Genetic variability of Polish serogroup B meningococci (2010–2016) including the 4CMenB vaccine component genes

Izabela Waśko, Agnieszka Golębiewska, Marlena Kiedrowska, Patrycja Ronkiewicz, Izabela Wróbel-Pawelczyk, Alicja Kuch, Eva Hong, Anna Skoczyńska

Vaccine 38 (2020) 1943–1952

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Genetic variability of Polish serogroup B meningococci (2010–2016) including the 4CMenB vaccine component genes

Izabela Waśko [a,*], Agnieszka Gołębiewska [a], Marlena Kiedrowska [a], Patrycja Ronkiewicz [a], Izabela Wróbel-Pawelczyk [a], Alicja Kuch [a], Eva Hong [b], Anna Skoczyńska [a]

[a] National Reference Centre for Bacterial Meningitis, National Medicines Institute, Warsaw, Poland
[b] Institute Pasteur, Invasive Bacterial Infections Unit, Paris, France

ARTICLE INFO

Article history:
Received 22 May 2019
Received in revised form 20 December 2019
Accepted 7 January 2020
Available online 21 January 2020

Keywords:
Neisseria meningitidis
invasive meningococcal disease
molecular epidemiology
Bexsero® Antigen Sequence Type

ABSTRACT

Neisseria meningitidis serogroup B (MenB) has recently become the major cause of invasive meningococcal disease in Poland. Therefore, the purpose of this study was to characterize MenB isolates, responsible for invasive meningococcal disease in 2010–2016, by MLST and sequencing of genes encoding proteins used as 4CMenB vaccine antigens. Two methods of coverage estimation were performed: extrapolation of MATS results of Polish meningococci 2010–2011 (exMATS) and gMATS, which combines genotyping and MATS results.

Among 662 isolates 20 clonal complexes (CC) were detected, of which the most frequent were CC32, CC41/44 and CC18, accounting for 31.9%, 16.5% and 12.7%, respectively. A total of 111 combinations of PorA variable regions (VR1/VR2) were found, with P1.7,16 (15.0%) and P1.22,14 (13.6%) being prevalent. Vaccine variant VR2:4 was detected in 7.3% of isolates, mainly representing CC41/44 and non-assigned CC. Eighty five *fHbp* alleles encoding 74 peptide subvariants were revealed. Subvariant 1.1, a component of 4CMenB, was prevalent (24.2%) and found generally in CC32. Typing of the *nhba* gene revealed 102 alleles encoding 87 peptides. The most frequent was peptide 3 (22.4%), whereas vaccine peptide 2 was detected in 9.8%, mostly among CC41/44. The *nadA* gene was detected in 34.0% of isolates and the most prevalent was peptide 1 (variant NadA-1; 71.6%), found almost exclusively in CC32 meningococci. Vaccine peptide 8 (variant NadA-2/3) was identified once. Consequently, 292 completed BAST profiles were revealed. Regarding vaccine coverage, 39.7% of isolates had at least one 4CMenB vaccine variant, but according to exMATS and gMATS the coverage was 83.3% and 86.6%, respectively.

In conclusion, Polish MenB (2010–2016) was highly diverse according to MLST and gene alleles encoding 4CMenB vaccine antigens. Some correlations between clonal complexes and variants of examined proteins/BAST profiles were revealed and a high coverage of 4CMenB vaccine was estimated.

1. Introduction

Neisseria meningitidis (meningococcus, Men) is a Gram-negative diplococcus responsible for invasive meningococcal disease (IMD), predominantly presenting as meningitis and/or septicemia, with a general notification rate of around 0.55 per 100,000 people in European countries [1]. Although IMD is present all over the world, the distribution of serogroups, clonal complexes and associated incidence and case fatality rates may differ significantly. The epidemiology of IMD has also been changing over time and differs in age groups, with the highest incidence in children under 1 year old [1–3]. IMD occurs mostly as sporadic cases but may suddenly appear as an outbreak or epidemic. The disease evolves rapidly and still presents high mortality in spite of appropriate treatment. Prevention against this human-associated bacterium is most efficient by vaccination. Polysaccharide-based vaccines and conjugate vaccines are effective against the most frequent serogroups (A, C, W, Y) except *N. meningitidis* serogroup B (MenB) strains [4]. Prevention of MenB IMD is still a challenge due to poor immunogenicity and similarity of MenB capsular polysaccharide to polysialic structures in human neural tissue. Therefore, vaccines including other, alternative antigens have been developed, such as the protein-based novel vaccines (Bexsero®, Trumenba®) that have been licensed in Europe, Australia and the USA in recent years. 4CMenB (tradename Bexsero) has been implemented into national immunization routine schedules in the UK, Ireland and Italy so far. Other countries, such as the USA, Canada, Ireland and France, have used these vaccines for the control of outbreaks [5–13]. 4CMenB

* Corresponding author.

https://doi.org/10.1016/j.vaccine.2020.01.021
0264-410X/

includes four meningococcal subcapsular proteins with antigenic activity: factor H binding protein (fHbp), Neisserial heparin binding antigen (NHBA), *Neisseria* adhesin A (NadA) and porin A (PorA). Due to the high variability of these protein antigens, 4CMenB does not cover all MenB isolates. To predict strain coverage by 4CMenB, the meningococcal antigen typing system (MATS) assay was developed. This combines PorA genotyping and enzyme-linked immunosorbent assay (ELISA) that compares the quality and quantity of the three remaining antigens with those of three corresponding reference strains, to generate a set of relative potencies (RPs). If a strain possesses PorA P1.4 and/or any of the three RPs exceed a corresponding threshold level (the positive bactericidal threshold) the isolate is likely to be covered by the vaccine [14]. However, MATS is performed only in a few laboratories selected by the 4CMenB producer, therefore estimates of coverage are often limited to those based on genotypic analysis of all four antigens. The Bexsero antigen sequence type (BAST) provides a means of indexing distinct combinations of 4CMenB antigens that may then be combined with chosen genotypic coverage criteria [15–17]. Recently, a genetic MATS assay (gMATS) was proposed that linked antigen genotyping and MATS data of 3481 MenB isolates from European and non-European countries. This led to the generation of a defined list of fHbp and NHBA peptides that were classed as covered, not covered or unpredictable. Only PorA P1.4 was considered covered while NadA was always regarded as not covered. When gMATS is applied to large national datasets, the proportion of isolates with indeterminate coverage is divided equally into covered and not covered [18].

Meningococci of serogroup B are the cause of the majority of IMD cases in many countries, although in recent years other serogroups have also been responsible for a high percentage of IMD cases (e.g. MenW in England, the Netherlands, Australia, Argentina and Chile; MenC in France and Italy) [19,20]. The epidemiology of serogroup B meningococci in Poland has changed over the last 20 years. According to the National Reference Centre For Bacterial Meningitis data, before 2005 MenB was responsible for approximately 75% of IMD, mainly meningitis, due to Polish surveillance system that obliged to report only meningitis but not septicaemia cases. After 2005 the system has changed and all cases of IMD had to be notified. Between 2006 and 2009, dissemination of MenC ST-11 isolates and their outbreaks were noted. All these led to decrease of the proportion of MenB to around 40–50% which was comparable to MenC. In the recent years due to decreasing of MenC, percentage of MenB increased up to 70% [21,22]. Consequently, the purpose of this study was the characterization of Polish MenB over a seven-year period in order to determine the molecular epidemiology and estimate the strain coverage by 4CMenB.

2. Materials and methods

The project included MenB isolates responsible for IMD in Poland between 2010 and 2016, sent to the National Reference Centre for Bacterial Meningitis (NRCBM) within a laboratory-based surveillance system. In the study period, the NRCBM received 698 MenB isolates; 36 could not be recovered and only confirmation of species and genogroup by polymerase chain reaction (PCR) was provided, so these were excluded from study. Therefore, 662 isolates were further analyzed (2010: n = 82; 2011: n = 114; 2012: n = 96; 2013: n = 113; 2014: n = 82; 2015: n = 100; 2016: n = 75). They were routinely re-identified and serogrouped by slide agglutination using serogroup-specific antisera (groups A, B, C, W, Y; Remel, UK). Multilocus sequence typing (MLST) and *por*A typing using standard procedures (http://pubmlst.org/neisseria/) were performed. Isolates were also characterized by sequencing of genes encoding surface proteins used as vaccine antigens (including *fHbp*, *nad*A and *nhba*) and generating BAST profiles [15,23,24]. The range of clonal complexes and tested peptides was measured by Simpson's index of diversity (value 0, no diversity; closer to 1, greater diversity) [25]. Phylogenetic analysis was carried out using BioNumerics (v7.6; Applied Maths, Sint-Martens-Latem, Belgium). Statistical analysis used the chi-square test (https://www.graphpad.com/quickcalcs/contingency2/) and the chi-square test for trend (http://epitools.ausvet.com.au/content.php?page=trend); a p value of $\leq$0.05 was considered to be significant.

The NRCBM provides routine PCR diagnosis of clinical materials and collects strains that, together with Polish National Census of Population data, were used for incidence calculation (presented in Section 3.1).

We used two calculations of strain coverage by 4CMenB. The first one (coverage/exMATS) was derived from extrapolation of Polish MenB 2010–2011 MATS analysis results [26] to all the MenB isolates tested in this study. Peptides that were 100% MATS positive were estimated as covered and those that were 100% MATS negative as not covered. For peptides that were MATS positive, but coverage was <100% and >0%, covered isolates between 2012 and 2016 were chosen randomly but with respect to their proportions in STs, clonal complexes and year of analysis. For example NHBA peptide 3 was covered by MATS in 24.6%, therefore 36 of 148 isolates between 2012 and 2016 were assumed as covered. Annual distribution was calculated as follows (number of isolates assumed as covered/all isolates with this peptide): in 2012: 5/21; 2013: 6/25; 2014: 4/16; 2015: 4/17 and in 2016: 3/12. Isolates with fHbp subvariants and NHBA variants that appeared for the first time after 2011, and therefore MATS data were not available, were defined as unknown. Among these, the 50% of isolates that were not estimated as covered by other antigens were assumed to be covered. The second calculation was based on gMATS presented by Muzzi et al. [18]. Detailed coverage of individual peptides identified among Polish meningococci is summarized in Table 1.

3. Results

3.1. Epidemiological background

During the study period (2010–2016), 1495 IMD cases were laboratory confirmed (1106 by culture and 389 by PCR) by the NRCBM and this was 93.9% of all IMD cases registered by the National Institute of Public Health–National Institute of Hygiene (NIPH-NIH) based on obligatory reports by physicians and microbiologists [27]. The majority of cases were caused by meningococci of serogroups B (61.7%: 698 culture and 224 non-culture cases) and C (32.0%: 361 culture and 118 non-culture cases). The distribution of MenB was temporally increasing, accounting for 54.9% in the first three years (2010–2012) and 68.2% in the following years (2013–2016) (χ^2 for trend, $p < 0.0001$). The highest percentage of MenB was found in infants (76.9% of all cases in this age group), in whom the incidence of MenB IMD was 9.7/100,000, whereas the overall MenB incidence was 0.34/100,000. The overall case fatality rate (CFR) was 11.2%, without any significant differences between serogroups B and C. However, taking age groups into consideration, in people aged 65 years and children aged 2–3 years the MenB CFR was twice the overall value (21.7% and 21.4%, respectively).

3.2. MLST analysis of MenB

MLST was performed for all 662 MenB isolates; 287 sequence types were determined, of which 217 (75.6%) were notified only once. These represented 32.7% of all MenB isolates. Only eight sequence types were represented by 10 or more isolates: the

Table 1
Two methods of coverage estimation for Polish MenB, 2010–2016.

Antigen	Calculation method	
	exMATS peptide – coverage – %* (number of covered/all isolates with the peptide)	gMATS
fHbp covered	1.1 (158), 1.4 (46), 1.14 (45), 1.144 (41), 1.15 (16), 1.232 (2), 1.234 (3), 1.245 (7), 1.66 (1), 1.510 (5), 1.590 (1), 1.591 (1), 1.592 (1), 1.594 (1), 1.664 (1), 1.665 (1), – 100% each peptide 1.37–94.3% (82/87) 1.13–60% (11/19) 1.321–43% (16/38) Unknown – 50% (10/20)	1.1, 1.2, 1.4, 1.14, 1.15, 1.37, 1.144, 1.232, 1.245, 1.249, 1.510 Unpredictable – 50%
fHbp not covered	1.213 (2), 1.260 (14), 1.539 (3), 1.593 (4) all variants of family 2 and 3 (135) Unknown (10/20) – 50%	1.213 all variants of family 2 and 3 Unpredictable – 50%
NHBA covered	10 (5), 12 (4), 243 (34), 384 (4), 408 (1), new_2 (2), 578 (1), 580 (1), 582 (1), 583 (1), new_6 (2) – 100%, 2–94.4% (61/65) 3–24.6% (36/148) 20–21.4%, (15/71) 21–25% (9/35) 81–50% (1/2) 54 – 50% (1/2) 6–3.8% (3/74) Unknown (27/54) – 50%	1, 2, 3, 10, 20, 21, 243 Unpredictable – 50%
NHBA not covered	17 (2), 18 (23), 24 (18), 29 (6), 42 (2), 47 (9), 49 (7), 53 (9), 58 (3), 63 (16), 89 (1), 114 (1), 115 (16), 118 (4), 188 (9), 249 (2), 253 (6), 502 (5), 579 (1), 581 (1), new_1 (1), new_3 (1), new_4 (1), new_5 (1), new_7 (1), Unknown (27/54) – 50%	6, 17, 18, 24, 30, 31, 43, 47, 58, 112, 114, 120, 187, 253 Unpredictable – 50%
NadA covered	3 (10) and 8 (1) – 100%	none
VR2 PorA covered	4 (48) – 100%	4
Overall coverage	83.3%	86.6%

Unknown – peptides for which Polish MATS data was not available.
Unpredictable – according to gMATS [18].
*- % of coverage of each peptide was calculated on the basis of MATS results for isolates 2010–2011 (n = 196). Accordingly, number of isolates assumed as covered was estimated.
MATS results for isolates 2010–2011 (number of MATS positive/all with the peptide) which are the basis of exMATS calculation is as follows:
fHbp peptides; 1.1 (57/57); 1.37 (33/35); 1.14 (15/15); 1.4 (9/9); 1.321 (3/7); 1.13 (3/5); 1.144 (5/5); 1.15 (4/4); 1.245 (3/3); 1.260 (0/3), 1.234 (2/2); peptides; 1.66, 1.232, 1.510, 1.590, 1.591, 1.592, 1.594, 1.664 and 1.665 – (1/1) per each; peptides; 1.213, 1.539, 1.593 – (0/1) per each; peptides belonging to family 2 and 3 (n = 38) all MATS negative.
NHBA peptides; 3 (14/57); 6 (1/26); 2 (17/18); 20 (3/14); 21 (2/8); 243 (5/5); 54 (1/2); 81 (1/2); new_2 (2/2); new_6 (2/2); peptides; 10, 12, 384, 408, 578, 580, 582 and 583 – (1/1) per each.
MATS negative peptides; 18 (6), 24 (6), 63 (6), 47 (3), 49 (3), 53 (3), 115 (3), 188 (3), 253 (3), 54 (2), 58 (2); 17, 29, 42, 89, 114, 249, 502, 579, 581, new_1, new_3, new_4, new_5, new_7 – 1 per each.
NadA peptides; 3 (2/2); all other MATS negative.

leading one was ST-32 (CC32) (n = 133, 20.1%), followed by ST-33 (CC32) (n = 43, 6.5%), ST-1194 (CC41/44) (n = 28, 4.2%), ST-9316 (non-assigned CC) (n = 21, 3.2%), ST-213 (CC213) (n = 19, 2.9%), ST-145 (CC18) (n = 17, 2.6%), ST-6765 (CC213) (n = 10) and ST-8203 (CC41/44) (n = 10). Distribution of sequence types among clonal complexes and Simpson's index of diversity of clonal complexes are shown in Table S1 (see Supplementary Material).

Twenty clonal complexes were detected, of which the most frequent were CC32, CC41/44, CC18, CC213 and CC269, accounting for 31.9%, 16.5%, 12.7%, 6.2% and 3.6% of isolates, respectively. Other clonal complexes were represented by less than 2% of isolates each. A significant part (20.2%) of MenB isolates were non-assigned to a well-defined clonal complex (Fig. 1). The yearly distribution of five major clonal complexes fluctuated in the range 66–77% without any statistical significance (χ^2 for trend, p = 0.6).

3.3. Genetic variability of fHbp, nhba, nadA and porA and their distribution among clonal complexes

3.3.1. fHbp

Typing of the *fHbp* gene was provided for 652 MenB isolates; for 10 isolates the peptide variant was not determined due to difficulty with obtaining the PCR product or sequencing. Eighty-five *fHbp* alleles encoding 74 peptide subvariants were revealed (Simpson's index of diversity = 0.9, Table S2, Table S4 see Supplementary Material). The majority of isolates represented variant 1 (79.2%), followed by variant 2 (14.1%) and variant 3 (6.6%). One isolate possessed an fHbp hybrid variant (allele 231, peptide 207, 0.1%). Among the determined peptide subvariants the leading one was 1.1 (variant 1, subvariant 1; the 4CMenB vaccine variant), accounting for 24.2%.

Variant 1 was predominant in all age groups, with the highest proportion among children 1–4 years of age, accounting for 89.6%, and the lowest among infants and adults aged >25 years (73.1% and 72.0%, respectively). It was also the prevalent variant in each year of the study, with proportions ranging from 74.0% to 82.7% (χ^2 for trend, p = 0.38). With regard to major subvariants, proportions of subvariants 1.1 and 1.37 were significantly descending (χ^2 for trend, p = 0.03 and 0.01, respectively). Conversely, an increasing trend was observed for subvariants 1.144 and 1.321 (χ^2 for trend, p = 0.001 and 0.007, respectively).

The vaccine subvariant 1.1 fHbp (n = 158) was mostly associated with CC32 (73.9% of all CC32, mainly ST-32, 123/133) but occurred also once in isolates of CC213 and CC269. The second

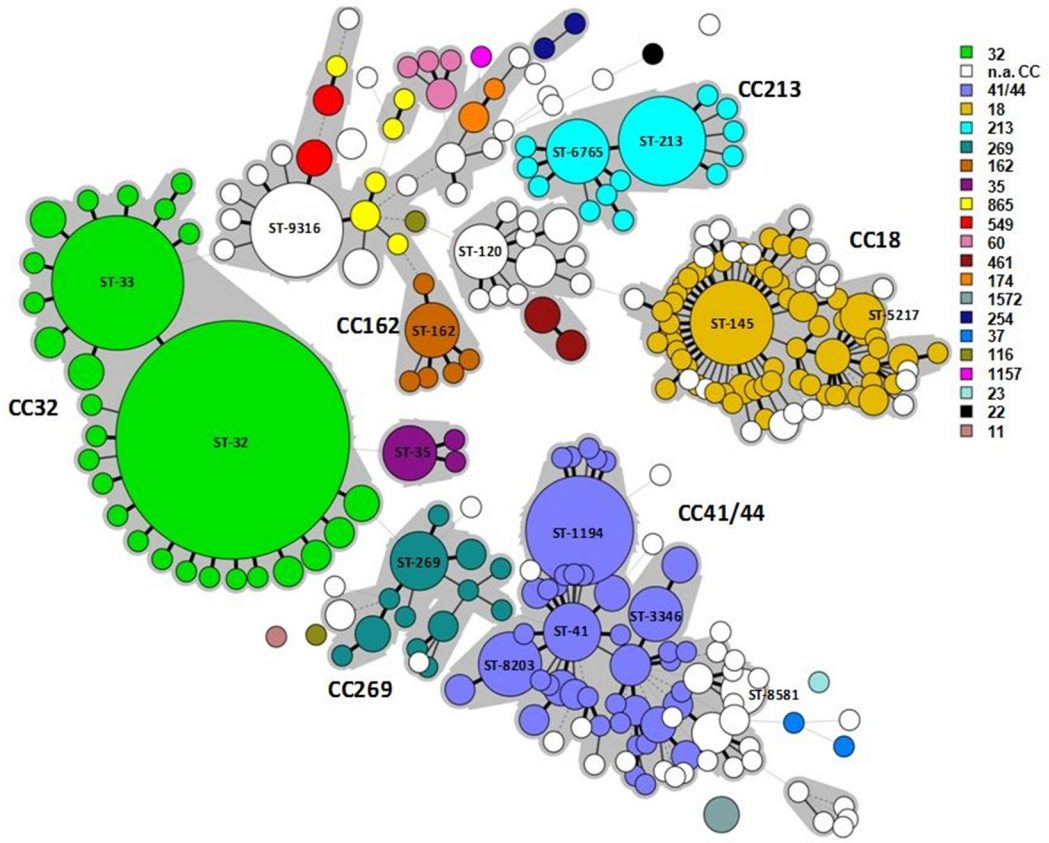

Fig. 1. Minimum spanning tree of the MLST data for the 662 *N. meningitidis* study isolates. Each circle represents one ST and the size of the circle is proportional to the number of isolates with that ST. CCs are marked with colors: white circles represent STs non assigned to CCs (n.a. CC). Connecting lines infer phylogenetic relatedness in terms of number of allelic differences (thick solid, 1: thin solid, 2: dashed, 3: dotted, 4). Gray zones surrounding groups of STs represent these with maximum distance of three between nodes. The ST numbers are indicated for those that are represented by five or more isolates.

most common CC32 peptide, subvariant 1.144, appeared in 19.4% of CC32 (mainly ST-33, 37/43). Subvariants 1.14 and 1.4 were commonly found among CC41/44, accounting for 38.5% and 35.8% of these isolates, respectively. In the group of non-assigned CC meningococci, major proportions were MenB carrying subvariants 1.321 (mostly ST-9316) and 1.37, accounting for 22.4% and 13.4%, respectively. However, the majority of subvariant 1.37 was associated with CC18: 80.9% of this clonal complex possessed this subvariant. Other most common subvariants 1.15 and 3.45 occurred almost exclusively in CC269 and CC213, respectively. The distribution of individual fHbp subvariants among major clonal complexes is shown in Fig. 2.

3.3.2. nhba

Typing of the *nhba* gene was completed for 661 isolates, revealing 102 alleles and 87 peptides (Simpson's index of diversity = 0.9, Table S4, see Supplementary Material). The PCR product was not obtained for one isolate, despite many attempts. The most frequent peptide was peptide 3 (n = 148, 22.4%), followed by peptides 6 (n = 74, 11.2%), 20 (n = 71, 10.7%), 2 (n = 65, 9.8%), 21 (n = 35, 5.3%), 243 (n = 34, 5.1%), 18 (n = 23, 3.5%), 24 (n = 18, 2.7%), 63 (n = 16, 2.4%) and 115 (n = 16, 2.4%). The other 78 peptides were each represented by less than 2.0% of all MenB, including 47 appearing only once. The yearly distribution of major peptides was changing slightly without statistical significance except for peptide 20, whose proportion was found to increase since 2013 (χ^2 for trend, p = 0.003). The 4CMenB vaccine peptide 2 appeared each year, accounting for 7.1–14.6% (χ^2 for trend, p = 0.5). Forty percent of MenB with peptide 2 occurred among children under 2 years of age (n = 26). The most common peptides were associated with individual clonal complexes or sequence types, for example peptides 3 and 20 were found mainly among CC32 (ST-32 and ST-33, respectively), peptides 6, 2 and 21 in CC18, CC41/44 and CC269, respectively, and peptides 18 and 115 were characteristic for ST-213 and ST-6765, both representing CC213. The vaccine peptide 2 was detected mostly among CC41/44, in five STs non-assigned to any clonal complex and once in CC60 and CC32 (Fig. 3).

3.3.3. porA

Typing of the *porA* gene was performed for 661 isolates. For one isolate (ST-9127, CC18) it was impossible to obtain the PCR product, despite many attempts. There were 37 variants of PorA variable region VR1 and 53 of VR2 detected, therefore 111 combinations of VR1/VR2 were found (Simpson's index of diversity = 0.9). The most common were P1.7,16 (n = 99, 15.0%), P1.22,14 (n = 90, 13.6%), P1.7–2,16 (n = 40, 6.1%) and P1.5–2,10–1 (n = 35, 5.3%), with the remaining combinations each represented by less than 5% of all MenB. The 4CMenB vaccine VR2 PorA variant (VR2:4) was detected each year, accounting for 1.8–12.2% in 48 isolates (7.3%), mainly among children under 2 years of age (n = 26, 54.2%). The most frequent combinations of P1.7,16 and P1.7–2,16 were found mostly among CC32, whereas P1.22,14 was found among all clonal complexes but mostly in CC213 (in 85.4% of isolates representing this clonal complex). The vaccine VR2:4 was found among CC41/44 (n = 24), followed by non-assigned CC (n = 12), CC162 (n = 5), CC269 (n = 4) and then CC18, CC32 and CC213 (n = 1 for each) (Fig. 4, Table S4, see Supplementary Material).

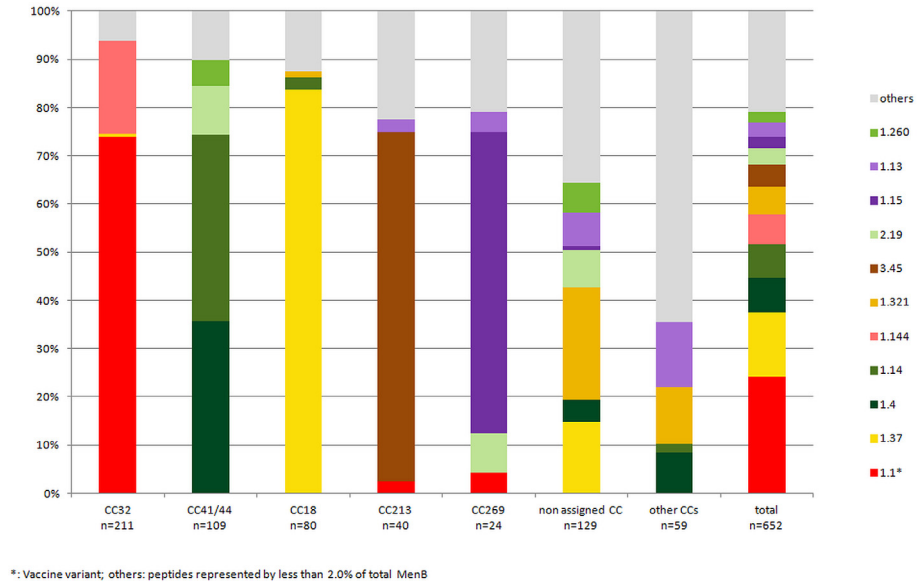

Fig. 2. Distribution of fHbp subvariants among major clonal complexes of Polish serogroup B meningococci, 2010–2016 (n = 652).

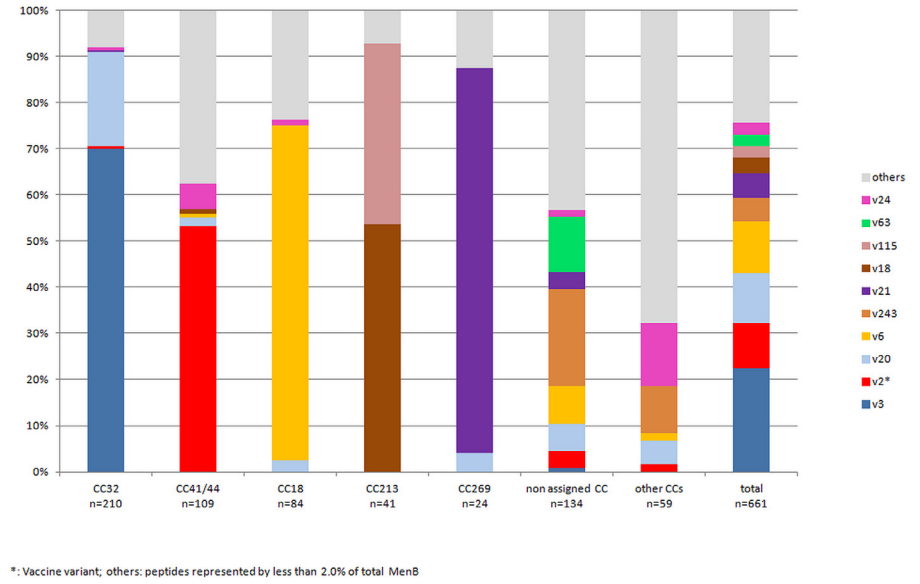

Fig. 3. Distribution of NHBA variants among major clonal complexes of Polish serogroup B meningococci, 2010–2016 (n = 661).

3.3.4. *nadA*

The *nadA* gene was detected in 225 (34.0%) isolates, of which 26 possessed a frameshift mutation and three possessed insertion sequences. For one isolate (ST-9316, non assigned CC) the result of PCR was unclear and needs further analysis. The most prevalent peptide was peptide 1 (variant NadA-1; 79.6%), followed by 21 (variant NadA-4/5; 13.3%) and 3 (variant NadA-2/3; 5.1%). Variant 1 was found almost exclusively in CC32 meningococci (mainly ST-32), whereas variant 4/5 was associated with CC213 and non-assigned CC (mostly ST-9316). Vaccine peptide 8 (variant NadA-2/3) was noticed once in CC18 (Table 2, Table S4, see Supplementary Material). Simpson's index of diversity for NadA was 0.45.

3.3.5. *BAST profiles*

A total of 292 completed BAST profiles were revealed, including 8 new ones (Table S3, see Supplementary Material). Thirteen isolates possessed incomplete BAST profiles due to lack of *fHbp* (n = 10), *nhba* (n = 1), *por*A (n = 1) and *nad*A (n = 1). Thus complete BAST profiles were assigned to 649 (98.0%) tested isolates. A significant number of detected BAST profiles (n = 221, representing 34.0% of all MenB) appeared only once and only a limited number were characterized by frequency $\geq$10, with the leading BAST-4 (fHbp 1.1; NHBA-3; NadA-1.1; PorA P1.7,16) occurring in 89 (13.7%) isolates. They were found exclusively in particular clonal complexes: for example, BAST-4, BAST-84, BAST-1701 and BAST-985 in CC32; BAST-239 and BAST-223 in CC/44; BAST-1243 in CC18; BAST-224 in CC213; and BAST-222 in CC269 (Fig. 5, Table S4, see Supplementary Material). The BAST-1 profile containing exact matches to the vaccine was not found.

3.4. Initial 4CMenB coverage estimation

Strain coverage by 4CMenB due to exact match was counted as 39.7% (258 isolates possessed at least one vaccine variant antigen).

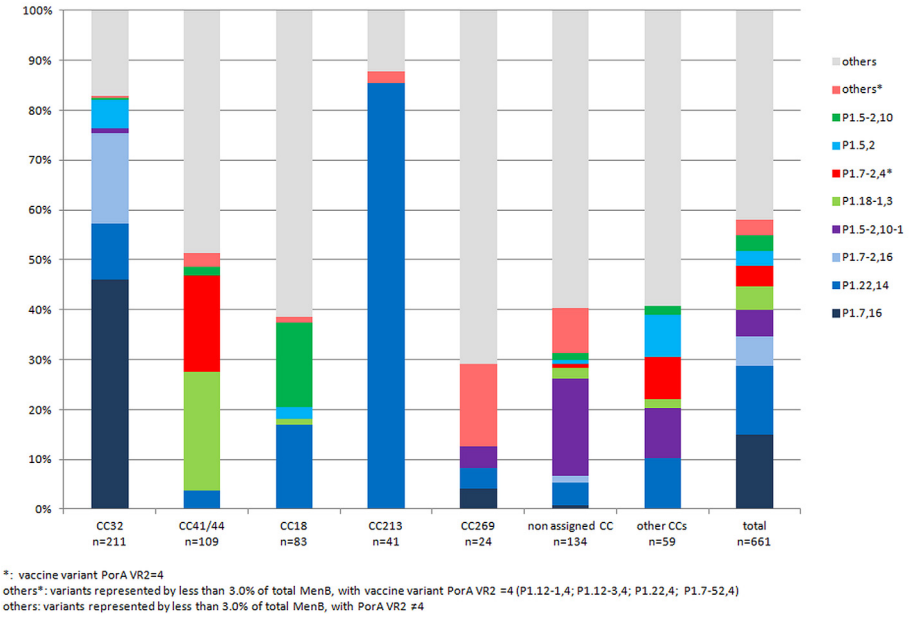

Fig. 4. Distribution of VR1/VR2 PorA combinations among major clonal complexes of Polish serogroup B meningococci, 2010–2016 (n = 661).

Table 2
Variability of NadA peptides among major clonal complexes of Polish serogroup B meningococci, 2010–2016.

Variant	Peptide		n	Clonal complex
Variant 1 (162, 72.0%)	1		156	CC32 (154 including 128 of ST-32); CC213 (1), non assigned CC (1)
	141		1	CC32
	frameshift (single deletion), allele 123		4	CC32
	NadA interrupted by insertion sequence, allele 259		1	CC32
Variant 2/3 (12, 5.3%)	3		10	CC213
	8		1	CC18
	frameshift, (FS:ΔA286 and deletion) allele 20		1	CC1157
Variant 4/5 (49, 21.8%)	21		26	non assigned CC (22 including 17 of ST-9316), CC213 (2), CC549 (2)
	79		1	CC213
	158		1	non assigned CC
	Frameshift (phase variable off)	allele 12	4	CC213
		allele 34	11	CC213 (10), CC18 (1)
		allele 38	1	CC213
		allele 39	1	CC213
		allele 135	1	CC213
		allele 210	1	CC41/44
		allele 261	1	CC213
	Frameshift (internal codon stop)	allele 258	1	CC865
NadA interrupted by insertion sequence* (0.9%)			2	CC32

* Two isolates for which size of PCR product confirmed presence of insertion sequence, but aligning consensus of sequenced fragments of gene failed.

However, taking into consideration two calculations (exMATS and gMATS), the results of coverage were 83.3% and 86.6%, respectively, without any significant difference (p = 0.1).

With regard to annual fluctuations, coverage was 79.0–89.7% for exMATS calculation (χ^2 for trend, p = 0.3) and 83.3–89.7% for gMATS calculation (χ^2 for trend, p = 0.9). Comparing both calculations in individual years, the biggest difference was in 2010 (79.0% vs. 88.9% for exMATS and gMATS, respectively) and in 2013 (80.4% vs. 87.9%) (Fig. 6).

With regard to individual antigens, coverage of fHbp was 68.8% and 70.9% (p = 0.2) in exMATS and gMATS calculations, respectively. The difference resulted mostly from the fact that 55 isolates that were considered as non-covered by exMATS/fHbp were determined as unpredictable (n = 43) or covered (n = 12) by gMATS/fHbp. The coverage of NHBA was 31.8% in exMATS and 66.1% in gMATS (p < 0.0001). This difference was due to the significant number of isolates that were interpreted in exMATS and gMATS as (i) non-covered and covered, respectively (n = 199), or (ii) non-covered and unpredictable, respectively (n = 86). Differences between exMATS and gMATS interpretations of fHbp and NHBA peptides are listed in Table 3.

The coverage of isolates of major clonal complexes is high and similar for both calculation methods, except for CC213 as shown on Fig. 7. Low coverage among this clonal complex was associated with dominated peptides; 75% of CC213 isolates possessed not-covered fHbp variant 3.45 and 45% of isolates not-covered NHBA variant 18. Additionally, 66% of CC213 meningococci lacked NadA peptide or had encoding gene with frameshift. Finally, coverage

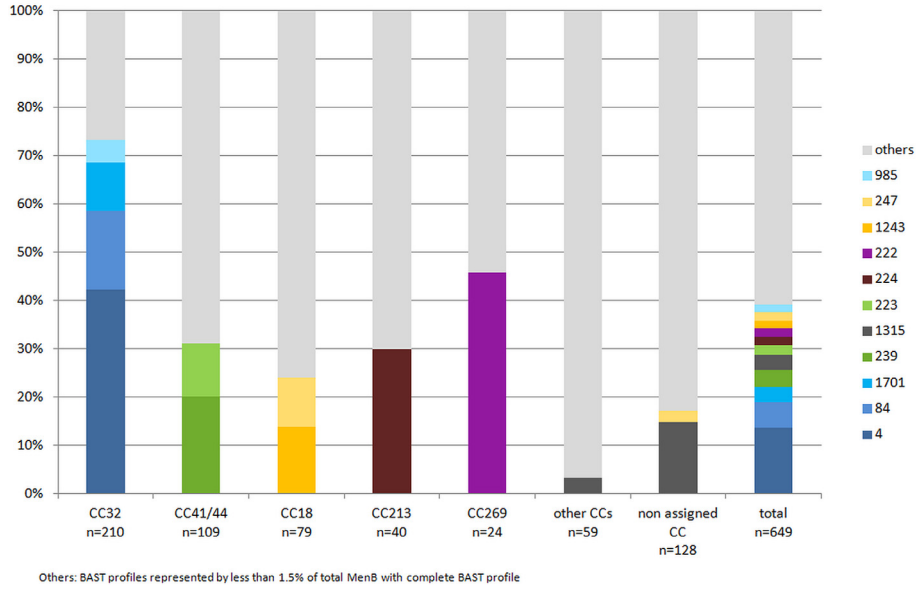

Fig. 5. Distribution of major BAST profiles among Polish serogroup B meningococci, 2010–2016 (n = 649).

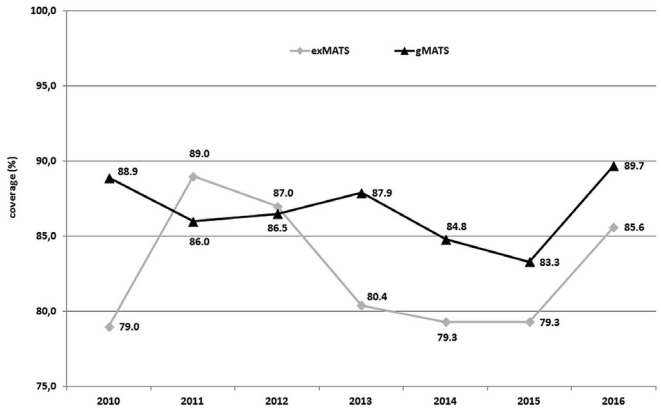

Fig. 6. Annual fluctuation of coverage among Polish MenB, 2010–2016, according to two different methods of calculation.

was linked in exMATS mainly to NadA peptide 3 assumed as covered, while in gMATS to half of unpredictable isolates regarded as covered.

4. Discussion

During 2010–2016, as well as in earlier years, MenB was the main cause of IMD in Poland, where population-based vaccination against meningococci had never been introduced [21].

MLST analysis revealed that the structure of the MenB population was heterogeneous, but major clonal complexes are well established in our country [21]. The distribution of clonal complexes among MenB in Poland is mostly similar to the Czech Republic, where the leading clonal complexes are CC32, CC41/44 and CC18 [28]. In other European countries, comparable to Poland, the most common are CC41/44, CC32, CC269, CC213 and CC162, although noticeable differences in their proportions have been shown. The main difference concerns CC18, which is common in Poland but very rare worldwide [29–34].

Polish isolates represented mainly fHbp variant 1 (79%), which predominated in each year of the tested period. Previous worldwide studies showed that the distribution of fHbp peptides/subvariants varies geographically. Murphy *et al.* studied 1837 MenB isolates from the USA, European countries, New Zealand and South Africa and found that the most frequent was variant 1 (called subfamily B, 70%), except in South Africa where variants 2 and 3 made up the majority [35]. Also, in a study of 1052 isolates from different parts of Europe by Vogel *et al.* variant 1 was the most prevalent and accounted for more than 60% [34]. The same proportion was presented in a recent study of MenB in the USA [31], and in Brazil the proportion of variant 1 was even higher, accounting for 80% [36]. Conversely, in a long-term analysis of Chinese isolates it was shown that invasive MenB possessed mainly those subvariants representing variant 2 (61.9%), with variant 1 observed less often (32.1%) [37]. Considering the distribution of subvariants among Polish MenB isolates, subvariant 1.1 (included in 4CMenB) was mostly observed, accounting for almost 31% of Men/variant 1 and a quarter of all tested MenB. This is quite similar to American (33%) [31], Spanish (19.3%) [29], French (21.5%) and German (18.9%) data [34] and much higher than in Greek (10.8%) [38], Norwegian (12.2%), English (6.4%) and Italian (9.3%) isolates [34]. Other subvariants that dominated in these countries (e.g. 1.4 in England and Norway; 1.14 in Italy and Germany [16,34]; 1.15 in Greece [38]) occurred with lower frequency in Poland.

With regard to NHBA, Polish meningococci showed very high diversity. Peptide 2, the 4CMenB component, was at 9.8% prevalence in our study, similar to the American (9.5%) [31] and Greek isolates (10.1%) [38], but this was at least half that in other European countries, where the proportion ranged between 22.0% in France and 31.7% in Norway [34]. Peptide 2 was also mostly found in MenB from Atlantic Canada (63.6%) [33], but not in Quebec and Western Canada (2.6% and 13.0%, respectively) [39,40]. Peptide 3, which was the most prevalent in Poland (22.4%), was much less prevalent (10.6%) in five European countries, but with the proportion ranging from 20.5% in France to 3.7% in England and Wales [34].

In this study a few correlations between clonal complexes and peptides of tested antigens were observed, although some of them were completely different in other countries. For example, vaccine subvariant 1.1 was mostly found in CC32, similar to worldwide results. Interestingly, subvariant 2.19 was mainly associated with CC41/44 but in Spain it dominated in CC269 [29], whereas in Polish MenB CC269, as well as that in England or some parts of Canada, subvariant 1.15 was the most prevalent [16,39,40]. With regard

Table 3
Differences of NHBA and fHbp peptides coverage between exMATS and gMATS calculation.

Antigen	Calculation method		Number of isolates	Peptide(s) (number of isolates)
	exMATS	gMATS		
fHbp	non-cov	UNP	43	1.321(22); 1.260(14); 1.593(4); 1.539(3)
	non-cov	cov	12	1.13(7); 1.37(5)
	cov	UNP	26	1.321(16); 1.234(3); 1.66, 1.590, 1.591, 1.1.592, 1.594, 1.664, 1.665–1 per each
	UNK	cov	3	1.2(2); 1.249(1)
NHBA	non-cov	cov	197	3(1 1 2); 20(56); 21(26), 2(3)
	non-cov	UNP	86	63(16); 115(16); 188(9); 53(9); 49(7); 29(6); 502(5); 118(4); 42 and 249–2 per each; 54, 81, 89, 576, 579, 581, 584, 585, 586, 588–1 per each
	UNK	non-cov	14	120(4); 31 and 187–3 per each; 43(2); 30, and 112–1 per each
	UNK	cov	4	1(2); 5(2)
	cov	UNP	19	12 and 384–4 per each, 577 and 587–2 per each; 54, 81, 408, 578, 580, 582, 583–1 per each
	cov	non-cov	3	6(3)

cov = covered.
non-cov = not covered.
UNK = unknown (MenB without Polish MATS data available, isolated after 2011; in exMATS calculation half of them assumed as covered.
UNP = unpredictable; according to gMATS half of them regarded as covered.

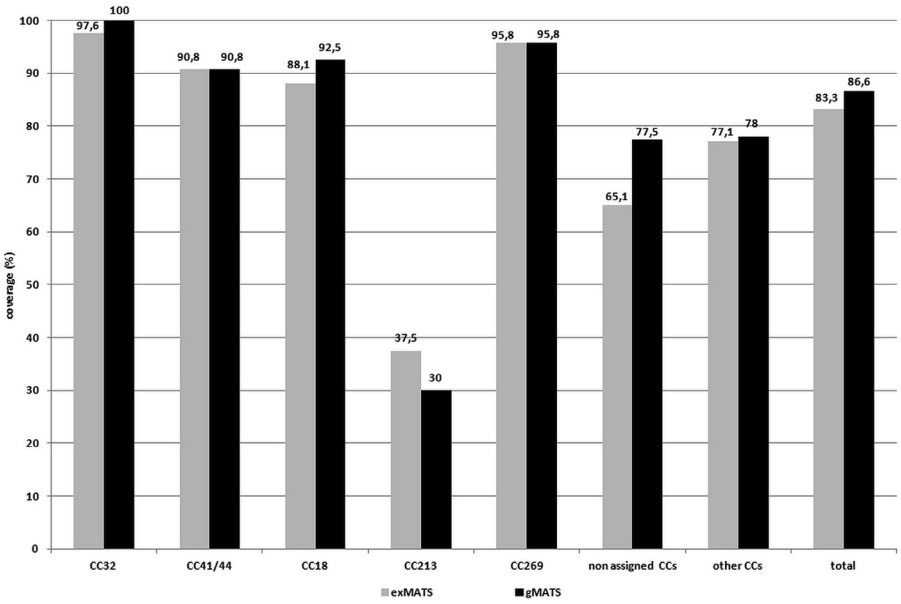

Fig. 7. Coverage among clonal complexes of Polish MenB, 2010–2016, according to two different methods of calculation.

to NHBA, peptides 3 and 20 were mostly detected in CC32, whereas in a Greek study peptide 20 dominated in CC162 [38]. Vaccine peptide 2 was associated with CC41/44, which was observed also in other countries [34]. It has been commonly described that *nad*A is usually noticed among specific clonal complexes such as CC32, CC8 and CC11 but not in CC41/44, CC162 or CC269 [16,17,29,38,41,42]. Similarly, Polish isolates that carried *nad*A represented mainly CC32 but also CC18, CC213 and non-assigned CC. Prevalent peptide 1 was found almost exclusively among CC32, peptide 21 among ST-9316 (non-assigned CC) and peptide 3 only in CC213. As well as peptide 3, alleles with a frameshift, poli C track resulting in phase variable off mutation, were also commonly found in CC213. Similar observations of peptide 1 corresponding to CC32 and frameshift peptides corresponding to CC213 were found in Spanish MenB isolates [29].

A very high number of BAST profiles were detected among Polish MenB isolates, as well as correlations between BAST profiles and clonal complexes. The variety was due to a huge range of fHbp and NHBA peptides. Only two profiles (BAST-4 and BAST-84) were each present in more than 5% of all MenB, and they were exclusively found in CC32. A similar observation of high diversity of BAST profiles was described for English isolates [15]. However, in our study the repertoire of major BAST profiles was different from that in the UK study, even within the same clonal complex. For example BAST-223 and BAST-239 were exclusively found in a third of Polish CC41/44 whereas for English CC41/44 BAST-220 was characteristic. Almost half of Polish CC269 represented BAST-222 whereas for English CC269 BAST-222 was the second profile after BAST-219 [15,17].

Overall coverage assessed by MATS extrapolation (exMATS) and gMATS between 2010 and 2016 is very similar, at 83.3% and 86.6%, respectively. However, temporal analysis showed some differences, especially in 2010 and 2013. This was mainly due to some NHBA peptides being estimated as non-covered by exMATS but as unpredictable or covered by gMATS. Generally, almost half of the isolates (n = 323) were considered differently by these two methods of calculation with regard to NHBA, whose coverage in gMATS double that in exMATS.

It is interesting that, despite significant differences in NHBA coverage, the overall coverage predicted by exMATS and gMATS

is very similar. This is because the isolate is considered as covered if it has at least one antigen estimated as covered. Therefore, although there were many isolates determined as non-covered by NHBA in exMATS, the majority of them were covered by other antigens.

These results confirm that genotyping may be very helpful in predicting 4CMenB strain coverage but it does have some limitations. Geographical distribution of individual peptides and their coverage performed by MATS may differ significantly and thus influence the final calculation in individual countries. Simultaneous temporal changes in distribution and constantly appearing new peptides for which MATS analysis is not yet available are additional limitations that may lead to significant false estimation of coverage. Thus, although surveillance of the meningococcal population and genes encoding 4CMenB components is indisputable, studies using MATS assay are needed, especially for new peptides.

In conclusion, our study provides a molecular characterization of the MenB responsible for IMD in Poland during the seven-year period of 2010–2016. The majority of MenB isolates represented five clonal complexes, the most prevalent being CC32 and CC41/44, which are also common worldwide, and also CC18, which is rarely observed in other European countries except the neighboring Czech Republic. Some correlations between clonal complexes and individual peptides of fHbp, NHBA and NadA were observed but a very diverse repertoire was noticed. Predicted strain coverage by 4CMenB in Poland ranged from 83.3% to 86.6% calculated by exMATS and gMATS, respectively, and was similar to other European countries. Estimation of strain coverage by 4CMenB based on sequencing of genes encoding vaccine antigens may be very useful, particularly in countries that are not able to perform MATS analysis. Additionally, sequencing captures the temporal changes and geographical differences in the distribution of peptides. However, the limitation appears to be the representativeness of existing MATS datasets, and therefore gMATS, when applied to different countries.

5. Financial support

The study was partially supported by The National Science Centre, Poland grant to IWa (Poland grant 2012/07/N/NZ7/04315), Ministry of Health within the framework of the National Program of Antibiotic Protection (NPOA), by the Ministry of Science and Higher Education within the framework of Mikrobank 2 Program and DS 5.24.

CRediT authorship contribution statement

Izabela Waśko: Conceptualization, Methodology, Funding acquisition, Investigation, Writing - original draft, Writing - review & editing, Formal analysis. **Agnieszka Gołębiewska:** Investigation, Writing - review & editing. **Marlena Kiedrowska:** Investigation, Writing - review & editing. **Patrycja Ronkiewicz:** Investigation, Writing - review & editing. **Izabela Wróbel:** Investigation, Writing - review & editing. **Alicja Kuch:** Investigation, Writing - review & editing. **Eva Hong:** Investigation, Writing - review & editing. **Anna Skoczyńska:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A. Skoczyńska; Assistance to attend scientific meetings and honoraria for lecturing funded from GlaxoSmithKline and Pfizer. Participation in Advisory Board of GlaxoSmithKline, Pfizer and Sanofi Pasteur.

Acknowledgements

We thank all BINet participants, physicians and microbiologists who provided isolates and epidemiological data to the NRCBM that were included and used in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.01.021.

References

[1] Whittaker R, Dias JG, Ramliden M, Kodmon C, Economopoulou A, Beer N, et al. The epidemiology of invasive meningococcal disease in EU/EEA countries, 2004–2014. Vaccine 2017;35:2034–41. https://doi.org/10.1016/j.vaccine.2017.03.007.

[2] Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine 2009;27(Suppl 2):B51–63. https://doi.org/10.1016/j.vaccine.2009.04.063.

[3] Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium Neisseria meningitidis. Vaccine 2009;27(Suppl 2):B71–7. https://doi.org/10.1016/j.vaccine.2009.04.070.

[4] Waśko I, Hryniewicz W, Skoczyńska A. Significance of Meningococcal Hyperinvasive Clonal complexes and their influence on vaccines development. Pol J Microbiol 2015;64:313–21.

[5] Caesar NM, Myers KA, Fan X. Neisseria meningitidis serogroup B vaccine development. Microb Pathog 2013;57:33–40. https://doi.org/10.1016/j.micpath.2013.02.003.

[6] Serruto D, Bottomley MJ, Ram S, Giuliani MM, Rappuoli R. The new multicomponent vaccine against meningococcal serogroup B, 4CMenB; immunological, functional and structural characterization of the antigens. Vaccine 2012;30(Suppl 2):B87–97. https://doi.org/10.1016/j.vaccine.2012.01.033.

[7] Panatto D, Amicizia D, Lai PL, Gasparini R. Neisseria meningitidis B vaccines. Expert Rev Vaccines 2011;10:1337–51. https://doi.org/10.1586/erv.11.103.

[8] Racloz VN, Luiz SJ. The elusive meningococcal meningitis serogroup: a systematic review of serogroup B epidemiology. BMC Infect Dis 2010;10:175. https://doi.org/10.1186/1471-2334-10-175.

[9] Ladhani SN, Campbell H, Parikh SR, Saliba V, Borrow R, Ramsay M. The introduction of the meningococcal B (MenB) vaccine (Bexsero(R)) into the national infant immunisation programme–new challenges for public health. J Infect 2015;71:611–4. https://doi.org/10.1016/j.jinf.2015.09.035.

[10] European ECDC. Centre for Disease Prevention and Control. Expert opinion on the introduction of the meningococcal B (4CMenB) vaccine in the EU/EEA. Stockholm 2017.

[11] O'Connor L, Ward M, Bennett D, Mulhall R, O'Lorcain P, Cunney R, et al. 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 2015:20.

[12] Watson PS, Turner DP. Clinical experience with the meningococcal B vaccine, Bexsero((R)): prospects for reducing the burden of meningococcal serogroup B disease. Vaccine 2016;34:875–80. https://doi.org/10.1016/j.vaccine.2015.11.057.

[13] Thabuis A, Tararbit K, Taha MK, Dejour-Salamanca D, Ronin V, Parent du Chatelet I, et al. Community outbreak of serogroup B invasive meningococcal disease in Beaujolais, France, February to June 2016; from alert to targeted vaccination. Euro Surveill 2018:23. https://doi.org/10.2807/1560-7917.ES.2018.23.28.1700590.

[14] Medini D, Stella M, Wassil J. MATS: Global coverage estimates for 4CMenB, a novel multicomponent meningococcal B vaccine. Vaccine 2015;33:2629–36. https://doi.org/10.1016/j.vaccine.2015.04.015.

[15] Brehony C, Rodrigues CMC, Borrow R, Smith A, Cunney R, Moxon ER, et al. Distribution of Bexsero(R) Antigen Sequence Types (BASTs) in invasive meningococcal disease isolates: implications for immunisation. Vaccine 2016;34:4690–7. https://doi.org/10.1016/j.vaccine.2016.08.015.

[16] Rodrigues CMC, Lucidarme J, Borrow R, Smith A, Cameron JC, Moxon ER, et al. Genomic surveillance of 4CMenB vaccine antigenic variants among disease-causing Neisseria meningitidis isolates, United Kingdom, 2010–2016. Emerg Infect Dis 2018;24:673–82. https://doi.org/10.3201/eid2404.171480.

[17] Mowlaboccus S, Perkins TT, Smith H, Sloots T, Tozer S, Prempeh LJ, et al. Temporal changes in BEXSERO(R) antigen sequence type associated with genetic lineages of Neisseria meningitidis over a 15-year period in Western Australia. PLoS ONE 2016;11:. https://doi.org/10.1371/journal.pone.0158315e0158315.

[18] Muzzi A, Brozzi A, Serino L, Bodini M, Abad R, Caugant D, et al. Genetic Meningococcal Antigen Typing System (gMATS): a genotyping tool that predicts 4CMenB strain coverage worldwide. Vaccine 2019;37:991–1000. https://doi.org/10.1016/j.vaccine.2018.12.061.
[19] ECDC. Annual Epidemiological Reports on meningococcal disease. https://ecdc.europa.eu/en/meningococcal-disease/surveillance-and-disease-data/aer. Accessed August 2019.
[20] Presa J, Findlow J, Vojicic J, Williams S, Serra L. Epidemiologic trends, global shifts in Meningococcal vaccination guidelines, and data supporting the use of MenACWY-TT vaccine: a review. Infect Dis Ther 2019;8:307–33. https://doi.org/10.1007/s40121-019-0254-1.
[21] Skoczynska A, Wasko I, Kuch A, Kadlubowski M, Golebiewska A, Forys M, et al. A decade of invasive meningococcal disease surveillance in Poland. PLoS ONE 2013;8:. https://doi.org/10.1371/journal.pone.0071943e71943.
[22] NRCBM. Invasive Meningococcal disease in Poland in 2018. koroun.edu.pl/wp-content/uploads/2019/05/ICHM2018.pdf. Accessed August 2019.
[23] Lucidarme J, Comanducci M, Findlow J, Gray SJ, Kaczmarski EB, Guiver M, et al. Characterization of fHbp, nhba (gna2132), nadA, porA, and sequence type in group B meningococcal case isolates collected in England and Wales during January 2008 and potential coverage of an investigational group B meningococcal vaccine. Clin Vaccine Immunol 2010;17:919–29. https://doi.org/10.1128/CVI.00027-10.
[24] Lucidarme J, Tan L, Exley RM, Findlow J, Borrow R, Tang CM. Characterization of Neisseria meningitidis isolates that do not express the virulence factor and vaccine antigen factor H binding protein. Clin Vaccine Immunol 2011;18:1002–14. https://doi.org/10.1128/CVI.00055-11.
[25] Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988;26:2465–6.
[26] Waśko I, Hong E, De Paola R, Stella M, Moschioni M, Taha MK, et al. High predicted strain coverage by the multicomponent meningococcal serogroup B vaccine (4CMenB) in Poland. Vaccine 2016;34:510–5. https://doi.org/10.1016/j.vaccine.2015.11.070.
[27] NIPH-NIH. Reports on cases of infectious diseases and poisonings in Poland. http://wwwold.pzh.gov.pl/oldpage/epimeld/index_a.html. Accessed August 2019.
[28] Jandova Z, Musilek M, Vackova Z, Kozakova J, Krizova P. Serogroup and clonal characterization of Czech Invasive Neisseria meningitidis strains Isolated from 1971 to 2015. PLoS ONE 2016;11:. https://doi.org/10.1371/journal.pone.0167762e0167762.
[29] Abad R, Medina V, Stella M, Boccadifuoco G, Comanducci M, Bambini S, et al. Predicted strain coverage of a new meningococcal multicomponent vaccine (4CMenB) in Spain: analysis of the differences with other European countries. PLoS ONE 2016;11:. https://doi.org/10.1371/journal.pone.0150721e0150721.
[30] Parikh SR, Newbold L, Slater S, Stella M, Moschioni M, Lucidarme J, et al. Meningococcal serogroup B strain coverage of the multicomponent 4CMenB vaccine with corresponding regional distribution and clinical characteristics in England, Wales, and Northern Ireland, 2007–08 and 2014–15: a qualitative and quantitative assessment. Lancet Infect Dis 2017;17:754–62. https://doi.org/10.1016/S1473-3099(17)30170-6.
[31] Rajam G, Stella M, Kim E, Paulos S, Boccadifuoco G, Serino L, et al. Meningococcal antigen typing system (MATS)-based Neisseria meningitidis serogroup B coverage prediction for the MenB-4C vaccine in the United States. mSphere 2017;2. https://doi.org/10.1128/mSphere.00261-17.
[32] Simoes MJ, Bettencourt C, De Paola R, Giuliani M, Pizza M, Moschioni M, et al. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Portugal. PLoS ONE 2017;12:. https://doi.org/10.1371/journal.pone.0176177e0176177.
[33] Tsang RS, Law DK, Gad RR, Mailman T, German G, Needle R. Characterization of invasive Neisseria meningitidis from Atlantic Canada, 2009 to 2013: With special reference to the nonpolysaccharide vaccine targets (PorA, factor H binding protein, Neisseria heparin-binding antigen and Neisseria adhesin A). Can J Infect Dis Med Microbiol 2015;26:299–304.
[34] Vogel U, Taha M-K, Vazquez JA, Findlow J, Claus H, Stefanelli P, et al. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect Dis 2013;13:416–25. https://doi.org/10.1016/s1473-3099(13)70006-9.
[35] Murphy E, Andrew L, Lee KL, Dilts DA, Nunez L, Fink PS, et al. Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B Neisseria meningitidis. J Infect Dis 2009;200:379–89. https://doi.org/10.1086/600141.
[36] de Filippis I, de Lemos AP, Hostetler JB, Wollenberg K, Sacchi CT, Dunning Hotopp JC, et al. Molecular epidemiology of Neisseria meningitidis serogroup B in Brazil. PLoS ONE 2012;7:. https://doi.org/10.1371/journal.pone.0033016e33016.
[37] Shi F, Zhang A, Zhu B, Gao Y, Xu L, Li Y, et al. Prevalence of factor H Binding Protein sub-variants among Neisseria meningitidis in China. Vaccine 2017;35:2343–50. https://doi.org/10.1016/j.vaccine.2017.03.057.
[38] Tzanakaki G, Hong E, Kesanopoulos K, Xirogianni A, Bambini S, Orlandi L, et al. Diversity of Greek meningococcal serogroup B isolates and estimated coverage of the 4CMenB meningococcal vaccine. BMC Microbiol 2014;14:111. https://doi.org/10.1186/1471-2180-14-111.
[39] Law DK, Lefebvre B, Gilca R, Deng S, Zhou J, De Wals P, et al. Characterization of invasive Neisseria meningitidis strains from Quebec, Canada, during a period of increased serogroup B disease, 2009–2013: phenotyping and genotyping with special emphasis on the non-carbohydrate protein vaccine targets. BMC Microbiol 2015;15:143. https://doi.org/10.1186/s12866-015-0469-6.
[40] Law DK, Zhou J, Deng S, Hoang L, Tyrrell G, Horsman G, et al. Determination of serotyping antigens, clonal analysis and genetic characterization of the 4CMenB vaccine antigen genes in invasive Neisseria meningitidis from Western Canada, 2009 to 2013. J Med Microbiol 2014;63:1490–9. https://doi.org/10.1099/jmm.0.079921-0.
[41] Comanducci M, Bambini S, Brunelli B, Adu-Bobie J, Arico B, Capecchi B, et al. NadA, a novel vaccine candidate of Neisseria meningitidis. J Exp Med 2002;195:1445–54.
[42] Bambini S, Muzzi A, Olcen P, Rappuoli R, Pizza M, Comanducci M. Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. Vaccine 2009;27:2794–803. https://doi.org/10.1016/j.vaccine.2009.02.098.