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Genetic Meningococcal Antigen Typing System (gMATS): A genotyping tool that predicts 4CMenB strain coverage worldwide ☆

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ABSTRACT

Background: The Meningococcal Antigen Typing System (MATS) was developed to identify meningococcus group B strains with a high likelihood of being covered by the 4CMenB vaccine, but is limited by the requirement for viable isolates from culture-confirmed cases. We examined if antigen genotyping could complement MATS in predicting strain coverage by the 4CMenB vaccine.

Abbreviations: Ca, Lin's coefficient of accuracy; IDs, identification numbers (protein variants); ELISA, enzyme-linked immunosorbent assays; fHbp, factor H-binding protein; hSBA, serum bactericidal antibody assay with human complement; IMD, invasive meningococcal disease; MATS, Meningococcal Antigen Typing System; NadA, *Neisseria* adhesin A; NIP, national immunization program; NHBA, Neisserial Heparin-Binding Antigen; OMV, outer membrane vesicles; PBT, positive bactericidal threshold; PorA, porin A protein; RP, relative potency; VE, vaccine effectiveness; VR2, variable region 2.

☆ *Keypoints:* The Genetic Meningococcal Antigen Typing System (gMATS), based on antigen genotyping, complements MATS in predicting 4CMenB strain coverage without requiring a cultivable isolate. Use of gMATS instead of MATS may simplify 4CMenB strain coverage prediction and accelerate 4CMenB protection surveillance.

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Methods: From a panel of 3912 MATS-typed invasive meningococcal disease isolates collected in England and Wales in 2007–2008, 2014–2015 and 2015–2016, and in 16 other countries in 2000–2015, 3481 isolates were also characterized by antigen genotyping. Individual associations between antigen genotypes and MATS coverage for each 4CMenB component were used to define a genetic MATS (gMATS). gMATS estimates were compared with England and Wales human complement serum bactericidal assay (hSBA) data and vaccine effectiveness (VE) data from England.
Results: Overall, 81% of the strain panel had genetically predictable MATS coverage, with 92% accuracy and highly concordant results across national panels (Lin's accuracy coefficient, 0.98; root-mean-square deviation, 6%). England and Wales strain coverage estimates were 72–73% by genotyping (66–73% by MATS), underestimating hSBA values after four vaccine doses (88%) and VE after two doses (83%). The gMATS predicted strain coverage in other countries was 58–88%.
Conclusions: gMATS can replace MATS in predicting 4CMenB strain coverage in four out of five cases, without requiring a cultivable isolate, and is open to further improvement. Both methods underestimated VE in England. Strain coverage predictions in other countries matched or exceeded England and Wales estimates.

1. Introduction

Neisseria meningitidis is the leading cause of bacterial meningitis and sepsis in children and young adults worldwide [1]. With licensure of 4CMenB (Bexsero, GSK), the first broad coverage meningococcus group B (MenB) vaccine, invasive meningococcal disease (IMD) has become a vaccine-preventable disease [2,3]. 4CMenB is a multicomponent vaccine that includes three recombinant protein antigens, *Neisseria* adhesin A (NadA), Neisserial Heparin-Binding Antigen (NHBA), and factor H-binding protein (fHbp), plus detergent-extracted outer membrane vesicles (OMV) obtained from the New Zealand outbreak strain, containing porin A protein (PorA) as main vaccine antigen [4].

Since the incidence of IMD is relatively low, effectiveness of meningococcal vaccines has been evaluated via an accepted surrogate of protection: the serum bactericidal antibody assay with human complement (hSBA) [5]. Assessing protein-based vaccines such as 4CMenB is complicated by diversity in the sequence and expression of MenB surface protein antigens [6]. Predicting efficacy would require hSBA investigation against a large panel of genetically diverse and geographically representative pathogenic strains, which is impractical due to the volumes of serum required [6,7].

The Meningococcal Antigen Typing System (MATS) was developed to predict strain coverage by 4CMenB from large panels of meningococcal isolates, independent of human sera [6,8]. MATS combines genotyping for PorA with three enzyme-linked immunosorbent assays (ELISA) for fHbp, NHBA, and NadA, that quantify in meningococcal strains the relative expression and cross-reactivity of antigenic variants with vaccine-induced antibody. Internationally-standardized MATS [9] was shown to provide a conservative prediction of hSBA results from pooled human sera [10] and indicated high rates of individual seroprotection [11], ranging from 66% to 91% in 14 countries [8,12–20].

The UK was the first country to introduce 4CMenB into its national immunization program (NIP). From September 2015, a reduced two-dose priming schedule was offered to infants aged 2 and 4 months, with a booster at 12 months, along with limited catch-up for those born after April 2015 [21]. In the first 10 months following introduction, two-dose vaccine effectiveness (VE) was 83% (95% confidence interval [95% CI]: 24–95%) against MenB cases, equivalent to 94% effectiveness against all cases predicted to be prevented by the vaccine [22].

A limitation of hSBA unresolved by MATS is the inability to use the assay in non-culture confirmed cases. In some countries, around half of cases are identified solely by PCR [23,24]. Also, MATS requires effort in maintaining the international standardization level, as for all immunoassays. A genome-based vaccine strain coverage predictor would provide a more easily accessible, rapid, portable, comprehensive, and cost-effective alternative to both hSBA and MATS assays.

Here, we present 4CMenB vaccine antigen genotyping and MATS data on over 3000 MenB invasive disease isolates, representing all notified meningococcal cases in England and Wales in three epidemiological years together with isolates collected in 16 other countries. We analyzed the correlation between individual antigen genotypes and coverage estimates by MATS to define gMATS, a new genetic Meningococcal Antigen Typing System.

Fig. 1 summarizes the research, clinical relevance and impact on the patient population.

2. Methods

2.1. Bacterial strains

MATS typing was collected for 3912 invasive MenB isolates from 13 European and 4 non-European countries (Table 1), recovered from the blood or cerebrospinal fluid of patients and representative of national epidemiology in each country at the time of testing. A subset of 3481 isolates was also typed using antigen genotyping.

2.2. Strain coverage prediction by MATS

Levels of expression and cross-reactivity of fHbp, NadA, and NHBA were analyzed using the MATS ELISA, according to the method described previously [6]. Vaccine coverage for these three components was predicted by defining a relative potency (RP) of the tested strain versus a reference strain for each antigen and then comparing the RP with a positive bactericidal threshold (PBT), the antigen-specific minimum RP value predictive of killing in the hSBA. If the RP exceeds the PBT for a given antigen, the strain is predicted as covered by that vaccine antigen. Contribution to coverage by the OMV component was estimated by genotyping, by sequencing part of the *porA* gene encoding variable region 2 (VR2) and checking identity to the variant present in the vaccine, i.e. PorA VR2 match with peptide 4 was defined as 'covered' (PorA VR2 = 4) and other cases as 'not covered' [6].

2.3. Strain coverage prediction by genotyping

For the 3481 isolates characterized by genotyping (Table 1), *fHbp* and *nhba* genes were PCR amplified and sequenced, or their sequences were extracted from the whole genome sequence when

Focus on the Patient

What is the context?

- 4CMenB is a 4-component, meningococcal group B (MenB) vaccine developed to protect against related invasive disease.
- The meningococcal Antigen Typing System (MATS) assay was developed to estimate the proportion of MenB strains against which 4CMenB vaccinees are protected in a given region.
- The MATS assay requires viable isolates and therefore cannot be applied to non-culture confirmed cases

What is new?

- We defined a genetic MATS (gMATS) for predicting strain coverage by associating antigen genotyping and MATS results, using European and non-EU MenB strain panels.
- In addition to viable isolates, gMATS can be applied to PCR confirmed non-culturable disease samples.
- We observed concordant results across national strain panels with gMATS, although both MATS and gMATS underestimated the effectiveness of the vaccine.

What is the take-home message?

- Our results suggest tha gMATS complement MATS in the estimation of protection provided by 4CMenB vaccination.
- This assay may help simplify estimation of vaccine strain coverage and accelerate surveillance of 4CMEnB vaccine protection.

Fig. 1. Focus on the Patient section.

Table 1
The global collection of 3912 invasive meningococcal B isolates characterized using the Meningococcal Antigen Typing System (MATS) to estimate 4CMenB vaccine coverage. A subset of 3481 isolates, collected in ten European countries and three non-European countries, was also characterized by antigen genotype.

Country (years)	Number of isolates	Predicted 4CMenB strain coverage by MATS, % (95% CI)	Reference
European countries			
Austria (2008–11)	118	68 (56–73)	Unpublished
Czech Republic (2007–10)	108	74 (58–87)	[12]
Finland (2010–14)	60[a]	78 (72–88)	[20]
France (2007–08)	200[a]	85 (69–93)	[12]
(2013–14)	172[a]	70 (60–80)	Unpublished
Germany (2007–08)	222[a]	82 (69–92)	[12]
Greece (2008–10)	52[a]	88 (60–96)	[13]
Ireland (2009–13)	105[a]	69 (65–85)	Unpublished
Italy (2007–08)	54[a]	87 (70–93)	[12]
Norway (2007–08)	41[a]	85 (76–98)	[12]
Poland (2010–11)	196[a]	84 (79–91)	[14]
Portugal (2011–15)	106	68 (56–81)	[15]
Spain (2009–10)	300[a]	69 (48–85)	[17]
UK (2007–08)	535[a]	73 (57–87)	[12]
(2014–15)	251[a]	66 (52–80)	[18]
(2015–16)	174[a]	73 (56–83)	Unpublished
Non-European countries			
Australia (2007–11)	520[a]	75 (61–86)	Unpublished
Brazil (2010)	99	81 (71–95)	[8]
Canada (2006–09)	157[a]	66 (46–78)	[16]
USA (2000–08)	442[a]	91 (72–96)	[19]

95% CI, 95% confidence interval.
[a] Isolates also characterized by antigen genotype.

available [25]; the *nadA* gene was PCR-amplified to determine gene presence/absence, but was sequenced only in 5% of cases. Alleles and corresponding peptide identification numbers (IDs; protein variants) were assigned using the PubMLST *Neisseria* multilocus sequence typing database (https://pubmlst.org/neisseria/).

Antigen-specific predicted strain coverage by gMATS was defined by identifying peptide IDs significantly associated with MATS coverage/non-coverage for that antigen. For fHbp and NHBA antigens, peptide IDs present in more than five isolates were considered. Peptide IDs for which the percentage of MATS-covered

strains was higher than 60% or lower than 40% were considered predictors of coverage or non-coverage, respectively, if a test of proportions rejected 50% as null hypothesis ($p < 0.05$ or <0.001). Peptide IDs not fulfilling these criteria were considered 'unpredictable'. The same approach was attempted for NadA, testing the association of *nadA* gene presence/absence and NadA-MATS coverage. Coverage for the OMV vaccine component PorA was genetically defined, as in MATS.

We defined a strain as gMATS 'covered' if one or more antigen-specific gMATS prediction for that strain was 'covered'. If all antigen-specific gMATS predictors were 'not covered', we defined the strain as gMATS 'not covered'. In remaining cases, the strain was defined as gMATS 'unpredictable'.

2.4. gMATS coverage predictions on national strain panels

Overall, 49% of gMATS 'unpredictable' strains were MATS covered. This observation suggests that empirically we could predict that half of the 'unpredictable' strains could be considered as covered even by the gMATS method. Therefore, when we applied the gMATS to estimate the coverage on national strain panels, we used the proportion of 'covered' strains plus half the proportion of 'unpredictable' strains as gMATS final estimation of strain panel coverage. We defined the lower limit of the estimate range as the proportion 'covered', the upper limit as the sum of the proportions 'covered' and 'unpredictable'.

Table 2
The genetic Meningococcal Antigen Typing System (gMATS) predictors identified via analysis of associations between antigen genotype and MATS coverage.

4CMenB antigen	gMATS predictors		
	Covered	Not covered	Unpredictable
fHbp	Peptides 1, 2, 4, 14, 15, 37, 89, 90, 110, 144, 224, 232, 245, 249, 252, 510	Peptide 213 and all variant 2 and 3 peptides	All other fHbp variant 1 peptides
NHBA	Peptides 1, 2, 3, 5, 10, 20, 21, 113, 243	Peptides 6, 13, 17, 18, 19, 24, 25, 30, 31, 43, 47, 58, 112, 114, 120, 122, 160, 187, 253	All other NHBA peptides
NadA	Never	Always	Not applicable
OMV	PorA VR2 = 4	PorA VR2 ≠ 4	Not applicable

fHbp, factor H binding protein; NHBA, Neisserial Heparin-Binding Antigen; NadA, *Neisseria* adhesin A; OMV, outer membrane vesicles; PorA VR2, porin A variant 2. Peptide numbers correspond to identification numbers in PubMLST *Neisseria* sequence typing database.

2.5. Comparisons across MenB strain panels

MATS and gMATS predictions for the different strain panels were compared against each other and against hSBA data from England and Wales and effectiveness data from England. The hSBA data were derived from a representative panel of 40 MenB isolates collected in 2007–2008 using sera from infants after the fourth 4CMenB vaccine dose, as described previously [10]. VE data were estimated for vaccine-eligible infants with laboratory-confirmed invasive MenB disease diagnosed in 2015–2016 after two doses administered as part of the NIP [22].

2.6. Statistical methods

Statistical analyses were performed using the *stats* and *Agreement* packages of R 3.3.1 [26,27]. Chi-squared statistical test for proportions was performed by testing the 50% proportion as null hypothesis. Fisher exact test for associations, Pearson correlation, and Poisson regression were performed using default parameters. Lin's coefficient of accuracy (C_a) was calculated with error = "const", TDI_a = 5, target = "random" parameters. MATS coverage 95% CIs were calculated as described previously [9].

3. Results

3.1. Individual 4CMenB antigen genotypes predict antigen-specific MATS coverage in 81–84% of strains analyzed

The association between antigen genotype and MATS coverage was analyzed to identify gMATS predictors. Table 2 reports the antigen-specific gMATS definitions and Table 3, their statistical properties as predictors of strain coverage. Since MATS coverage is already genetically defined for the OMV component, gMATS and MATS for OMV coincide. The NadA antigen was present in 27% of the strain panel, as demonstrated by *nadA* gene PCR, but it is known to be artificially under-expressed under MATS assay conditions [28]. Consequently, only 1.6% of strains (N = 57) were NadA-MATS covered. Since the gMATS objective was to reproduce MATS results, the best predictor of NadA-MATS was the *a priori* 'not covered' prediction, independent of gene presence/absence or sequence.

3.1.1. Specific fHbp peptides predict fHbp-MATS coverage for 84% of strain panel

In the strain panel investigated, 285 different fHbp peptides were identified, 42 of which were present in more than five isolates (Supplementary Table 1), representing 88% of isolates. Fig. 2A shows the percentage of strains covered or not covered by fHbp-MATS for each of the 42 fHbp peptides. Sixteen peptides,

Table 3
Single antigen genotyping as predictor of 4CMenB strain coverage estimated using the Meningococcal Antigen Typing System (MATS) and combined genetic Meningococcal Antigen Typing System (gMATS). The distribution in 2 × 2 contingency tables of positive/negative antigen genotyping predictions and MATS outcome was always significantly non-random (Fisher exact test p-value $<10^{-16}$).

4CMenB antigen (predictor)	Predicted MATS outcome	Prediction of MATS MenB strain coverage by genotyping (%)					
		Isolates that can be predicted	Accuracy	Positive predictive value	Negative predictive value	Sensitivity	Specificity
fHbp peptide	Coverage by fHbp	84.5	93.6	90.7	97.9	98.4	87.9
NHBA peptide	Coverage by NHBA	81.5	82.5	79.9	87.7	92.7	69.1
nadA gene presence	Coverage by NadA	100	98.4	Indet.	98.4	0	100
PorA VR2 match	Coverage by PorA	100	100	100	100	100	100
Combined gMATS	Coverage by any antigen	81.3	92.4	93.7	85.3	97.1	72.4

Indet., indeterminate.
fHbp, factor H binding protein; NHBA, Neisserial Heparin-Binding Antigen; NadA, *Neisseria* adhesin A; PorA VR2, porin A variant 2.

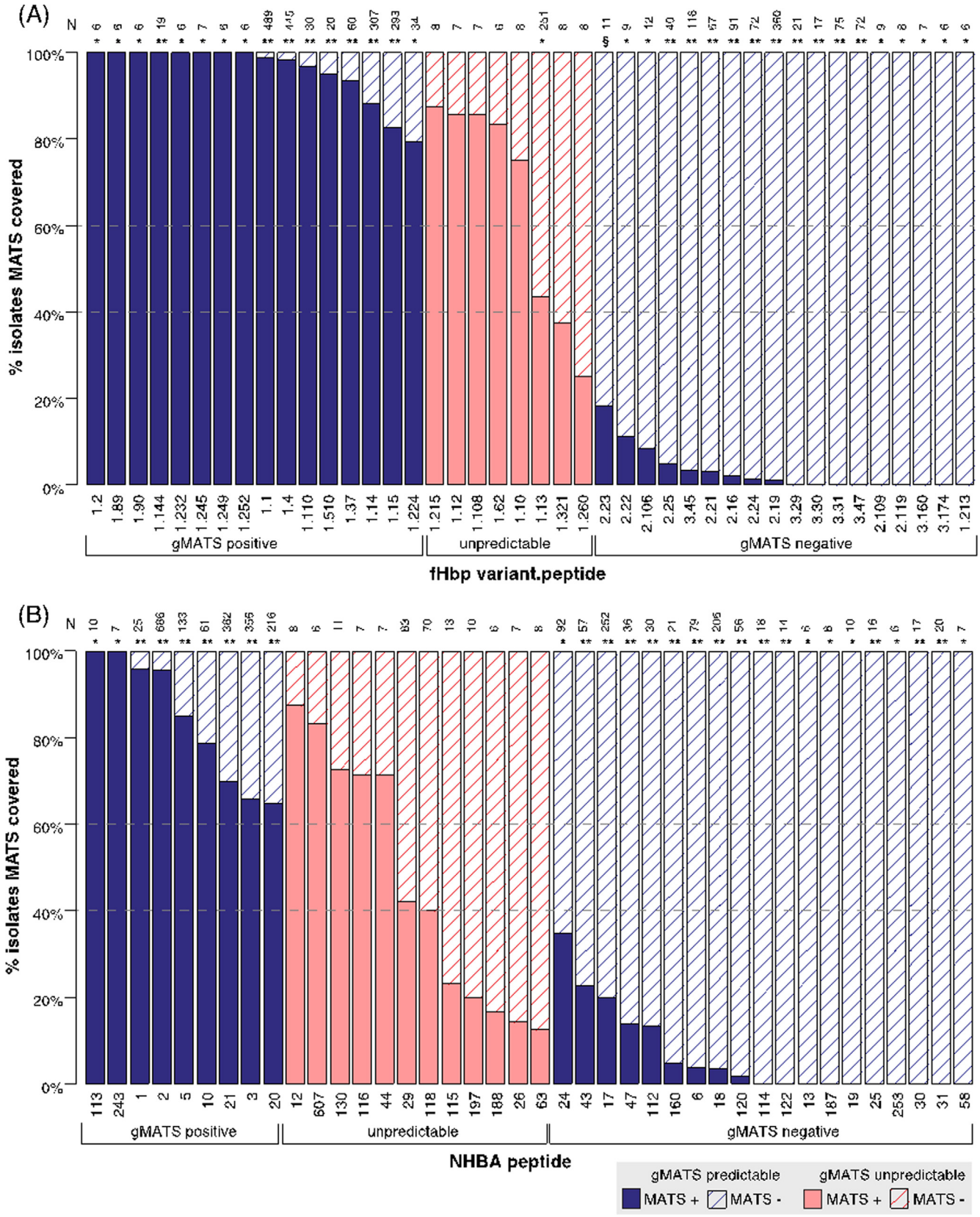

Fig. 2. Proportion of the 3481 MenB isolates covered by 4CMenB, as estimated using the Meningococcal Antigen Typing System (MATS), stratified by fHbp (A) or NHBA (B) antigen genotypes. Genotypes for which MATS coverage can/cannot be predicted are depicted in blue pink respectively. The plots show data for fHbp or NHBA peptides present in more than five isolates (peptide numbers are identification numbers in PubMLST *Neisseria* sequence typing database). *Footnotes*: $^{*}p < 0.05$; $^{**}p < 0.001$; $^{\S}p = 0.07$. fHbp, factor H binding protein; NHBA, Neisserial Heparin-Binding Antigen; gMATS, genetic Meningococcal Antigen Typing System.

all belonging to fHbp variant 1, showed coverage of at least 79% and were significantly ($p < 0.05$) or highly significantly ($p < 0.001$) associated with fHbp-MATS coverage. Seventeen peptides belonging to fHbp variants 2 and 3, and variant 1 peptide ID 213, had coverage of 18% or lower and were significantly associated with fHbp-MATS non-coverage ($p = 0.07$ for ID 23; considered significant and indicative of non-coverage). Eight peptides, all belonging to variant 1, were neither positively nor negatively associated with fHbp-MATS coverage because of small sample size (frequency 6–8), intermediate coverage or both, and were considered 'unpredictable', along with peptide IDs with population frequency ≤ 5. Fourteen frameshifted proteins (0.4% of isolates) were considered 'not covered'. With these definitions, 84% of the strain panel had genetically predictable fHbp-MATS coverage, with 94% predictive accuracy (Table 3).

3.1.2. Specific NHBA peptides predict NHBA-MATS coverage for 81% of the strain panel

A total of 288 NHBA peptides were identified, 40 of which were present in more than five isolates (Supplementary Table 2). As shown in Fig. 2B, nine peptides showed coverage of at least 65% and were significantly ($p < 0.05$) or highly significantly ($p < 0.001$) associated with NHBA-MATS coverage. Nineteen peptides had coverage of 35% or lower and were associated with NHBA-MATS non-coverage. Twelve peptide IDs were neither positively nor negatively associated with NHBA-MATS coverage because of small sample size (frequency 6–13) or intermediate coverage. NHBA-gMATS for these and other peptide IDs with population frequency ≤ 5 were defined as 'unpredictable'. Ten frameshifted proteins (0.3% of isolates) were considered 'not covered'. Overall, 81% of the strain panel had genetically predictable NHBA-MATS coverage, with 82% predictive accuracy (Table 3).

3.2. gMATS predicts overall MATS strain coverage for 81% of the strains analyzed

When the four antigen-specific gMATS predictors were combined, 81% of strains in the global panel were predictable by gMATS (covered or not covered), with 92% prediction accuracy versus MATS (Table 3). Per national panel, 68–88% of isolates were gMATS predictable (Supplementary Table 3). Fig. 3 shows the comparison of MATS and gMATS predictions for gMATS-predictable subsets of each panel. The two methods generated highly correlated results (Pearson correlation coefficient 0.88, $p < 10^{-5}$). The most discordant panels were from Ireland and Spain (10% over- and underestimation by gMATS, respectively). Across all countries, gMATS versus MATS root-mean-square deviation was 5%, indicating strong concordance. The regression analysis showed gMATS slightly exceeded MATS for coverage values >80%, although the 95% dispersion area of the regression model always included the identity, indicating an absence of statistically significant bias.

3.3. gMATS reproduces MATS strain coverage predictions on national panels

Fig. 4 shows the results of the empirical gMATS strain coverage method applied to the 16 national strain panels, along with corresponding MATS results (see also Supplementary Table 4). gMATS accurately reproduced MATS point estimates across panels (Lin's accuracy coefficient, C_a, 0.98; root-mean-square deviation, 6%), with similar uncertainty ranges (average width 25% for MATS and 19% for gMATS) that largely overlapped.

3.4. gMATS underestimates England and Wales pooled hSBA results and VE, and predicts similar or higher strain coverage in other countries

Within a dataset of 40 isolates representative of meningococcal disease in England and Wales [10], 100% of gMATS covered strains, 57% of gMATS negative strains, and 75% of gMATS unpredictable strains were killed in hSBA (Table 4), indicating that gMATS substantially underestimates killing in hSBA from infant pooled sera.

Fig. 4 also shows that, as with MATS estimates (66–73%), gMATS (72–73%) underestimated both the hSBA estimate of strain coverage in England and Wales (88%, 95% CI: 72–95%) [10] and VE (83%, 95% CI: 24–95%) in England [22], although with overlapping ranges.

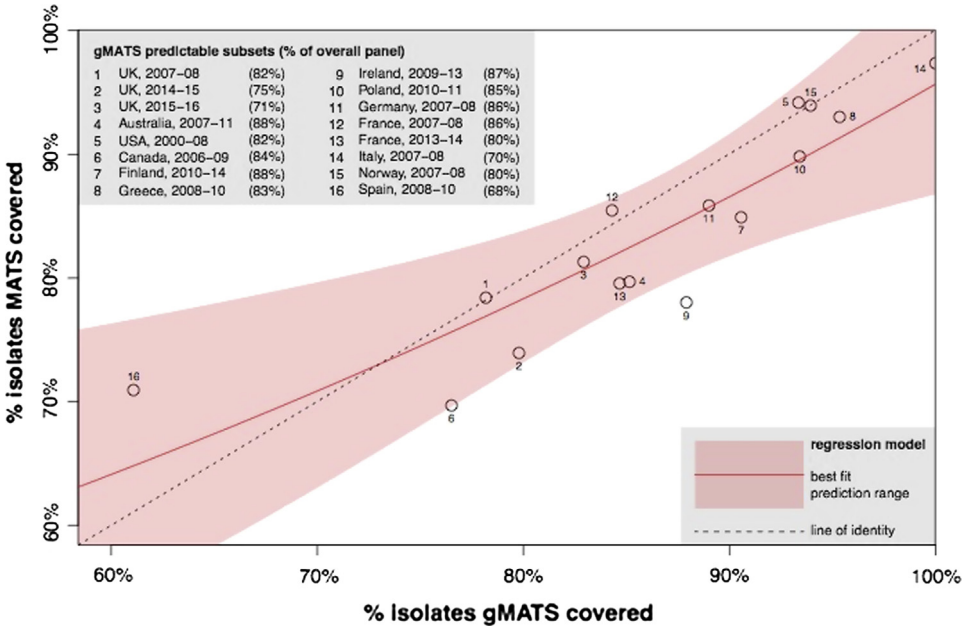

Fig. 3. Regression analysis of predicted coverage of 4CMenB by MATS and gMATS on subset of gMATS-predictable strains, in each national strain panel. Predicted gMATS coverage was determined by combining predictions for three vaccine antigens (fHbp, NHBA and PorA). *Foonotes*: MATS, Meningococcal Antigen Typing System; gMATS, genetic Meningococcal Antigen Typing System; fHbp, factor H bonding protein; NHBA, Neisserial Heparin-Binding Antigen; NadA, *Neisseria* adhesin A; PorA, Porin A.

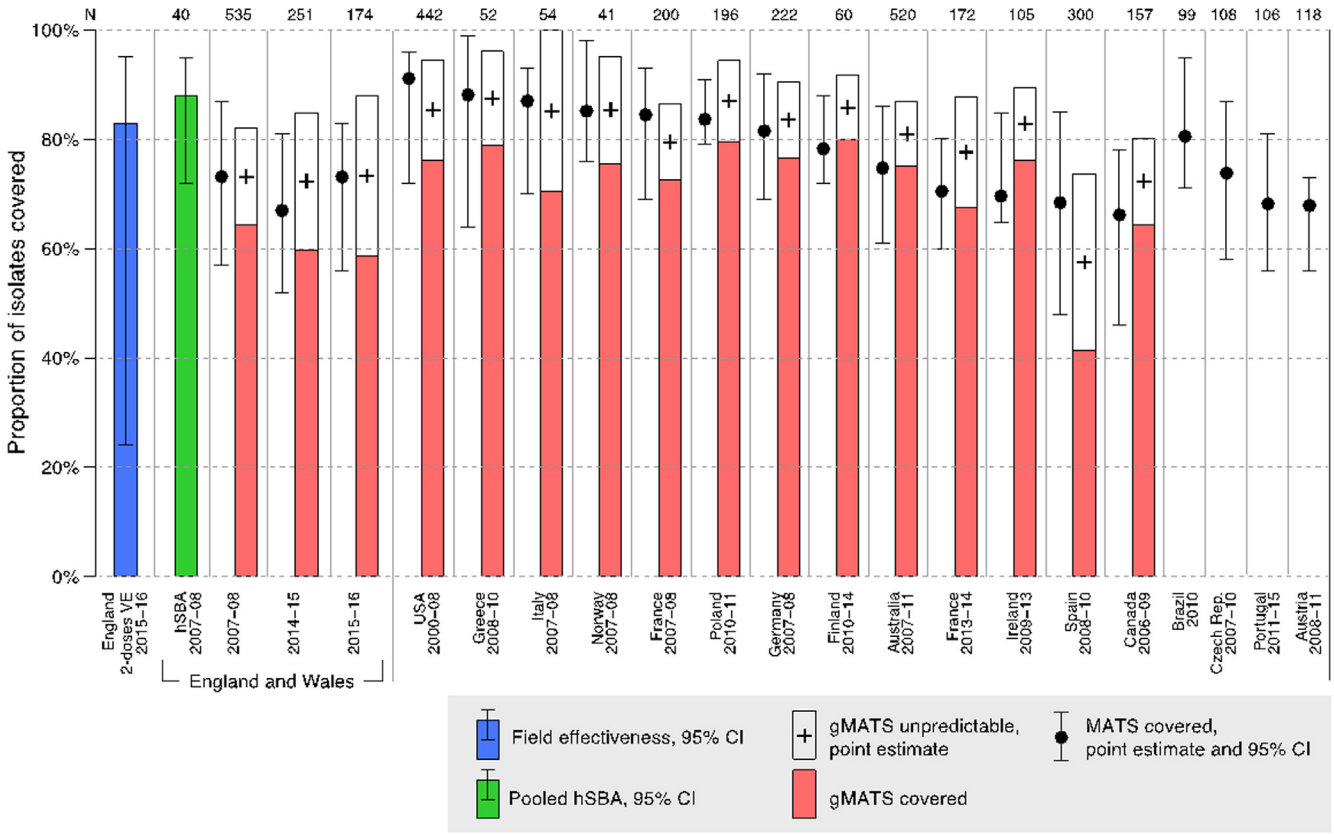

Fig. 4. Predicted strain coverage of 4CMenB by MATS and gMATS in England and Wales and 16 other countries. The blue bar represents the England 4CMenB vaccine effectiveness after two doses, 83% (95% CI, 24–95%) [22]. The green bar represents the hSBA coverage estimate 88% (95% CI, 72–95%) for a panel of 40 isolates tested with pooled sera from infant and adolescent vaccines and representative of the England and Wales meningococcal epidemiology [10]. The red and white portions of histograms represent respectively the proportion of gMATS 'covered' and 'unpredictable' strains for all country-specific panels analyzed. Point estimates of gMATS coverage ('covered' + 50% 'unpredictable' proportions) are shown as crosses. The proportion of MATS covered strains (point estimates with 95% confidence intervals [CI]) are shown as black circles for all country-specific strain panels analyzed. The panels of 4 countries (Brazil, Czech Republic, Poland and Austria) were not characterized by genotyping of antigens and gMATS prediction was not applicable. *Foonotes*: MATS, Meningococcal Antigen Typing System; gMATS, genetic Meningococcal Antigen Typing System; hSBA, human complement serum bactericidal assay; VE, vaccine effectiveness.

Table 4
Comparison of bacterial killing in the human complement serum bactericidal assay (hSBA) with gMATS coverage prediction on 40 representative isolates from England and Wales [10]. gMATS predicts hSBA with 88% accuracy for 80% of isolates for which a prediction can be made. Positive and negative predictive values are, respectively, 100% and 43%.

gMATS coverage prediction	Infant pooled hSBA result	
	Killed	Not killed
Positive	25	0
Negative	4	3
Unpredictable	6	2

gMATS, genetic Meningococcal Antigen Typing System.

Comparison of gMATS and MATS coverage estimates from England and Wales with results from other countries showed percentages ranged from 58% in Spain to 88% in Greece for gMATS, and from 66% in Canada to 91% in USA for MATS (Fig. 4). 4CMenB strain coverage in England and Wales, estimated by gMATS or MATS, is therefore at the lower end of the spectrum of estimates from the countries investigated so far.

4. Discussion

A correlation between MATS and antigen genotype was initially noted in a previous assessment of predicted strain coverage of 4CMenB [12]. Here, we extended the analysis to a global panel of over 3900 isolates from MenB disease cases to establish the level of correlation between MATS and antigen genotyping and define a new genetic predictor of 4CMenB strain coverage, gMATS.

We found that gMATS could replace MATS in predicting 4CMenB strain coverage in more than 80% of isolates, with 92% accuracy and 0.98 concordance. The least concordant strain sets were from Ireland, where gMATS overestimated MATS by 10% and Spain, where gMATS underestimated MATS by 10%. The reason of this underestimation in Ireland is mainly related to NHBA MATS relative potencies close to, but below, the positive bactericidal threshold for peptides 20 and 21, that are peptides considered 'covered' by gMATS. In Spain, the reason is still related to NHBA MATS relative potencies close to, but above, the positive bactericidal threshold for peptide 17, which is 'not covered' by gMATS. Also worth noting, Spain had the largest proportion of 'unpredictable' strains (32%) due to the specific strain epidemiology in this country [12]. Comparison with hSBA results from England and Wales [10] and VE data from England [22] confirmed that MATS and gMATS are conservative predictors of strain coverage. MATS and gMATS predicted equivalent or higher strain coverage in another 16 countries, highlighting the potential impact of 4CMenB outside of the UK.

A major difference between gMATS and MATS is the 'unpredictable' strains category in gMATS, allowing for two uses of the method. When testing a single strain, the gMATS result is either clear ('covered'/'not-covered') or clearly absent, in which case

another method must be used to determine strain coverage, such as MATS or hSBA. When estimating strain coverage at a country level, we considered the proportion of 'unpredictable' strains as the uncertainty of the genetic predictor. Supported by the observation that, globally, 49% of unpredictable strains by gMATS were covered by MATS, we defined the gMATS point estimate for national strain panels as the proportion of gMATS 'covered' plus half the 'unpredictable' proportion. This practical, though simplistic, use of gMATS reproduced MATS predictions at a national level for the 17 countries investigated (accuracy coefficient, C_a, 0.98; root-mean-square deviation, 6%).

Genotype-phenotype modelling had already shown some promise in analyses of two subsets of the strain panels investigated here, as reported by Brehony and Mowlaboccus [29,30] and summarized in Supplementary Table 5. With a larger sample size and broader geographic representation, overall, gMATS confirms and significantly extends the associations that were identified previously.

Compared to immunoassay-based methods, gMATS has the distinct advantage of not requiring a bacterial isolate and is therefore not limited to culture-positive cases only. gMATS can be performed using antigen PCR or methods that allow whole genome sequencing of *N. meningitidis* directly from clinical specimens [31]. In the England study of 4CMenB effectiveness, 70% of MenB cases in vaccine-eligible infants were confirmed by PCR only [22] indicating that gMATS, although currently applicable to only 80% of isolates, has the potential to duplicate MATS's basis for strain coverage assessment and accelerate surveillance of 4CMenB VE. Also, gMATS can substantially simplify laboratory operations as technical implementation of MATS requires sufficient numbers of samples to be processed to maintain laboratory proficiency and remain cost-effective [32].

Current implementation of gMATS has limitations. Like MATS, gMATS (*i*) provides a conservative estimate of killing in the hSBA because it does not consider cooperative effects among antigens that can mediate killing together but not individually [33,34], (*ii*) ignores the effects of minor constituents of OMV [6], and (*iii*) underestimates the contribution of NadA antigen to coverage [28]. gMATS also has specific limitations connected with the genotype-phenotype association approach: (*iv*) low-frequency antigenic variants, including new variants that may appear in the future, cannot be assessed until enough isolates are collected, *v)* future changes in regulatory elements or elsewhere in the genome – possibly promoted by immune selection and recombination [35] – may alter the genotype-phenotype association, and (*vi*) the threshold-based approach to define 'covered'/'not covered' and the heuristic imputation of 'unpredictable' variants, albeit practical, is ameneable to statistical improvements.

In contrast to immunoassay-based methods, gMATS is open to potentially unlimited improvement with the accumulation of prospective genotypic/phenotypic data, including hSBA or VE estimates from countries implementing 4CMenB. Cooperative effects among antigens could be detected by identifying di-, tri-, or tetra-antigenic profiles associated with killing in hSBA, or disappearing upon mass vaccination. As previously observed [29], the frequency of multi-antigen profiles drops dramatically with the number of antigens included, suggesting that efforts focused on the most frequent combinations, complementary to single-antigen predictors, could have higher chances of achieving statistical significance. Coverage mediated by minor OMV components could be defined using recently developed, OMV-specific typing schemes [36]. The NadA contribution could be completely re-assessed using hSBA or VE estimates for NadA-specific isolates. Accumulation of phenotypic data on low-frequency variants will progressively reduce the fraction of 'unpredictable' isolates, and the introduction of typing schemes for regulatory elements [37,38] could clarify the peptide IDs whose phenotype is highly affected by variability in expression levels. Finally, a probabilistic error model applied to the joint probabilities of multiple antigens, both for predictable and unpredictable isolates, would likely further improve the accuracy and robustness of the method. As previously proposed [39], we envision a community effort – possibly supported by a centralized resource for genotypic and phenotypic data [40] – whereby accumulation of prospective data from 4CMenB implementation and surveillance programs worldwide fuels continuous improvements of gMATS, simplifying 4CMenB strain coverage prediction and accelerating surveillance of 4CMenB vaccine protection.

In conclusion, gMATS, a new approach based on antigen genotyping, can accurately complement MATS in predicting 4CMenB strain coverage. Both methods underestimated field VE in England, while strain coverage predictions in 16 other countries matched or exceeded England and Wales coverage estimates. These results suggest that gMATS can become a suitable alternative to MATS for predicting vaccine strain coverage and monitoring vaccine implementation, and point to a positive potential impact of 4CMenB on IMD globally.

Trademark statement

Bexsero is a trademark owned by the GSK group of companies.

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Conflict of interest

MC was and AM, AB, LS, MB, MN, KV, PW, and DM are employees of the GSK groups of companies. MN, PW, RR, and KV hold shares in the GSK group of companies as part of their employee remuneration. RA reports grants from Novartis. APL reports personal fees from Pfizer and Sanofi-Pasteur. HN is the secretary of the Finnish NITAG making recommendations on the use/purchase of vaccines, including MenB, to the Ministry of Health, Finland. HN's institution received a grant from the GSK group of companies. GR's institution has a Cooperative Research and Development Agreement (CRADA) with Novartis Vaccine Development and the GSK groups of companies. AS reports grant from the Polish National Science Centre and Novartis, and participated in the National Program for Antibiotic Protection (Ministry of Health) and the Mikrobank Program (Ministry of Science and Higher Education). AS reports personal fees from Baxter and the GSK group of companies, grant from Pfizer and Novartis and, personal fees and non-financial support from Pfizer. PS reports grants from

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Authors' contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors reviewed and commented critically drafts of the manuscript for important intellectual content and gave final approval to submit for publication. The corresponding author had final responsibility to submit for publication. Drafts were developed by a professional publication writer according to the recommendations, documentation, and outline provided by the lead author. KV, RB, PW, DM, LS, AM, AB, MKT, JAV, MT, GT, RA, MC, and PS were involved in the conception or the design of the study. All authors (except PW and KV) participated in the collection or generation of the study data. HN, RB, DM, LS, AM, AB, MKT, JAV, AS, MT, GT, RA, CM, MB, MC, GR, APL, MCOG, and PS performed the study. PK, HN, RB, LS, AM, AB, MKT, JAV, AN, MT, RM, GT, RA, MJS, CM, MB, DC, and PS contributed to the study with materials/-analysis tools. KV, HN, RB, PW, DM, LS, AM, AB, MKT, JAV, AS, MT, RM, GT, RA, CM, MB, MC, GR, and PS were involved in the analyses or interpretation of the data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.12.061.

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