Vaccine 31 (2013) 4968–4974

Contents lists available at ScienceDirect

Vaccine

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

Bactericidal antibody against a representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage☆

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ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form 31 July 2013
Accepted 2 August 2013
Available online 14 August 2013

Keywords:
Neisseria meningitidis serogroup B
4CMenB vaccine strain coverage
Serum bactericidal antibody assay
MATS
Stratified proportional sampling

ABSTRACT

Background: 4CMenB (Bexsero), a vaccine developed against invasive meningococcal disease caused by capsular group B strains (MenB), was recently licensed for use by the European Medicines Agency. Assessment of 4CMenB strain coverage in specific epidemiologic settings is of primary importance to predict vaccination impact on the burden of disease. The Meningococcal Antigen Typing System (MATS) was developed to predict 4CMenB strain coverage, using serum bactericidal antibody assay with human complement (hSBA) data from a diverse panel of strains not representative of any specific epidemiology.
Objective: To experimentally validate the accuracy of MATS-based predictions against strains representative of a specific epidemiologic setting.
Methods and results: We used a stratified sampling method to identify a representative sample from all MenB disease isolates collected from England and Wales in 2007–2008, tested the strains in the hSBA assay with pooled sera from infant and adolescent vaccinees, and compared these results with MATS. MATS predictions and hSBA results were significantly associated ($P = 0.022$). MATS predicted coverage of 70% (95% CI, 55–85%) was largely confirmed by 88% killing in the hSBA (95% CI, 72–95%). MATS had 78% accuracy and 96% positive predictive value against hSBA.
Conclusion: MATS is a conservative predictor of strain coverage by the 4CMenB vaccine in infants and adolescents.

Abbreviations: CC, clonal complex; ELISA, enzyme-linked immunosorbent assay; fHBP, factor H binding protein; FN, false negatives; FP, false positives; HPA, Health Protection Agency; hSBA, serum bactericidal antibody assay with human complement; IMD, invasive meningococcal disease; MATS, Meningococcal Antigen Typing System; MenB, capsular group B meningococcus; MLST, multilocus sequence typing; NadA, Neisserial adhesin A; NHBA, Neisserial heparin binding antigen; OMV, outer membrane vesicle; PorA, porin A; PBT, positive bactericidal threshold; RP, relative potency; SBA, serum bactericidal antibody; ST, sequence type; TN, true negatives; TP, true positives.

☆ Portions of the data have been presented at the Meningitis Research Foundation conference: Meningitis & septicaemia in children & adults 2011, November 8–9, 2011, London, UK and the XVIIIth International Pathogenic Neisseria Conference (IPNC) 2012, September 9–14, 2012, Würzburg, Germany.

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0264-410X
http://dx.doi.org/10.1016/j.vaccine.2013.08.006

1. Introduction

Neisseria meningitidis capsular groups A, B, and C are responsible for 90% of invasive meningococcal disease (IMD) [1], with the remainder accounted for chiefly by groups Y, W, and X [2]. Although effective vaccines against capsular groups A, C, W, and Y are available, the structural similarity of the capsular group B polysaccharide to that of a constitutively expressed human fetal molecule has precluded the development of polysaccharide vaccines against capsular group B meningococci (MenB) [3]. A genome-based approach, reverse vaccinology, was applied to develop a recombinant protein-based vaccine named 4CMenB, recently approved for use by the European Medicines Agency [4]. 4CMenB contains 4 main components: Neisserial adhesin A (NadA) [5,6], Neisserial heparin binding antigen (NHBA) [7], factor H binding protein (fHbp) [8,9], and porin A (PorA, presented as part of an outer membrane vesicle [OMV] derived from strain NZ98/254) [10,11].

Serologic markers have been used to determine efficacy of glycoconjugate vaccines against IMD, which were licensed based on immunogenicity and safety data in the absence of formal efficacy studies [12]. The serum bactericidal antibody (SBA, or, when using human complement, hSBA) assay is an accepted surrogate of protection [13] used to determine the ability of serum antibody to lyse a specific meningococcal strain. Because of diversity in sequence and expression level of the surface proteins used in 4CMenB, determining vaccine strain coverage could require performing the hSBA against many diverse *N. meningitidis* strains endemic to each geographic region [14]. This approach is labor intensive, time consuming, and ethically unfeasible in infants, owing to the limited volume of serum that can be collected.

To address this issue, the Meningococcal Antigen Typing System (MATS) [15] combines conventional genotyping for PorA with a specialized sandwich enzyme-linked immunosorbent assay (ELISA) that quantifies the relative expression and cross-reactivity of antigenic variants by vaccine-induced antibody. The MATS ELISA readout is a relative potency (RP) against fHbp, NadA, and NHBA on individual strains. To mitigate the need to perform hSBA on large panels of isolates, the MATS method established the positive bactericidal threshold (PBT), a minimum level of RP for fHbp, NadA, and NHBA, which predicts whether a given MenB isolate would be susceptible to killing in the hSBA assay by 4CMenB-induced antibodies [15]. PBTs were derived by comparing MATS RPs and hSBA titers from pooled postvaccination sera against a panel of 57 MenB disease-causing isolates, covering a broad range of non-endemic multilocus sequence typing (MLST) and antigen genotypes from varying geographic regions [16].

Experimental validation of PBT predictions on disease strains relevant for a specific epidemiology is crucial to confirm that the relationship between MATS and hSBA can be used to assess 4CMenB strain coverage, defined as the proportion of IMD-causing isolates susceptible to killing in the hSBA by postvaccination sera. The major factor limiting extensive validation of PBT predictions is the practicality of performing large numbers of hSBA assays using sera from individuals immunized with 4CMenB. We developed an approach for selecting a sufficient number of representative MenB clinical isolates which allows experimental validation of PBT coverage predictions by comparing MATS and hSBA results from pooled sera on an epidemiologically relevant collection of disease-causing isolates independent of the strain panel used originally to derive the PBT.

In a recently published study [17], 1460 culture-confirmed IMD isolates identified from 7 European countries were tested in MATS to assess strain coverage of 4CMenB. The meningococcal isolates from England and Wales had the largest representation in that sample, 535 isolates, with a predicted strain coverage of 73% (95% CI, 57–87%) [17]. In this report, we describe a stratified proportional random sampling procedure that has been used to select a representative panel of 40 MenB isolates from the overall panel of 535 England and Wales isolates. The selected isolates were tested to experimentally validate the accuracy of MATS-based predictions of vaccine strain coverage.

2. Materials and methods

2.1. Definition of MATS phenotype

Each of the 4 antigens tested in MATS can score positive ("+"; RP > PBT or PorA = 1.4) or negative ("−"; RP ≤ PBT or PorA ≠ 1.4). Combining 2 possible states ("+" and "−") for 4 antigens, we obtain $2^4 = 16$ MATS phenotypes (Table S1). For example, an isolate scoring positive for fHbp (RP > PBT) and PorA (=1.4), but not for NHBA and NadA, would be assigned the phenotype "fHbp+, NHBA−, NadA−, PorA+". The MATS phenotype "fHbp−, NHBA−, NadA−, PorA−" represents isolates predicted to be not covered by 4CMenB (i.e., MATS−). The other 15 phenotypes represent strains predicted covered by ≥1 antigens (i.e., MATS+).

2.2. Definition of genotypic profiles

Sequence type (ST) and clonal complex (CC) defined by MLST, genotypes for the *fHbp*, *nhba*, *nadA* genes and the *porA* variable region 2 (VR2) were assigned as previously described [17].

For each isolate we concatenated CC: *fHbp* genotype/*nhba* genotype/*nadA* genotype/*porA* VR2 to generate a "complete genotypic profile." We used genotypic properties to substratify isolates within each MATS phenotype stratum. Because the genetic variant of an antigen is relevant to the susceptibility of the strain to killing in the hSBA only if a sufficient amount of the antigen is expressed by the strain, for each strain we defined a genotypic profile by removing the genotype of the antigen/antigens that were MATS negative in the MATS profile for that strain. For example, a strain belonging to the MATS phenotype "fHbp+, NHBA+, NadA−, PorA−" with complete genotypic profile CC32:1.p1/3/1/16 was assigned the genotypic profile CC32:1.p1/3/−/−.

2.3. Description of overall panel of isolates from England and Wales

The 535 MenB isolates collected from England and Wales by the Health Protection Agency from July 2007 to June 2008 have been previously characterized as part of a larger study [17]. Briefly, 50% and 23% of strains scored positive for ≥2 and 1 antigens, respectively, for an overall predicted coverage of 73%. Of these, 64% and 55% of strains were fHbp+ and NHBA+, respectively, and 20% were matched for PorA+. Few strains achieved NadA RP values above the PBT (NadA+, 0.6%) despite 16% of isolates having intact alleles for NadA by gene sequence analysis. Of the 16 possible MATS phenotypes, only 10 were observed (Table S1). The 6 most frequent MATS phenotypes were "fHbp+, NHBA+, NadA−, PorA−" (30%); all antigens negative (27%); "fHbp+, NHBA+, NadA−, PorA+" (16%); fHbp+ only (15%); NHBA+ only (7.9%); and "fHbp+, NHBA−, NadA−, PorA+" (3.2%). Four other MATS phenotypes had negligible frequencies (<1%).

2.4. Stratified proportional sampling to select a subset of 40 strains

We adapted stratified sampling methods [18] to experimentally validate the predictions of vaccine coverage provided by the MATS/PBT approach. For logistical reasons a maximum of 40 strains could be tested in the hSBA assay; the characteristics of interest of the strain population to be sampled were (1) the MATS phenotype

and (2) the strain genotypic profile. Because the genetic variant of an antigen is relevant to the susceptibility of the strain to killing in the hSBA only if a sufficient amount of the antigen is expressed by the strain, we adopted a 2-stage, hierarchical stratification scheme.

As the first stage of stratification, strains were grouped in subsets defined by their MATS phenotype. The number n_i of isolates to be sampled for each of the $i = 1, \ldots, 6$ most frequent MATS phenotypes (strata) was calculated as $n_i = 40/535 \times N_i$, where N_i was the number of strains belonging to the i-th stratum in the overall England and Wales epidemiologic strain panel (Table S1).

Within each stratum defined by MATS phenotype, strains were further grouped into substrata defined by their genotypic profile, considering only the genotypes of antigens positive in MATS for that particular stratum. The number n_{ij} of strains to be sampled in the j-th substratum of the i-th stratum was calculated, as for the primary strata, proportionally to the size of the substrata (Table S2).

Within each of the stratifications defined by these 2 criteria, strains were sampled at random. Finally, 3 isolates belonging to the "fHbp+, NHBA+, NadA−, PorA+" stratum, substratum CC41/44, fHbp 1.4, NHBA 2, NadA−, PorA 1.4 were replaced with 3 equivalent invasive England and Wales isolates from 2009 and 2011 because of the unavailability of the bacterial culture.

2.5. Human serum samples

Healthy human volunteers were immunized under informed consent. The 4CMenB vaccine contained 50 μg each of GNA2091-fHbp, NHBA-GNA1030, and NadA and 25 μg of outer membrane vesicles from the strain NZ98/254 which were adsorbed to aluminum hydroxide. Serum samples before and after immunization were obtained from the following clinical trials.

Study 1 was a phase 2b/3 clinical trial conducted in healthy adolescents, aged 11–17 years [19]. Two pooled sera preparations were derived from 13 subjects (1) before vaccination and (2) 30 days after the second dose of 4CMenB vaccine, administered 2 months after the first dose. In the clinical trial, positive pre-vaccination titers were observed against 4 strains tested. To favor a proper assessment of vaccine effect, the 13 sera used in the present study were randomly selected among the sub-set of subjects whose pre-vaccination sera had shown negative hSBA titers in the clinical trial.

Study 2 was a phase 2b clinical study in which 4CMenB was administered to infants at 2, 4, and 6 months of age or 3, 5, and 7 months of age [20]. Extensions of this clinical study investigated a fourth (booster) dose at 12, 18, or 24 months of age. Pooled sera were derived from (1) 109 subjects at 13 months of age randomly selected among those who received only the routine vaccination (no 4CMenB, control group) and (2) from 69 subjects randomly selected among those who received the primary series of 3 doses of 4CMenB in the first year of life, plus a booster in the second year of life, 30 days after the booster dose was administered.

2.6. Serum bactericidal antibody assay with human sera

Serum bactericidal antibody with human complement assays were performed as previously described [21] with minor modifications. Human plasma obtained from volunteer donors under informed consent was selected for use as complement source with a particular MenB strain only if it did not significantly reduce the number of colony-forming units of that strain when added to the assay at a concentration of 50%. The final assay mixture contained 25% human plasma. The activity of plasma complement was reconstituted by the addition of divalent cations immediately before use.

A strain was considered hSBA+, i.e. experimentally covered by the 4CMenB vaccine, if the postvaccination hSBA titer was ≥1:8 and ≥4-fold higher than the pre-immune titer (adolescents) or the control group (infants) [15].

2.7. Statistical analyses

Statistical analyses were performed using R, version 2.14.0 (www.r-project.org). Equivalence of genotypic and phenotypic properties in the two panels ($n = 535$ isolates; $n = 40$ isolates) was verified with proportion tests (R prop.test routine). Equivalence of the medians for MATS RP distributions in the two panels was tested for each antigen with the Wilcoxon test [22].

MATS-based predictions of 4CMenB strain coverage were compared with hSBA results from the infant post-fourth-dose and adolescent post-second-dose pools by classifying the 40 strains tested with both assays into 2 × 2 contingency tables. Using MATS +/− as predictor and hSBA +/− as outcome we defined true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). Using values in the contingency tables, we calculated positive predictive value [TP/(TP + FP)], negative predictive value [TN/(TN + FN)], and accuracy [(TP + TN)/(TP + FP + TN + FN)] [23] and tested the statistical significance of the MATS–hSBA association with the Fisher exact test [24].

3. Results

3.1. Stratified proportional sampling identifies an unbiased and representative 40-strain sample of the original panel of strains

No significant bias was detected between the 40-strain subset and the overall 535-strain panel comparing the frequency distributions of the MATS antigen phenotypes ($P = 0.999$; Fig. 1A), the distribution of MLST genotypes ($P = 0.972$; Fig. 1B), fHbp genotypes ($P = 0.576$; Fig. 1C), and NHBA genotypes ($P = 0.619$; Fig. 1D). Comparison of the MATS RP distributions for each antigen (fHbp, NHBA, and NadA) also showed no bias between the 2 strain panels (Wilcoxon text $P = 0.828$, 0.685, and 0.413 for fHbp, NHBA and NadA, respectively; Fig. 2).

Vogel et al. [17] reported that MATS predicts 73% (95% CI, 57–87%) strain coverage of 4CMenB on the overall England and Wales panel. The 4CMenB strain coverage predicted by MATS for the selected 40-strain subset is 70% (95% CI, 55–85%), consistent with coverage prediction on the overall England and Wales panel (test for equality of 2 proportions, $P = 0.830$).

No significant difference was observed between the 2 panels for the empirical frequencies of MATS strata and genetic substrata ($P > 0.7$ for all; Table 1), indicating that the selected sample of 40 strains is representative of the overall 535 strain panel.

3.2. MATS predicts conservatively killing in the hSBA

Prevaccination (adolescents) or control (infants) hSBA titers against the 40 strains tested were negative (<1:4), with the exception of 2 strains that had high prevaccination responses against the adolescent sera (>1:32) with similar postvaccination titers (1:64) and were therefore considered hSBA− in this age group (<4-fold increase over prevaccination). The same strains had negative prevaccination titers against infant sera (<1:4): 1 of 2 had a titer of 1:8 with post-fourth-dose infant sera and was considered hSBA+, the other had 1:4 and was considered hSBA−. High and low prevaccination titers with adolescent and infant serum, respectively, suggest previous carriage of similar strains by some of the adolescents composing the pool.

All of the other strains with negative/low prevaccination hSBA titers had postvaccination titers either negative and classified as hSBA− (4 and 3 strains with infant and adolescent sera, respectively), or positive (≥1:8 and ≥4-fold higher than the respective

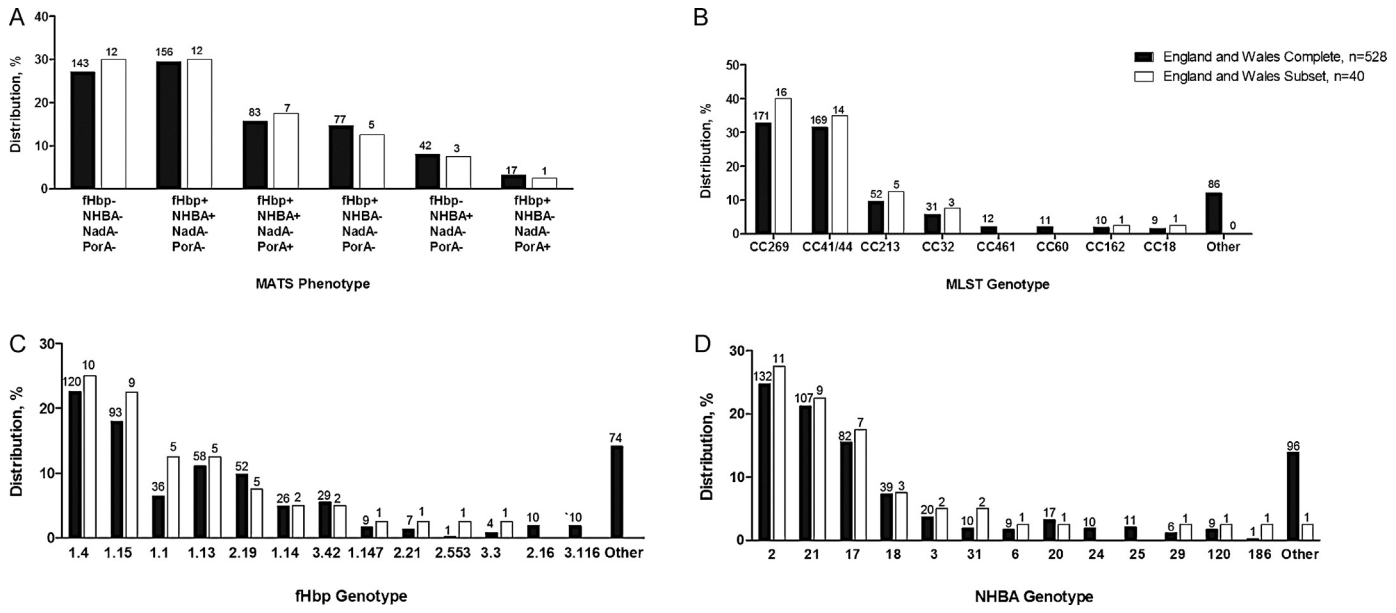

Fig. 1. Comparisons between distributions of (A) MATS antigen phenotypes, (B) MLST genotypes, (C) fHbp genotypes, and (D) NHBA genotypes in the England and Wales complete (black) and subset (white) strain panels. fHbp = factor H binding protein; MATS = meningococcal antigen typing system; MLST = multilocus sequence typing; NHBA = Neisserial heparin binding antigen.

prevaccination titer) and classified as hSBA+ (34 and 35 strains with infant and adolescent sera, respectively).

Overall, for both age groups, 35 of the 40 strains were classified as hSBA+, for a measured strain coverage of 88% (95% CI, 72–95%) (Fig. 3). hSBA responses were highly concordant between the 2 age groups, with all but 2 strains being positive or negative with both infant and adolescent sera.

MATS-based predictions of coverage were largely confirmed by hSBA results, with 27 TPs and 4 TNs (Table 2). One strain belonging to the "fHbp+, NHBA−,NadA−, PorA−" stratum, CC18-fHbp1.147 substratum was predicted positive by MATS but not confirmed by hSBA in either age group (1 FP). Eight strains predicted negative by MATS, belonging to the CC269, CC41/44 or CC213, tested positive in hSBA (8 FNs).

As a predictor of pooled hSBA, MATS had a 96% positive predictive value, a 33% negative predictive value, and an overall accuracy of 78%, indicating that in this sample MATS underestimated strain coverage. Despite the underestimation observed, the association between the 2 assays was statistically significant (Fisher exact test, $P = 0.022$). MATS-predicted coverage was consistently lower than coverage measured by hSBA using pooled sera from infants and adolescents, but the difference was not statistically significant ($P = 0.10$).

Overall, results confirmed that, for infants and adolescents, MATS is an accurate and conservative predictor of strain susceptibility to killing in the hSBA.

4. Discussion

Initial comparisons of the relationship between MATS and SBA relied on results from hSBA analysis of 57 MenB isolates using pooled sera from infants who had received 4 immunizations with 4CMenB [15]. Because the chosen isolates were not representative of IMD in any specific epidemiologic setting, confirmation of the relationship between MATS and hSBA assay using isolates that are representative of those endemic to a particular geographic area during a defined period was necessary.

Using a stratified proportional sampling method, we were able to select a sample of 40 MenB isolates epidemiologically representative of IMD in England and Wales for the years 2007–2008. MATS predicted 70% (95% CI, 55–85) coverage for this panel, and hSBA from pooled infant and adolescent sera measured 88% (95% CI, 72–95) coverage based on the accepted surrogate of protection. Sixty-six percent of the strains predicted not covered by MATS were killed in the hSBA assay (FNs) possibly owing to synergy of antibody raised against multiple antigens, each of which was below the PBT.

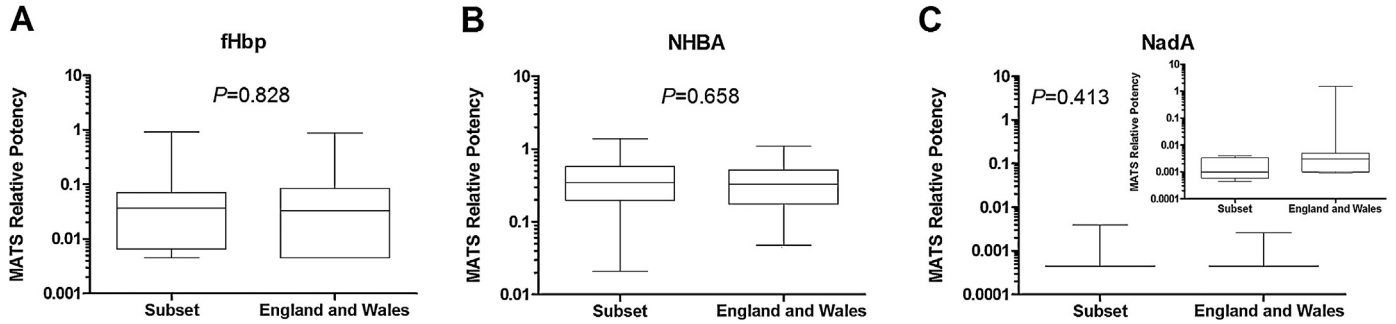

Fig. 2. Comparison of MATS relative potencies in the England and Wales complete and subset strain panels. (A) fHbp, (B) NHBA, (C) NadA (all strains); Insert: NadA positive strains. fHbp = factor H binding protein; MATS = meningococcal antigen typing system; NadA = Neisserial adhesin A.

Table 1
Comparison of MLST/genotype distribution in overall panel and selected subpanel.

Strata (MATS phenotype)	Substrata (genotypic profile)	England and Wales panel (*n*, % of stratum)	40 strains subpanel (*n*, % of stratum)
fHbp−, NHBA−, NadA−, PorA−	CC269:−/−/−/−	48 (33.3)	5 (41.7)
E&W full set = 144 strains	CC213:−/−/−/−	43 (29.9)	5 (41.7)
E&W subset = 12 strains	CC41/44:−/−/−/−	11 (7.6)	2 (16.6)
	Other profiles (11)	42 (29.2)	0 (0.0)
fHbp+, NHBA+, NadA−, PorA−	CC269: 1.p15/21/−/−	74 (46.3)	7 (58.4)
E&W full set = 160 strains	CC41/44: 1.p4/2/−/−	35 (21.9)	3 (25.0)
E&W subset = 12 strains	CC32: 1.p1/3/−/−	15 (9.4)	1 (8.3)
	CC41/44: 1.p14/2/−/−	6 (3.8)	1 (8.3)
	Other profiles (27)	30 (18.8)	0 (0.0)
fHbp+, NHBA+, NadA−, PorA+	CC41/44: 1.p4/2−/+	62 (75.0)	6 (85.7)
E&W full set = 84 strains	CC41/44: 1.p14/2−/+	12 (14.3)	1 (14.3)
E&W subset = 7 strains	Other profiles (9)	9 (10.7)	0 (0.0)
fHbp+, NHBA−, NadA−, PorA−	CC269: 1.p13/−/−/−	14 (17.9)	1 (20.0)
E&W full set = 78 strains	CC32: 1.p1/−/−/−	14 (17.9)	2 (40.0)
E&W subset = 5 strains	CC269: 1.p15/−/−/−	10 (12.8)	1 (20.0)
	CC18: 1.p147/−/−/−	8 (10.3)	1 (20.0)
	Other profiles (21)	32 (41.0)	0 (0.0)
fHbp−, NHBA+, NadA−, PorA−	CC269:−/21/−/−	8 (19.0)	1 (33.3)
E&W full set = 42 strains	CC162:−/20/−/−	5 (11.9)	1 (33.3)
E&W subset = 3 strains	CC269:−/17/−/−	5 (11.9)	1 (33.4)
	Other profiles (17)	24 (57.1)	0 (0.0)
fHbp+, NHBA−, NadA−, PorA+	CC41/44: 1.p4/−/−/+	13 (75.5)	1 (100)
E&W full set = 17 strains	Other profiles (3)	4 (23.5)	0 (0.0)
E&W subset = 1 strain			

fHbp = factor H binding protein; MATS = meningococcal antigen typing system; MLST = multilocus sequence typing; NadA = Neisserial adhesin A; NHBA = Neisserial heparin binding antigen; PorA = porin A.

Only 1 of the 28 strains predicted positive by MATS was resistant to killing in the hSBA assay for a proportion of FPs lower than in the 57-strain panel (3% vs. 11%, respectively). This difference was not surprising because the proportion of strains predicted covered by ≥2 antigens was higher in the England and Wales panel than in the 57-strain panel (50% vs. 35%) and the rate of FPs has been shown to be lower in such strains [15]. The high proportion of strains positive in MATS for ≥2 antigens might also account for the similar killing in hSBA observed for infants and adolescents, whereas previous studies reported lower hSBA coverage in infants compared with adolescents [15]. Overall, these effects might explain the smaller value of strain coverage predicted by MATS compared with pooled hSBA results and validate the rationale for a multicomponent vaccine.

Comparison of MATS predictions with hSBA results for each strain confirmed a statistically significant association between the 2 assays with good accuracy and positive predictive power demonstrating that, as recently suggested, MATS could be

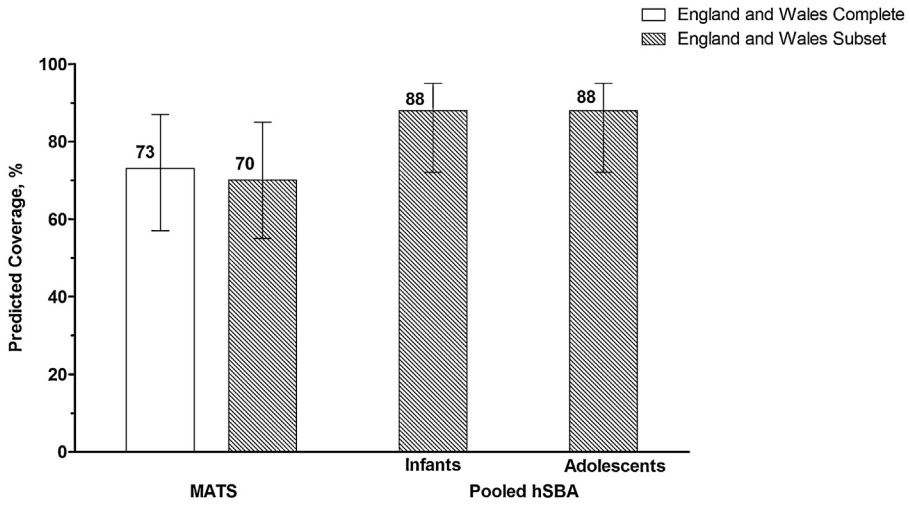

Fig. 3. MATS yields conservative predictions of 4CMenB strain coverage as measured by pooled hSBA with infant and adolescent sera. MATS coverage intervals were estimated according to the MATS interlaboratory standardization study [33] using the PBTs and a log-normal approximation based on overall assay reproducibility. hSBA CIs were constructed on the basis of the binomial approximation (Clopper–Pearson method). Results of the hSBA assay were tested with pooled sera. For infants the endpoint was post dose 4; for adolescents the endpoint was post dose 2. fHbp = factor H binding protein; hSBA = serum bactericidal antibody assay with human complement; MATS = meningococcal antigen typing system; NadA = Neisserial adhesin A; NHBA = Neisserial heparin binding antigen; PBT = positive bactericidal threshold; SBA = serum bactericidal antibody.

Table 2
Contingency table for MATS coverage vs. hSBA coverage comparison.

MATS	hSBA[a]	
	Positive	Negative
Positive	27	1
Negative	8	4

hSBA, serum bactericidal antibody assay with human complement; MATS, meningococcal antigen typing system; PBT, positive bactericidal threshold; PorA, porin A; RP, relative potency.

[a] Pooled sera from infant vaccinees post fourth dose and adolescent vaccinees post second dose of the 4CMenB vaccine (same results for both age groups). MATS positive: RP > PBT for one or more antigens and/or PorA = P1.4. hSBA positive: post-immune titer ≥1:8 and ≥4-fold increase over preimmune.

used as a relevant tool for post-implementation surveillance [25].

The complete set of 535 England and Wales strains from which the 40-strain sample was selected is currently the largest panel of invasive MenB strains isolated in a single country typed in MATS [17,26–32]. The size of the panel and the similar predictions of vaccine strain coverage in other epidemiologic panels studied support the relevance of the results obtained in this study to the MATS-based predictions of 4CMenB strain coverage in other countries.

Lacking in these studies are hSBA data using sera from individuals; an impractical undertaking, especially among infants from whom limited sample quantities are available. To complement the results seen here overcoming this limitation, the general relationship between pooled and individual sera from different age groups may be investigated. Also, additional studies may be conducted to determine the fewest number of isolates necessary to retain the overall diversity in an epidemiologic panel of clinical isolates.

In summary, results of the present study show that MATS provides accurate and conservative estimates of strain coverage by the 4CMenB vaccine for a strain panel representative of IMD in a specific geographic setting and epidemiologic year. This further illustrates the value of the MATS approach, which may be tailored for use with other pathogens with the potential of providing additional epidemiologic and efficacy data geared toward optimizing treatment.

Acknowledgments

We thank Philip E. Boucher for critical reading of the manuscript, and Gerard Johnson, PhD, of Complete Healthcare Communications, Inc. (Chadds Ford, PA, USA) for the precious editorial support.

Author contributions: GF and AB participated in the conception and design of the study, and the acquisition of data. MLS,SG,JL, JF, and RB participated in the acquisition of data. MP, MMG, and DM participated in the conception and design of the study. All authors participated in the analysis and interpretation of data and in the drafting and revision of the manuscript and have approved the manuscript as submitted.

Conflict of interest statement: GF and MLS were employed by Novartis Vaccines and Diagnostics at the time of the study; AB, FR, MP, MMG and DM are currently employed by Novartis Vaccines and Diagnostics. JF, JL, RB and SG have performed contract research on behalf of the Health Protection Agency, now Public Health England, for Novartis Vaccines and Diagnostics. JL was undertaking a PhD sponsored by Novartis Vaccines and Diagnostics at the time of the study.

Funding: This work was supported by Novartis Vaccines. Novartis Vaccines was involved in the conception and design of the study, acquisition, analysis, and interpretation of data, and drafting, revision, and final approval of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.08.006.

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