Journal of Infection 84 (2022) 136–144

Contents lists available at ScienceDirect

Journal of Infection

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

Invasive serogroup B meningococci in England following three years of 4CMenB vaccination – First real-world data

Jay Lucidarme[a,*], Xilian Bai[a], Aiswarya Lekshmi[a], Stephen A. Clark[a], Laura Willerton[a], Sonia Ribeiro[b], Helen Campbell[b], Laura Serino[c], Rosita De Paola[c], Ann Holland[d], Jennifer Louth[d], Mary E. Ramsay[b], Shamez N. Ladhani[b,e], Ray Borrow[a,d]

[a] Meningococcal Reference Unit, Public Health England, Floor 2 Clinical Sciences Building 2, Manchester Royal Infirmary, Manchester, M13 9WL, United Kingdom
[b] Immunisation and Countermeasures Division, Public Health England, London, NW9 5EQ, United Kingdom
[c] GlaxoSmithKline Vaccines, via Fiorentina 1, 53100, Siena, Italy
[d] Vaccine Evaluation Unit, Public Health England, Floor 2 Clinical Sciences Building 2, Manchester Royal Infirmary, Manchester, M13 9WL, United Kingdom
[e] Paediatric Infectious Diseases Research Group, St. George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom

ARTICLE INFO

Article history:
Accepted 12 November 2021
Available online 25 November 2021

Key Words:
Meningococcal disease
Serogroup B
4CMenB
Meningococcal antigen typing system
MATS
Vaccine coverage
England

SUMMARY

Objectives: In 2015 the UK became the first country to implement the meningococcal B (MenB) vaccine, 4CMenB, into the national infant program. 4CMenB is expected to cover meningococci expressing sufficient levels of cross-reactive proteins. This study presents clonal complex, 4CMenB antigen genotyping, and 4CMenB coverage data for all English invasive MenB isolates from 2014/15 (1 year pre-vaccine) through 2017/18 and compares data from vaccinated and unvaccinated ≤3 year olds.

Methods: Vaccine coverage of all invasive MenB isolates from 2014/15 to 2017/18 ($n = 784$) was analysed using the Meningococcal Antigen Typing System. Genotyping utilised the Meningococcus Genome Library.

Results: Among ≤3 year olds, proportionally fewer cases in vaccinees (1, 2 or 3 doses) were associated with well-covered strains e.g. cc41/44 (20.5% versus 36.4%; $P<0.01$) and antigens e.g. PorA P1.4 (7.2% versus 17.3%; $P = 0.02$) or fHbp variant 1 peptides (44.6% vs 69.1%; $P<0.01$). Conversely, proportionally more cases in vaccinees were associated with poorly-covered strains e.g. cc213 (22.9% versus 9.6%; $P<0.01$) and antigens e.g. variant 2 or 3 fHbp peptides (54.2% versus 30.9%; $P<0.01$).

Conclusions: 4CMenB reduces disease due to strains with cross-reactive antigen variants. No increase in absolute numbers of cases due to poorly covered strains was observed in the study period.

Introduction

Neisseria meningitidis (the meningococcus) is a major global cause of meningitis and/or septicaemia. Six serogroups (A, B, C, W, X and Y) cause the majority of invasive meningococcal disease (IMD). In western countries serogroup B (MenB) disease predominates[1] peaking among infants and young children with a smaller peak in young adults.[1] Polysaccharide-conjugate vaccines exist against serogroups A, C, W and Y but not MenB, owing to poor immunogenicity of the capsular polysaccharide.[1,2] Outer membrane vesicle (OMV) based MenB vaccines have mostly been used against strain-specific outbreaks owing to the high diversity and poor immunological cross-reactivity of the immunodominant porin A (PorA) antigen.[3] Two MenB vaccines containing recombinant proteins (4CMenB [Bexsero] and MenB-fHBP [Trumenba]) are licensed in multiple countries/regions.[4,5]

In September 2015, the UK became the first country to implement 4CMenB into the national infant immunization program at a reduced 8 week, 16 week and 1 year schedule.[6] After 3 years, MenB cases declined by 75% in fully-eligible cohorts, with an estimated vaccine effectiveness (2 doses plus booster) of 71.2% against strains predicted to be covered.[7]

4CMenB includes three recombinant antigens, factor H-binding protein (fHbp; variant 1, peptide 1), neisserial heparin-binding antigen (NHBA; peptide 2) and *Neisseria* adhesin A (NadA; peptide 8), and OMVs from a New Zealand outbreak strain possessing PorA subtype P1.4.[4] fHbp, NHBA and PorA are widely distributed among meningococci while only certain strains possess a *nadA* gene. Unlike PorA, the three recombinant antigens each exhibit im-

* Corresponding author at: Meningococcal Reference Unit, Public Health England, Manchester Medical Microbiology Partnership, Floor 2 Clinical Sciences Building II, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, United Kingdom.
E-mail address: jay.lucidarme@phe.gov.uk (J. Lucidarme).

https://doi.org/10.1016/j.jinf.2021.11.015

munological cross-reactivity between closely-related variants. Thus, the fHbp component may afford protection against meningococci expressing variant group 1, but not variant group 2 or 3 peptide variants.[4] The NadA component may afford protection against meningococci possessing variant group NadA-2/3 or NadA-1 peptides, but not NadA-4/5 or NadA-6 peptides, or isolates lacking the gene. NHBA is also diverse but cross-reactivity is not restricted to discreet phylogenetic groups.[4]

A meningococcus must sufficiently express a cross-reactive peptide on its surface to trigger antibody-mediated killing. The serum bactericidal antibody (SBA) assay is the gold-standard for establishing antibody titres against a standard isolate but is impractical for routine use against large panels of IMD isolates.[8] The Meningococcal Antigen Typing System (MATS) was, therefore, developed to determine vaccine coverage among such panels.[9] An isolate is considered covered by the vaccine (MATS-positive) if it possesses PorA P1.4 and/or at least one of the other antigens has a relative potency (RP; as determined by enzyme-linked immunosorbent assay), versus a reference strain, that exceeds the so-called positive bactericidal threshold (PBT). MATS can only be performed on viable cultures which are obtained in approximately 50% of cases in the UK. These are, nonetheless, considered broadly representative of prevailing IMD.[10,11] Genotypic analysis of 4CMenB antigens supplements MATS by characterizing antigenic diversity.[12]

Here we present strain (clonal complex and 4CMenB antigen genotyping) and MATS data for all English invasive MenB isolates from one year pre- through three years post-4CMenB introduction in England, and compare the data from vaccinated and unvaccinated ≤3 year olds.

Materials and methods

Surveillance

The Public Health England Meningococcal Reference Unit (MRU) and immunization and Countermeasures Division conduct IMD enhanced surveillance in England. The MRU receives cultures for >90% of culture-confirmed cases from microbiology laboratories in England for species confirmation and characterization.[13] Laboratory-confirmed cases are routinely followed up for vaccination status, disease presentation, and outcome.[14] In the present study an individual was counted as having had a specific dose of vaccine if disease occurred ≥14 days thereafter.

Isolates

The study included one isolate each for all English MenB IMD cases (n = 784) that were culture confirmed by the MRU during the 2014/15 (n = 220), 2015/16 (n = 195), 2016/17 (n = 189) and 2017/18 (n = 180) academic years (1st September to 31st August) (table S1).

Genotypic analyses

Genotypic data (vaccine antigen genotypes and multilocus sequence typing) were obtained from the Meningitis Research Foundation Meningococcus Genome Library (MGL; https://pubmlst.org/bigsdb?db=pubmlst_neisseria_mrfgenomes&page=projects) which contains fully annotated draft genome sequences for all English IMD isolates received by the MRU dating back to July 2010, inclusive.[15]

MATS analysis

Genotypic PorA data were obtained from the MGL as described above. Phenotypic PorA data were routinely obtained using a dot-blot ELISA as previously described.[16] The MATS ELISA was performed as previously described[9] using the following PBTs: fhbp 0.012, NHBA 0.294 and NadA 0.009.[17]

Human serum bactericidal antibody assays

Serum bactericidal antibody (SBA) assays utilizing an exogenous source of human complement were performed as previously described against target strains NZ98/254 (covered for PorA), 44/76-SL (covered for fHbp) and 5/99 (covered for NadA). A titre of 4 or above was considered protective.[8]

Statistics

Comparisons were made using Chi squared or Fisher's exact tests with Bonferroni correction.

Results

MenB epidemiology

The age group distribution of culture-confirmed MenB IMD cases (n = 784) was: ≤3 years (n = 355, 45.3%), 4 to 9 years (n = 88, 11.2%), 10 to 14 years (n = 17, 2.2%), 15 to 19 years (n = 74, 9.4%), 20 to 24 years (n = 40, 5.1%), 25 to 44 years (n = 36, 4.6%), 45 to 64 years (n = 66, 8.4%) and ≥65 years (n = 108, 13.8%).

The clonal complex distribution varied with age group (Fig. 1a). Overall, the most common clonal complex was cc41/44 (n = 272/784, 34.7%) followed by cc269 (n = 167/784, 21.3%), cc213 (n = 92/784, 11.7%), cc32 (n = 76/784, 9.7%), cc461 (n = 25/784, 3.2%), cc162 (n = 23/784, 2.9%), cc35 (n = 16/784, 2.0%), cc60 (n = 10/784, 1.3%) and ten other ccs each representing <10 isolates. The greatest diversity in terms of the number of clonal complexes was observed among younger children and older adults.

Overall MenB mats coverage

MATS coverage was relatively low among ≤3 year olds (67.0%), increasing to 94.1% among 10 to 14 year olds and then decreasing to 66.7% among ≥65 year olds (Fig. 1b). Overall, 72.7% (n = 570/784) of isolates were predicted to be covered by 4CMenB; 34.1% (n = 267/784) by a single antigen, 28.4% (n = 223/784) by two antigens, and 10.2% (n = 80/784) by three antigens. The predominant combinations of MATS-positive antigens varied with age group (Fig. 1c). The most common combinations overall were fHbp alone (24.0%, n = 188/784), fHbp;NHBA (20.7%, n = 162/784), fHbp;NHBA;PorA (9.9%, n = 78/784), NHBA alone (8.8%, n = 69/784) and fHbp;PorA (4.5%, n = 35/784).

MATS coverage by clonal complex and peptide variant

Among the predominant clonal complexes, the majority of isolates belonging to cc41/44 (93.0%, n = 253/272), cc32 (93.4%, n = 71/76) and cc162 (91.3%, n = 21/23) were MATS covered. This figure was lower for cc269 (64.1%, n = 107/167). A minority of isolates belonging to cc213 (29.3%, n = 27/92) and cc461 (28.0%, n = 7/25) were covered (Fig. 2a).

None of the n = 232/784 (29.6%) isolates possessing fHbp variant 2 or 3 alleles were MATS-positive for fHbp. Among the n = 550/784 (70.2%) isolates with fHbp variant 1 alleles, 86.3% (n = 475/550) were MATS-positive for fHbp including most or all isolates with alleles for peptides 1.4 (99.5%, n = 195/196), 1.1 (100.0%, n = 57/57), 1.14 (95.5%, n = 42/44), 1.510 (100%, n = 8/8), 1.110 (100%, n = 5/5), 1.483 (100%, n = 5/5) and 1.69 (100%,

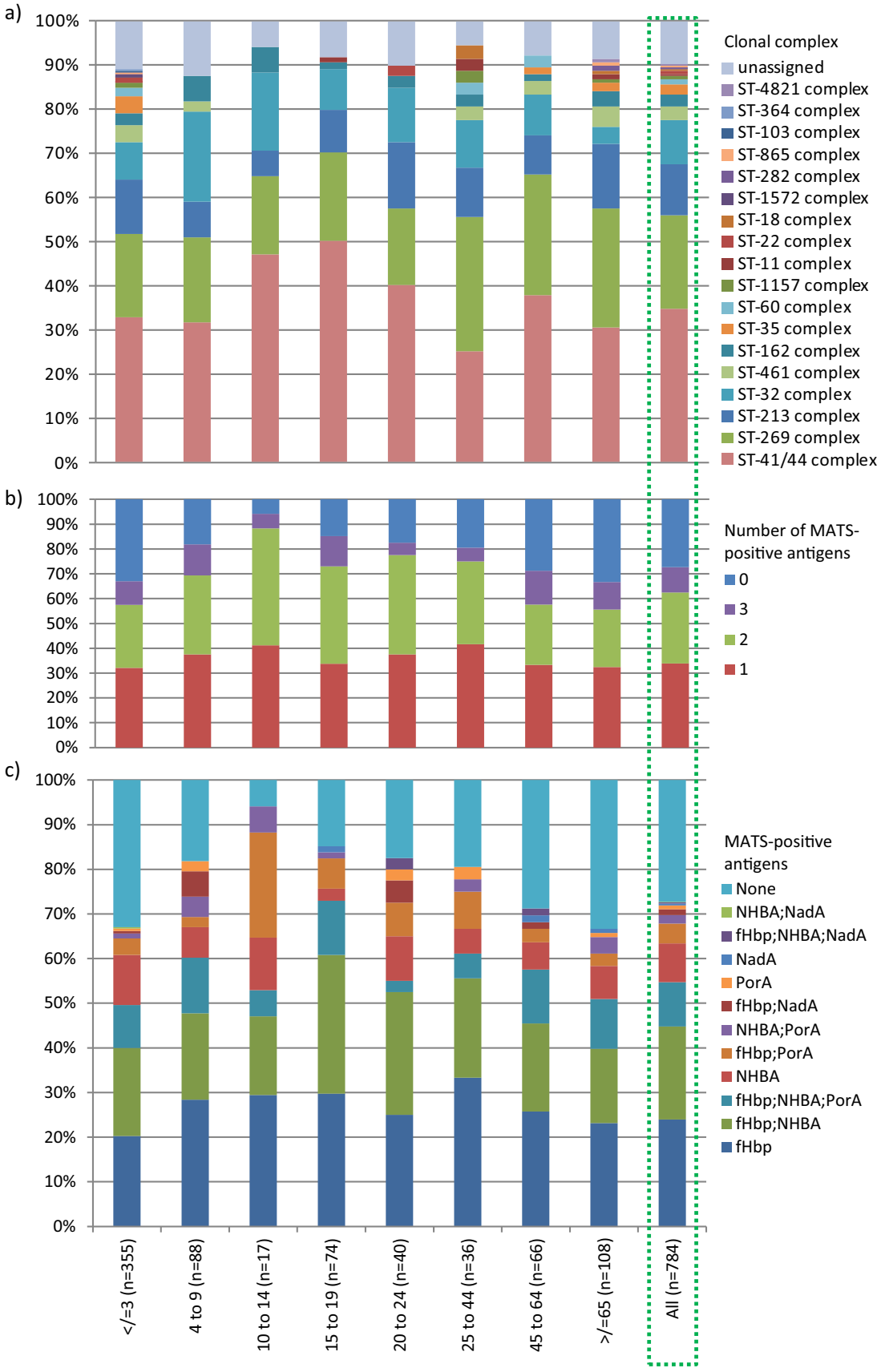

Fig. 1. Distribution of clonal complex (a), MATS positivity by number of antigens (b), and MATS positivity by antigen combination (c) versus age group for invasive MenB isolates from culture confirmed cases in England, 2014/15 to 2017/18, inclusive.

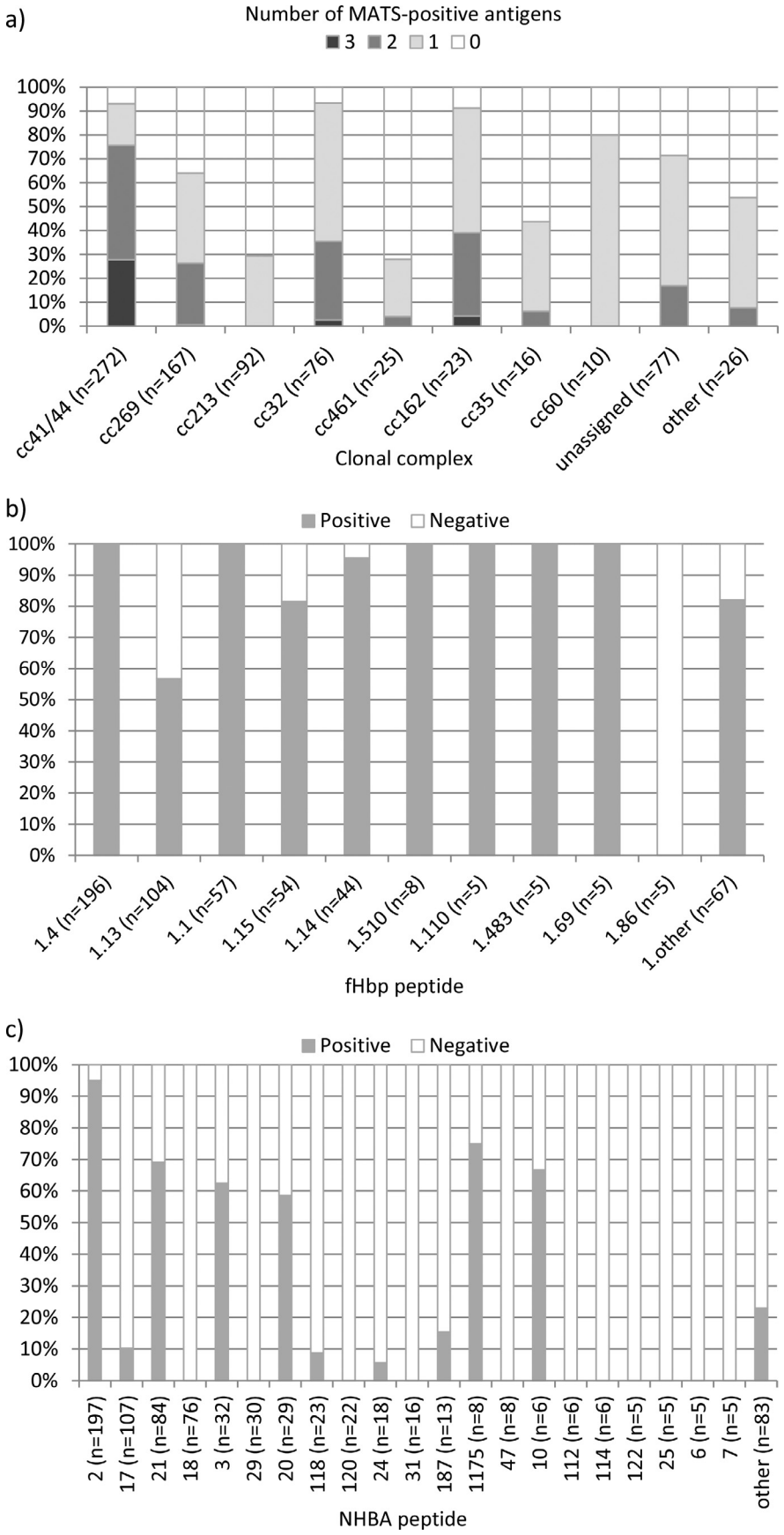

Fig. 2. MATS positivity by clonal complex (a), fHbp peptide (b) and NHBA peptide (c) among invasive MenB isolates from culture confirmed cases in England, 2014/15 to 2017/18, inclusive.
NB. All fHbp variant 2 peptides were MATS negative.

Table 1
Clonal complex distribution versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

Clonal complex	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value[a]
	n	%	n	%	n	%	n	%	n	%	n	%		
cc103									1	0.4	1	0.3	n/a	n/a
cc11									1	0.4	1	0.3	n/a	n/a
cc1157	1	8.3					1	1.2	3	1.1	4	1.1	1.09	n/a
cc1572									2	0.7	2	0.6	n/a	n/a
cc162			1	3.3	1	2.4	2	2.4	7	2.6	9	2.5	0.94	n/a
cc18									1	0.4	1	0.3	n/a	n/a
cc213	2	16.7	9	30.0	8	19.5	19	22.9	26	9.6	45	12.7	2.39	**0.0014**
cc22									3	1.1	3	0.8	n/a	n/a
cc269	5	41.7	6	20.0	9	22.0	20	24.1	47	17.3	67	18.9	1.39	0.1647
cc282					1	2.4	1	1.2			1	0.3	n/a	n/a
cc32			3	10.0	2	4.9	5	6.0	24	8.8	29	8.2	0.68	0.4150
cc35					1	2.4	1	1.2	12	4.4	13	3.7	0.27	n/a
cc364									1	0.4	1	0.3	n/a	n/a
cc41/44	2	16.7	5	16.7	10	24.4	17	20.5	99	36.4	116	32.7	0.56	**0.0068**
cc461	1	8.3	2	6.7	4	9.8	7	8.4	8	2.9	15	4.2	2.87	0.0295
cc60			1	3.3			1	1.2	6	2.2	7	2.0	0.55	n/a
cc865									1	0.4	1	0.3	n/a	n/a
UA	1	8.3	3	10.0	5	12.2	9	10.8	30	11.0	39	11.0	0.98	n/a

Blank boxes = 0. [a]P values taken for five most prevalent clonal complexes. Bold type = significant (<0.01).

n = 5/5) (Fig. 2b). This proportion was lower for isolates with peptides 1.15 (81.5%, n = 44/54) and 1.13 (56.7%, n = 59/104). All isolates with peptide 1.86 (100%, n = 5/5) were MATS-negative for fHbp. Among the minor variant 1 peptides (representing <5 isolates each) most isolates (82.1%, n = 55/67) were MATS-positive for fHbp.

Most isolates possessing alleles for NHBA peptide 2 (94.9%, n = 187/197) were MATS-positive for NHBA (Fig. 2c). This dropped to between 58.6% and 75.0% for peptides 21 (69.0%, n = 58/84), 3 (62.5%, n = 20/32), 20 (58.6%, n = 17/29), 1175 (75.0%, n = 6/8) and 10 (66.7%, n = 4/6), and then dropped further to between 5.6% and 15.4% for peptides 17 (10.3%, n = 11/107), 118 (8.7%, n = 2/23), 24 (5.6%, n = 1/18), and 187 (15.4%, n = 2/13). No isolates with peptides 18 (n = 76), 29 (n = 30), 120 (n = 22), 31 (n = 16), 47 (n = 8), 112 (n = 6), 114 (n = 6), 122 (n = 5), 25 (n = 5), 6 (n = 5) or 7 (n = 5) were MATS-positive for NHBA. Among the isolates with minor NHBA peptides (<5 isolates per peptide) 22.9% were MATS-positive.

Among the isolates possessing alleles for potentially covered NadA peptides, 16/70 (22.9%) were MATS-positive for NadA including 20.6% (n = 13/63) with peptide 1 (NadA-1), 1/1 with peptide 3 (NadA-2/3), 1/1 with peptide 6 (NadA-2/3), and 1/1 with peptide 146 (NadA-2/3). No isolates with alleles for potentially covered peptides 118 (NadA-1; n = 2), 8 (NadA-2/3; n = 1), or 127 (NadA-2/3; n = 1) were MATS-positive.

Differences between MenB isolates from vaccinated (1, 2 or 3 doses) and unvaccinated ≤3 year olds

4CMenB vaccine status among the ≤3 year olds was as follows: n = 272/355 unvaccinated (including those not eligible), n = 41/355 one dose, n = 30/355 two doses and n = 12/355 three doses. Owing to small numbers of cases, in this section, 'vaccinees' refers to individuals that have had one, two or three doses.

Table 1 shows the clonal complex distribution versus number of doses among ≤3 year olds. Among the five most prevalent clonal complexes, cc41/44 (relatively well-covered) accounted for a significantly lower proportion (20.5% versus 36.4%; P = 0.0068), and cc213 (relatively poorly-covered) accounted for a significantly higher proportion (22.9% versus 9.6%; P = 0.0014), of cases among vaccinees versus unvaccinated individuals. cc32 (relatively well covered) accounted for a lower (not significant) proportion of cases among vaccinees (6.0% versus 8.0%; P = 0.415). cc461 (relatively poorly covered) accounted for a higher (not significant) proportion of cases among vaccinees (8.4% versus 2.9%; P = 0.0295). cc269, despite being relatively well-covered, accounted for a higher (not significant) proportion of cases among vaccinees (24.1% vs 17.3; P = 0.165).

Table 2 shows MATS-coverage by number of antigens versus number of doses among ≤3 year olds. MATS-covered isolates (one, two or three antigens, collectively) accounted for a lower (not significant) proportion of cases among vaccinees (61.4% versus 68.8%; P = 0.215). Collectively, non-covered isolates and isolates covered by a single antigen accounted for a significantly higher proportion of cases among vaccinees (80.7% versus 60.7%; P = 0.00077), and isolates covered for two or three antigens accounted for a significantly lower proportion (19.3% versus 39.3%; P=<0.00001), of cases among vaccinees.

Table 3 shows the relatively common fHbp peptides (representing >30 isolates each, overall) versus number of doses for ≤3 year olds. Among these, isolates with peptide 1.4, or isolates with any fHbp variant 1 peptide, accounted for a significantly lower proportion of cases among vaccinees (10.8% versus 26.1%; P = 0.0036 and 44.6% versus 69.1%; P = 0.000049, respectively). Isolates with fHbp 1.1 also accounted for a lower (not significant) proportion of cases among vaccinees (0% versus 6.3%, P = 0.0161). Isolates with variant 2 or 3 peptides accounted for a significantly higher proportion of cases among vaccinees (54.2% versus 30.9%, P = 0.0001).

Table 4 shows the relatively common NHBA peptides (representing >30 isolates each, overall) versus number of doses for ≤3 year olds. Among these, several relatively well-covered peptides accounted for a non-significantly lower proportion of cases among vaccinees including peptides 2 (15.7% versus 29.0%; P = 0.015) and 3 (1.2% versus 4.4%; P = 0.173). Several relatively poorly covered peptides accounted for a higher proportion of cases among vaccinees including peptides 18 (14.5% versus 9.2%; P = 0.169) and 29 (9.6% versus 2.9%; P = 0.010). Peptide 21 accounted for a non-significantly higher proportion of vaccinees despite being relatively well-covered (3.3% versus 9.2%; P = 0.283).

Table 5 shows the distribution of PorA P1.4 versus number of doses for ≤3 year olds. Isolates with PorA P1.4 accounted for a significantly lower proportion of cases among vaccinees (7.2% versus 17.3%; P = 0.02). Table 6 shows the distribution of NadA variants versus number of doses for ≤3 year olds. Isolates with potentially-covered NadA peptides accounted for a similar proportion of cases among vaccinees (6.0% versus 7.0%; P = 0.760).

Table 2
Number of MATS positive antigens versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

Number of MATS-positive antigens	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value[a]
	n	%	n	%	n	%	n	%	n	%	n	%		
0	5	41.7	11	36.7	16	39.0	32	38.6	85	31.3	117	33.0	1.23	0.22
1	4	33.3	14	46.7	17	41.5	35	42.2	80	29.4	115	32.4	1.43	0.03
2	3	25.0	2	6.7	7	17.1	12	14.5	77	28.3	89	25.1	0.51	0.01
3	0	0	3	10.0	1	2.4	4	4.8	30	11.0	34	9.6	0.44	0.09
0 or 1	9	75.0	25	83.3	33	80.5	67	80.7	165	60.7	232	65.4	1.33	**0.0008**
2 or 3	3	0.25	5	16.7	8	19.5	16	19.3	107	39.3	123	34.6	0.49	**<0.00001**
1, 2 or 3	7	58.3	19	63.3	25	61.0	51	61.4	187	68.8	238	67.0	0.89	0.215

Bold type = significant (<0.007).

Table 3
fHbp peptide distribution versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

fHbp peptide[a]	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value
	n	%	n	%	n	%	n	%	n	%	n	%		
1.4	1	8.3	2	6.7	6	14.6	9	10.8	71	26.1	80	22.5	0.42	**0.0036**
1.13	2	16.7	6	20.0	6	14.6	14	16.9	40	14.7	54	15.2	1.15	0.6312
1.1									17	6.3	17	4.8	n/a	0.0161
1.15	1	8.3	1	3.3	1	2.4	3	3.6	15	5.5	18	5.1	0.66	0.7748
1.14			2	6.7	2	4.9	4	4.8	14	5.1	18	5.1	0.94	1
2.19			2	6.7	4	9.8	6	7.2	19	7.0	25	7.0	1.03	0.939294
3.45	1	8.3	2	6.7	3	7.3	6	7.2	12	4.4	18	5.1	1.64	0.305830
1.any	5	41.7	16	53.3	16	39.0	37	44.6	188	69.1	225	63.4	0.64	**0.000049**
2/3.any	7	58.3	13	43.3	25	61.0	45	54.2	84	30.9	129	36.3	1.76	**0.0001**
fhbp⁻			1	3.3			1	1.2			1	0.3	n/a	ND

Blank boxes = 0. ND = not done. [a]Translated genotype. Bold type = significant (<0.006).

Table 4
NHBA peptide distribution versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

NHBA peptide[a]	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value
	n	%	n	%	n	%	n	%	n	%	n	%		
2	1	8.3	3	10.0	9	22.0	13	15.7	79	29.0	92	25.9	0.54	0.015
17	1	8.3	5	16.7	4	9.8	10	12.0	39	14.3	49	13.8	0.84	0.597
21	4	33.3	3	10.0	4	9.8	11	13.3	25	9.2	36	10.1	1.44	0.283
18	2	16.7	5	16.7	5	12.2	12	14.5	25	9.2	37	10.4	1.57	0.169
3					1	2.4	1	1.2	12	4.4	13	3.7	0.27	0.173
29			4	13.3	4	9.8	8	9.6	8	2.9	16	4.5	3.28	0.010
other	4	33.3	10	33.3	14	34.1	28	33.7	84	30.9	112	31.5	1.09	ND

Blank boxes = 0. ND = not done. [a]Translated genotype.

Table 5
PorA distribution versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

PorA VR2	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value
	n	%	n	%	n	%	n	%	n	%	n	%		
Other	12	100.0	26	86.7	39	95.1	77	92.8	225	82.7	302	85.1	1.12	**0.02**
P1.4	0	0.0	4	13.3	2	4.9	6	7.2	47	17.3	53	14.9	0.42	**0.02**

Bold type = significant (<0.05).

Isolates from three-dose vaccinees

None of the 12 three-dose case isolates had alleles for PorA P1.4 or full-length cross-protective NadA peptides. Five were MATS-negative, four were MATS-positive for NHBA only, and three were MATS-positive for both fHbp and NHBA (table S2).

All four patients with isolates that were MATS positive for NHBA were vaccinated per schedule (i.e. within 7 days) for dose one, three were vaccinated per schedule for dose two (one was vaccinated at ~19 weeks), and all received their booster at between 53 and 61 weeks of age.

The three patients with isolates that were MATS positive for fHbp and NHBA were each vaccinated slightly off schedule. One (isolate MATS-positive for fhbp 1.69 and nhba 21) was vaccinated at ~10, 34 and 57 weeks of age and exhibited non-protective hSBA antibody titres to fHbp, PorA, and NadA in convalescent serum (>3

Table 6
NadA distribution versus number of 4CMenB doses among ≤3 year olds in England (2014/15 to 2017/18).

NadA Peptide variant[a]	3 doses (n = 12)		2 doses (n = 30)		1 dose (n = 41)		1, 2 or 3 doses (n = 83)		0 doses (n = 272)		All (n = 355)		Rate ratio 1, 2 or 3 doses versus 0 doses	P value
	n	%	n	%	n	%	n	%	n	%	n	%		
None/not covered	12	100.0	28	93.3	38	92.7	78	94.0	253	93.0	331	93.2	1.01	0.760
NadA-1	0	0.0	2	6.7	2	4.9	4	4.8	17	6.3	21	5.9	0.77	0.629
NadA-2/3	0	0.0	0	0.0	1	2.4	1	1.2	2	0.7	3	0.8	1.64	0.683
Any potentially covered	0	0.0	2	6.7	3	7.3	5	6.0	19	7.0	24	6.8	0.86	0.760

[a] Translated genotype.

months following disease). Another (isolate MATS-positive for fHbp 1.15 and NHBA 21) was vaccinated at ~10, 22 and 54 weeks of age (no available SBA titres). The other (isolate MATS-positive for fHbp 1.4 and NHBA 2) appeared to have been given a 16 weeks + 12 month regimen (as per the catch up campaign) with a second booster ~21 weeks later. In convalescent serum (>1 month following disease) this patient exhibited protective hSBA titres for fHbp (titre = 16), PorA (titre = 64) and NadA (titre = 16).

Notably, MATS-positive NHBA peptide 21 occurred four times among isolates from the three dose vaccinees, three of which had RPs well above the PBT (0.452, 0.575, 0.508 vs PBT of 0.294).

Isolates from two-dose vaccinees

Among the isolates from the two-dose cases, 12/30 were MATS-negative, 14/30 were MATS-positive for a single antigen (NHBA n = 5; fHbp n = 9), 2/30 were MATS-positive for two antigens (fHbp;PorA n = 1; fHbp;NHBA n = 1) and 3/30 were MATS-positive for three antigens (all fHbp;NHBA;PorA). The latter three all belonged to cc41/44 and had alleles for fHbp peptide 1.4 (n = 2, RPs 0.024 and 0.046) or 1.14 (RP 0.016). All three had high NHBA RPs (0.740 to 0.808 for peptide 2 and 0.808 for peptide 805). MATS-positive antigens observed multiple times among the two-dose cases included fHbp peptides 1.13 (n = 5, RP 0.013 to 0.018) and 1.14 (n = 2, RP 0.016 to 0.021), and NHBA peptides 2 (n = 2, RP 0.765 to 0.808) and 21 (n = 3, RP 0.376 to 0.793) (table S3). There were three cc32 isolates among the two-dose cases none of which had fHbp peptide 1.1, while two had alleles for full-length potentially covered NadA peptides.

Isolates from one-dose vaccinees

Among the isolates from the one-dose cases, 16/41 were MATS-negative, 17/41 were MATS-positive for a single antigen (n = 9 NHBA, n = 7 fHbp, n = 1 PorA), 7/41 were MATS-positive for two antigens (n = 6 fHbp;NHBA, n = 1 NHBA;NadA), and 1/41 was MATS-positive for three antigens (fHbp;NHBA;PorA). There were eight MATS-positive cc269 isolates, seven of which had fHbp peptide 1.13 (n = 4 RP 0.013 to 0.019) or variant 2/3 peptides. There were nine MATS-positive cc41/44 isolates, only one of which possessed PorA P1.4. Six of these possessed fHbp peptide 1.4 (RPs 0.031 to 0.056) and seven possessed NHBA peptide 2 (RPs 0.410 to 1.167). MATS-positive antigens observed multiple times among the one-dose cases included fHbp peptides 1.13 (n = 5, RP 0.013 to 0.019) and 1.4 (n = 6, RP 0.031 to 0.056), and NHBA peptides 2 (n = 8, RP 0.410 to 1.167) and 21 (n = 4, RP 0.350 to 0.778) (table S4). There were two cc32 isolates among the one-dose cases, neither of which had fHbp peptide 1.1. Both had alleles for full-length potentially covered NadA peptides albeit with RPs<PBT.

Discussion

We have presented strain (clonal complex and 4CMenB genotyping) and MATS data for all English invasive MenB isolates, and compared the data from vaccinated (1, 2 or 3 doses) and unvaccinated ≤3 year olds in a period spanning 1 year pre- and three years post-4CMenB introduction.

As previously observed[15], the predominant clonal complexes were distributed, in varying proportions, throughout all age groups. The greater diversity observed at the higher and lower age extremes includes strains rarely seen in disease in other age groups, perhaps reflecting the naivety of the infant immune system and waning of the elderly immune system as previously proposed.[18]

As expected, the best covered clonal complexes/antigens and the isolates with relatively more (two or three) MATS-positive antigens accounted for a lower proportion of cases among vaccinees. The less well-covered clonal complexes/antigens, and the isolates with one or no MATS-positive antigens, conversely, accounted for a higher proportion. The differences were not always significant, but this likely reflects the low numbers, especially when focussing on subsets of isolates. One exception was NHBA peptide 21 which was well covered according to MATS but accounted for a higher proportion of cases among vaccinees. More data and further analysis are needed to understand if this is a genuine trend and, if so, why. Indeed, the role of anti-NHBA antibodies in SBA activity is complex and not well understood raising questions over the suitability of MATS for predicting NHBA mediated coverage.[19,20]

Similar observations were made at the sub-strain level. For example, none of the five cc32 isolates among vaccinees possessed fHbp peptide 1 as compared with 64.4% (n = 49/76) of cc32 isolates overall. Interestingly, 80% (n = 4/5) of two-dose cc41/44 case isolates possessed PorA P1.4. This compared with only 10% (n = 1/10) of one-dose cases, 0% of three dose cases, and 41.2% (n = 112/272) of cc41/44 cases overall. As these cases occurred between 188 and 248 days after the second dose, this likely reflects waning of anti-PorA immunity in the period post-dose 2 as previously observed.[21]

There was no increase in absolute numbers of cases due to poorly covered CCs among ≤3 year olds. For example, there were 11 or 12 cc213 cases, and 4, 5, 2 and 4 cc461 cases, in ≤3 year olds over the four years studied. This is expected, however, since (i) there is no evidence of a vaccine impact on MenB carriage[22,23], and (ii) the main reservoir of carriage is in late-teenagers/young adults in any case.[24] Nonetheless, the increase of cc213 among invasive MenB in Spain from 3.6% in 2007 to 33% in 2018 demonstrates the need for ongoing vigilance regarding natural fluctuations in meningococcal epidemiology.[25] Following the study period, IMD cases have decreased to unprecedentedly low levels since early 2020 when COVID-19 containment measures were first introduced.[26] If, when, and how much IMD case numbers will increase following relaxation of these measures, and the distribu-

tion/potential vaccine coverage of the prevailing strains, all remain to be seen.

None of the three-dose case isolates possessed alleles for protective NadA peptides. This compared with 7.4% (n = 3/41) and 6.5% (n = 2/31) among one and two-dose cases, respectively. This is consistent with an earlier phase II trial assessing the immunogenicity of a 2, 4, 6 and 12 month schedule in infants whereby only 12% of primary course and 75% of post-booster vaccinees exhibited protective titres against a moderately expressing NadA-2/3 isolate, one month post-vaccine.[27] This suggests that a booster may be important for NadA-mediated protection. Accordingly, the collective proportion of one and two dose cases with potentially covered NadA peptides (6.0%) was similar to that observed among the unvaccinated ≤3 year olds (7.0%).

Submission of additional serum for hSBA assessment of vaccine responses (currently for PorA, fHbp and NadA) among vaccinated cases is encouraged in England.[14] hSBA data were available for 4/12 three-dose cases, one of whom had sub-protective titres against all three antigens, and another of whom had a sub-protective titre against fHbp. In the former case, the isolate was MATS-positive for fHbp and NHBA indicating that at least some vaccine-failure cases are due to poor antibody responses by the individual. One vaccine-breakthrough case exhibited a protective titre against fHbp which was strongly MATS-positive in the corresponding isolate. This does not rule out a complement deficiency as the hSBA assay uses exogenous complement. At present, however, PHE does not recommend immune testing in these patients. It is noteworthy that none of the three-dose cases with isolates that were MATS positive for >1 antigen were vaccinated in strict accordance with the 8, 16 weeks and one year schedule.

Other possible reasons for IMD due to MATS-positive isolates occurring in 3-dose vaccinees include (i) that MATS-positivity is only indicative of ≥80% and 96% probability of being killed by post-vaccine sera for one or two-plus antigens, respectively[9], (ii) that MATS was developed based on a 3 + 1 dose schedule while the UK employs 2 + 1 dose schedule, and (iii) pooled sera were used during development of MATS and so individual responses may be, in some cases, overlooked. It should be stated, however, that pooled sera have been shown to accurately predict seroprotection rates in infants.[28] Furthermore, MATS is considered conservative since it does not account for synergistic effects of targeting multiple antigens. Nonetheless, overestimation of coverage by MATS e.g. due to a 2 + 1 schedule, may have occurred where, in several cases, MATS-positive RPs were very close to the PBT. Any such overestimations may be peptide specific. NHBA peptide 21, for example, occurred multiple times among three-dose vaccinees often with relatively high RPs. Incidentally, MATS coverage due to NHBA peptide 21 in England was already reduced in 2014/15 (63%) versus 2007/08 (86%), possibly reflecting strain-related changes in expression.[12] This highlights the need for ongoing surveillance.

Vaccine breakthrough cases were small in number. In time, and with an improved understanding of individual vaccine responses, the reasons these occur may become clearer. Work is also ongoing to compare sequelae among vaccinees and age-matched unvaccinated individuals. The data presented here are encouraging as they show the vaccine is working as expected with regard to strain coverage. MATS coverage was highest among teenagers, however, routine vaccination in this age group is not cost-effective due to the lack of an effect on MenB carriage and the low case numbers.[22] Interestingly, recent evidence points to an impact of meningococcal OMV-containing vaccines against gonococcal disease which may significantly increase cost effectiveness of vaccinating teenagers who may therefore benefit from an IMD perspective if such a vaccine were introduced to also combat gonococcal disease.[29,30] As previously observed, predicted strain coverage was lowest among ≤3 year olds as a whole.[12] This is likely to improve as next generation vaccines incorporating relatively poorly-covered antigenic variants are introduced.

Funding

Material and funding for the MATS assay used in this study were provided by GlaxoSmithKline Biologicals SA. GlaxoSmithKline Biologicals SA was provided the opportunity to review a preliminary version of the manuscript for factual accuracy, but the authors are solely responsible for final content and interpretation. The authors received no financial support or other forms of compensation related to the development of the manuscript.

Declaration of competing interests

JL, XB, AL, SAC, LW, AH, JLo and RB perform contract research on behalf of Public Health England for GlaxoSmithKline, Pfizer, and Sanofi Pasteur. SNL performs contract research for vaccine manufacturers (including GSK, Pfizer, and Sanofi Pasteur) on behalf of St George's University of London and Public Health England but receives no personal remuneration. LS and RDP are employed by the GSK group of companies. LS holds shares in the GSK group of companies. SR, HC and MER have no interests to declare. The immunization and Countermeasures Division at PHE has provided GSK, Pfizer, and Sanofi Pasteur with postmarketing surveillance reports on meningococcal, Haemophilus influenzae, and pneumococcal infections, which the companies are required to submit to the UK Licensing Authority in compliance with their Risk Management Strategy. A cost recovery charge is made for these reports.

Acknowledgments

This publication made use of the Neisseria Multi Locus Sequence Typing website (https://pubmlst.org/organisms/neisseria-spp/) sited at the University of Oxford. The development of this site has been funded by the Wellcome Trust and the European Union.[31] It also made use of the Meningitis Research Foundation Meningococcus Genome Library (http://www.meningitis.org/research/genome) developed by Public Health England, the Wellcome Trust Sanger Institute and the University of Oxford as a collaboration. The project is part funded by Meningitis Research Foundation.

The authors would like to thank Paul Charter for his support with the IMD surveillance and all those who have contributed from PHE HPTs and General Practice.

Supplementary materials

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.015.

References

1. Parikh SR, Campbell H, Bettinger JA, et al. The everchanging epidemiology of meningococcal disease worldwide and the potential for prevention through vaccination. *J Infect* 2020;**81**(4):483–98.
2. Wyle FA, Artenstein MS, Brandt BL, et al. Immunologic response of man to group B meningococcal polysaccharide vaccines. *J Infect Dis* 1972;**126**(5):514–21.
3. Holst J, Oster P, Arnold R, et al. Vaccines against meningococcal serogroup B disease containing outer membrane vesicles (OMV): lessons from past programs and implications for the future. *Hum Vaccin Immunother* 2013;**9**(6):1241–53.
4. 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.
5. Perez JL, Absalon J, Beeslaar J, et al. From research to licensure and beyond: clinical development of MenB-FHbp, a broadly protective meningococcal B vaccine. *Expert Rev Vaccines* 2018;**17**(6):461–77.

6. 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**(6):611–14.
7. Ladhani SN, Andrews N, Parikh SR, et al. Vaccination of Infants with Meningococcal Group B Vaccine (4CMenB) in England. *N Engl J Med* 2020;**382**(4):309–17.
8. Lucidarme J, Louth J, Townsend-Payne K, Borrow R. Meningococcal serogroup A, B, C, W, X, and Y serum bactericidal antibody assays. *Methods Mol Biol* 2019;**1969**:169–79.
9. Donnelly J, Medini D, Boccadifuoco G, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci U S A* 2010;**107**(45):19490–5.
10. Heinsbroek E, Ladhani S, Gray S, et al. Added value of PCR-testing for confirmation of invasive meningococcal disease in England. *J Infect* 2013;**67**(5):385–90.
11. Clark SA, Lekshmi A, Lucidarme J, et al. Differences between culture & non-culture confirmed invasive meningococci with a focus on factor H-binding protein distribution. *J Infect* 2016;**73**(1):63–70.
12. Parikh SR, Newbold L, Slater S, 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**(7):754–62.
13. Ladhani SN, Waight PA, Ribeiro S, Ramsay ME. Invasive meningococcal disease in England: assessing disease burden through linkage of multiple national data sources. *BMC Infect Dis* 2015;**15**:551.
14. Public Health England (2015) Meningococcal disease enhanced surveillance plan. https://www.gov.uk/government/publications/meningococcal-disease-enhanced-surveillance-plan.
15. Hill DM, Lucidarme J, Gray SJ, et al. Genomic epidemiology of age-associated meningococcal lineages in national surveillance: an observational cohort study. *Lancet Infect Dis* 2015;**15**(12):1420–8.
16. Gray SJ, Trotter CL, Ramsay ME, et al. Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit. *J Med Microbiol* 2006;**55**(Pt 7):887–96.
17. Boccadifuoco G., Stella M., De Paola R., et al. *Re*-assessment of MATS ELISA specifications. 13th Congress EMGM 2015, Amsterdam.
18. Brehony C, Trotter CL, Ramsay ME, et al. Implications of differential age distribution of disease-associated meningococcal lineages for vaccine development. *Clin Vaccine Immunol* 2014;**21**(6):847–53.
19. Rossi R, Beernink PT, Giuntini S, Granoff DM. Susceptibility of meningococcal strains responsible for two serogroup B outbreaks on U.S. University campuses to serum bactericidal activity elicited by the MenB-4C vaccine. *Clin Vaccine Immunol* 2015;**22**(12):1227–34.
20. Partridge E, Lujan E, Giuntini S, Vu DM, Granoff DM. The role of anti-NHba antibody in bactericidal activity elicited by the meningococcal serogroup B vaccine, MenB-4C. *Vaccine* 2017;**35**(33):4236–44.
21. Snape MD, Voysey M, Finn A, et al. Persistence of bactericidal antibodies after infant serogroup B meningococcal immunization and booster dose response at 12, 18 or 24 months of age. *Pediatr Infect Dis J* 2016;**35**(4):e113–23.
22. Marshall HS, McMillan M, Koehler AP, et al. Meningococcal B vaccine and meningococcal carriage in adolescents in Australia. *N Engl J Med* 2020;**382**(4):318–27.
23. Marjuki H, Chang HY, Topaz N, et al. Genetic diversity of meningococcal serogroup B vaccine antigens among carriage isolates collected from students at three universities in the United States, 2015-2016. *MBio* 2021;**12**(3).
24. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**(12):853–61.
25. Abad R, Garcia-Amil C, Navarro C, Martin E, Martin-Diaz A, Vazquez JA. Molecular characterization of invasive serogroup B Neisseria meningitidis isolates from Spain during 2015-2018: evolution of the vaccine antigen factor H binding protein (FHbp). *J Infect* 2021;**82**(4):37–44.
26. Brueggemann AB, Jansen van Rensburg MJ, Shaw D, et al. Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data. *Lancet Digit Health* 2021;**3**(6):e360–70.
27. Findlow J, Borrow R, Snape MD, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant Meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis* 2010;**51**(10):1127–37.
28. Budroni S, Kleinschmidt A, Boucher P, Medini D. Pooled-sera hSBA titres predict individual seroprotection in infants and toddlers vaccinated with 4CMenB. *Vaccine* 2016;**34**(23):2579–84.
29. Petousis-Harris H, Radcliff FJ. Exploitation of Neisseria meningitidis Group B OMV Vaccines Against N. gonorrhoeae to inform the development and deployment of effective gonorrhea vaccines. *Front Immunol* 2019;**10**:683.
30. Gottlieb SL, Jerse AE, Delany-Moretlwe S, Deal C, Giersing BK. Advancing vaccine development for gonorrhoea and the Global STI Vaccine Roadmap. *Sex Health* 2019;**16**(5):426–32.
31. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: bIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018;**3**:124.