context, high-throughput-based methods may be a powerful alternative or complement to conventional testing.[33] Whilst WGS does not provide direct information on antigen reactivity, the experimental data available allowed us to predict the reactivity for the vast majority of the collected strains. WGS has the added advantage of being easy to scale-up, and allows us to fully characterize the circulating strains, providing valuable insight into potential relationships between strains and other relevant genomic features.

In conclusion, this study presents the clinical data of all 311 strains collected by the NRC in Belgium between 2016 and 2022. For 281 strains, high-quality WGS data was generated which was made publicly available. The WGS data revealed a high genetic diversity in antigen variants, which was reflected in a large number of different BAST profiles. Despite this diversity, both MenDeVAR and gMATS predicted high coverage of the 4CMenB vaccine. We show that circulating antigenic variants change over time, which may affect vaccine coverage, highlighting the need for surveillance programs. Finally, we show that phenotypic data are currently still required for accurate vaccine coverage predictions based on genomic data.

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Author contributions

CRediT: **Nathalie Goeders:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing; **Kevin Vanneste:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing; **Nancy H.C. Roosens:** Conceptualization, Funding acquisition, Writing – review & editing; **Bert Bogaerts:** Conceptualization, Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing; **Wesley Mattheus:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Disclosure statement

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Wesley Mattheus is responsible for the unit of Gastrointestinal Diseases and Bacterial Meningitis within Sciensano. He has over 15 years of experience in the molecular surveillance of the bacterial pathogens *Listeria*, *Salmonella*, *Shigella*, *Yersinia* and *Neisseria meningitidis*. His scientific work mainly focuses on molecular surveillance of IMD within the framework of the tasks of the National Reference Centres. He participates in several national and international research projects and has authored numerous peer reviewed international scientific publications.

Data availability statement

The WGS data used in this study are available in the Sequence Read Archive (SRA) under the BioProject PRJNA1127336. Metadata and SRA accession numbers are provided in Supplementary Table S5. Metadata and assemblies are available in the PubMLST *Neisseria* database.

Ethics statement

This study does not involve human participants and so no ethical approval was required.

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