**A qualitative and quantitative assessmentof meningococcal serogroup B strain coverage of the multicomponent 4CMenB vaccine with corresponding regional distribution and clinical characteristics: England, Wales and Northern Ireland, 2007/08 and 2014/15**

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**Abstract**

**Background:** The UK introduced 4CMenB (Bexsero®), a multicomponent vaccine against serogroup B meningococcal (MenB) disease, into the national infant immunisation programmein September 2015. In 2007/08, the Meningococcal Antigen Typing System (MATS) estimated 73% (391/535) of invasive MenB isolates in England and Wales would be covered by 4CMenBThis study aimed to (i) repeat the survey for invasive MenB isolates during 2014/15, prior to 4CMenB introduction, (ii) compare strain coverage between 2007/08 and 2014/15, and (iii) investigate relationships between MATS coverage, age, region and disease outcomes.

**Methods:** InvasiveMenB isolates from cases in England, Wales and Northern Ireland during 2014/15 (n=251) were assayed using MATS and compared with 2007/08 data (n=535). MATS coverage was evaluated by geographical region and age-group. Clinical characteristics, risk factors and outcomes were assessed according to MATS coverage for 2014/15 English cases (n=231).

**Findings:** MATS coverage in 2014/15 was 65.7% (165/251; 95% CI, 52-80%) compared to 73.1% (391/535; 95% CI, 57-87%) in 2007/08. The proportion of MATS-positive isolates with one vaccine antigen increased from 22.8% (122/535) to 31.1% (78/251), while the proportion with >1 antigen fell from 50.3% (269/535) to 35% (87/251). This reflected changes in circulating strains, particularly the ST-269 clonal complex strains. MATS coverage increased with age, varied by region and was associated with more severe disease.

**Interpretation:** In 2014/15, two-thirds of MenB isolates were predicted to be covered by 4CMenB. Temporal changes in MATS coverage underscore the need for continued monitoring of antigen expression and diversity, especially in countries with 4CMenB programmes.

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# Introduction

In September 2015, the United Kingdom introduced a novel multi-component, vaccine (4CMenB; Bexsero®) against serogroup B meningococcal disease (MenB) into the nationally-funded, infant immunisation programme.1 Like in most European countries, MenB is the main serogroup responsible for invasive meningococcal disease (IMD) in the UK, with the highest incidence in infants.1 Currently-licensed glycoconjugate vaccines that target the capsular polysaccharides of serogroups A, C, W and Y meningococci do not provide any cross-protection against other meningococcal serogroups.2 The development of polysaccharide-based vaccines against MenB has been hampered because of structural similarities to foetal neural tissue, rendering the polysaccharide poorly immunogenic.2

Relatively conserved and cross-protective MenB surface proteins were identified by reverse vaccinology, and three recombinant proteins (factor H-binding protein [fHbp] variant 1.1, Neisserial heparin-binding antigen [NHBA] peptide 2, and Neisserial adhesin A [NadA] variant 3) were included in 4CMenB, along with PorA P1.4-containing outer membrane vesicles (OMV) from the New Zealand outbreak strain. Unlike capsular polysaccharides which tend to be abundantly expressed and antigenically relatively uniform in invasive meningococci, surface proteins may be sparse and antigenically diverse. Poorly-expressed surface proteins and antigenic variability resulting from different mechanisms, such as mutation or recombination,3 may result in the binding of insufficient antibodies to promote complement-mediated lysis. Therefore, the ability of 4CMenB to protect against MenB strains and the breadth of protection depends on the degree of surface expression and the extent to which vaccine-induced antibodies recognise and bind to these proteins.4

The Meningococcal Antigen Typing System (MATS) is a qualitative and quantitative ELISA that quantifies fHbp, NHBA and NadA expression in combination with the ability of 4CMenB-induced antibodies to recognise these proteins on individual meningococcal isolate.5 For an isolate to be considered vaccine-preventable (MATS-positive), the Relative Potency (RP) of ≥1 protein must be greater than the respective positive bactericidal thresholds (PBT), which were assigned on the basis of killing using post-vaccination pooled sera from infants after their 12-month booster, or the isolate must possess homologous PorA (P1.4).4

The first application of MATS to a large-scale epidemiological survey involved 1,052 MenB isolates from five European countries during 2007/08.6 The study found that all MenB isolates possessed at least one gene encoding a major vaccine antigen. MATS predicted that 78% of MenB isolates from patients across Europe (including 73% in England and Wales) would be killed by post-vaccination sera. Since this survey is now almost a decade old, we conducted a MATS survey of isolates from patients with invasive MenB disease in England, Wales and Northern Ireland during 2014/15, the last epidemiological year (01July to 30 June) prior to 4CMenB introduction. MATS coverage and regional distribution were then compared with the corresponding 2007/08 data. We also assessed age distribution, clinical characteristics and outcomes of IMD in patients with MATS-positive and MATS-negative MenB isolates.

**Methods**

MenB isolates

Public Health England (PHE) conducts enhanced national IMD surveillance and provides a national reference service for IMD confirmation and characterisation of invasive meningococci (both culture and non-culture).7 Confirmed cases in England are routinely followed-up with a short questionnaire sent to the patient’s general practitioner, requesting information on comorbidities, clinical presentation, intensive care admission and outcomes. Invasive MenB isolates from patients in England, Wales and Northern Ireland, diagnosed during 2014/15 (n=251) were subjected to MATS-testing and the results were compared to published data from 2007/08 (n=535).6

Sequencing and multilocus sequence typing (MLST)

Isolates were genotypically characterised by MLST and each of the four main 4CMenB antigens. Genotypic characterisation of 2007/08 isolates was performed using Sanger sequence analysis.6 Genotypic characterisation of 2014/15 isolates was performed using the Illumina platform,7 and data extracted from the Meningitis Research Foundation Meningococcus Genome Library (<http://www.meningitis.org/genome-library>), which contains genome sequences for all English, Welsh and Northern Irish invasive isolates received by the PHE Meningococcal Reference Unit (MRU) since July 2010 and is populated on an on-going basis.8

MATS

MATS data were generated for both epidemiological years using the same methodology, as described previously.4,9,10 PorA subtypes were determined by phenotyping7 and genome sequencing.11 Predicted strain coverage is defined as the proportion of MenB isolates with MATS relative potency greater than the PBT for ≥1 antigen or the presence of PorA P1.4.

Estimating 2007/2008 and 2014/2015 strain coverage

For 2007/08 isolates, log-normal approximation estimates of the 95% CIs were calculated for all PBTs and were based on overall assay reproducibility (0.014-0.031 for fHbp, 0.169-0.511 for NHBA and 0.004-0.019 for NadA) as described in the MATS laboratory standardisation study.9 The new rabbit sera used to manufacture fhbp MATS plates used in the 2014/15 analysis, required reassignment of a new PBT, as they showed a lower affinity for genetically distant and middle/low fHbp expressing strains. New specifications were set using previously described standardisation methods.9 For 2014/15 isolates, fHbp PBT was 0.012 (0.008-0.018) compared to 0.021 in 2007/2008, while NHBA and NadA PBTs did not change.12 The fHbp and NHBA peptides were stratified by their RPs and plotted against the PBTs (fHbp 2007/08:0.021; fHbp 2014/15:0.012; NHBA: 0.294; NadA: 0.009) in MATS, with 95% CIs. Isolates with RP<Lower Limit of Quantification (LLOQ) were assigned half of the LLOQ (0.00045 for 2007/08 fHbp isolates; 0.002 for 2014/15 fHbp isolates) and those with peptide frequencies of <5 (either dataset) were not included.

Statistical Analysis

Data were analysed using Stata SE v.13.1 (Statcorp, TX) and are mainly descriptive. Data that did not follow a normal distribution are described as medians with interquartile ranges, and compared using the Mann Whitney U test. Proportions were compared using chi-squared or Fisher’s exact test, as appropriate. The 95% CI for MATS coverage were based on overall assay reproducibility using a log-normal scale to estimate 95% CI limits around the PBT for the different antigens, and then calculating the proportion of RPs falling within the upper and lower limitsThe same analysis was performed for both epidemiological years. Logistic regression was used to assess any association between adverse outcomes (intensive care admission/death) and MATS positivity (yes/no) after adjusting for age (<1y/1-2y/3-4y/≥5y), underlying comorbidity (present/absent), and clinical presentation (meningitis/septicaemia/both/other).

Role of the funding source

The study was jointly funded by GlaxoSmithKline Vaccines and Public Health England. The authors had sole responsibility for the study design, data collection, data analysis, data interpretation, and writing of the report. Public Health England is an executive agency of the Department of Health. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

In 2014/15, 764 IMD cases were confirmed in England, Wales and Northern Ireland, including 440 (57.6%) MenB cases, of which 251 (57.0%) were culture-confirmed. This compares with 1,289, 1,123 (87.1%) and 535 (47.6%) cases, respectively, in 2007/08. In 2014/15, 38% of isolates had at least one protein homologous to a vaccine antigen compared to 36% in 2007/08.

Overall MATS coverage

MATS coverage of all MenB isolates in 2014/15 was 65.7% (165/251 isolates; 95% CI, 52-80%) compared to 73.1% (391/535; 95%CI, 57-87%) in 2007/08 (Table 1). For both years, MATS-positivity was most frequently due to coverage by fHbp alone and in combination with NHBA. None of the MATS-positive isolates in either year were covered by all four vaccine antigens.

The proportion of isolates covered by >1 vaccine antigen fell from 50.3% to 34.7%, whilst the proportion covered by one antigen increased from 22.8% to 31.1%. This was mainly due to a shift in the proportion of isolates covered by both NHBA and fHbp in 2007/08 to a higher frequency of isolates covered by fHbp only in 2014/15.

Clonal complexes

The clonal complex cc269, cc41/44 and cc213 accounted for more than two-thirds of MenB isolates for both years (Table 2). The proportion of cc269 isolates decreased by 9% from 32.9% in 2007/08 to 23.9% in 2014/15 (**Table 2**). MATS coverage for cc269 also decreased (72.7% to 53.3%), mainly through loss of coverage by NHBA both individually and in combination with fHbp (**Table 4** cc41/44 accounted for around a third of isolates and retained very high MATS coverage in both years (93.5% and 93.9%, respectively) (**Figure 1; Appendix, page 1**). cc32 representation increased by over 3%, but MATS coverage for this clonal complex declined by 6.9%. MATS coverage for cc213 increased by 5.8% (17.3% to 23.1%) because of a proportional increase in isolates possessing protective fHbp variant 1.

cc269 and cc-unassigned isolates

cc269 is composed of two major clusters, centred around ST-269 and ST-275, respectively. A large proportion of cc-unassigned isolates share ≥4 MLST loci with ST-275 but <4 with the founder ST, ST-269, and are thus unassigned to cc269. In the 2007/08 analysis, these isolates (n=6) formed part of the unassigned group. If these isolates had been included in cc269/ST-275 cluster in 2007/08 this would have increased the proportion of the ST-275 cluster from 39.2% (69/176) to 41.2% (75/182) of overall cc269 isolates. In 2014/15, the proportion would have increased from 56.4% (35/62) to 59.7% (40/67). ST-269 cluster isolates typically have NHBA peptide 21 which is more likely to have an RP > PBT, while ST-275 cluster isolates harbour NHBA peptide 17 which is less likely to have an RP >PBT. Compared to 2007/08, the proportion of isolates with NHBA peptide 21 was lower in 2014/15, while those with NHBA peptide 17 remained stable (**Figure 3**). NHBA peptide 21 coverage decreased by 49.1% between the two years (85.8% [113/535]in 2007/08 to 36.7% [11/30] in 2014/15).Similar trends were observed with fHbp peptide sub-variants 1.15 and 1.13 (**Appendix, page 3**), which are associated with the ST-269 and ST-275 clusters, respectively. These changes resulted in a decrease in MATS-coverage for cc269 in 2014/15.

The contribution of individual vaccine antigens

In 2014/15, 39.4% of isolates (99/251) had ≥1 protein homologous to a vaccine antigen compared to 35.3% (189/535) in 2007/08. Coverage by each individual vaccine antigen declined between the two years except for NadA, which increased (0.56% to 1.99%) (Figure2).

fHbp

In 2007/08, the *fHbp* gene was absent in only 1/535 isolates (0.19%), while two others (0.37%) had frame-shift mutations. There were no deletions or frameshifts among 2014/15 isolates. The overall distribution of fHbp variants remained the same, with variant 1 being the most prevalent (66% and 68%; Appendix, page 3). MATS coverage by fHbp decreased between the two years, from 62.6% (333/532) to 59.0% (148/251). There was a 2% increase in the proportion of isolates with the vaccine-homologous fHbp sub-variant 1.1. None of the variant 2 or 3 isolates were above the fHbp PBT for either year.

**NadA**

In 2007/08, 18% of MenB isolates (97/535) possessed *nadA* alleles including all cc11 (n=6), cc32 (n=31) and cc1157 (n=1) isolates and a proportion of cc213 (47/52, 90.4%), cc269 (4/176, 2.3%), cc364 (1/2, 50%), cc41/44 (1/169, 0.6%) and unassigned (6/35, 17.1%) isolates. Of the 97 *nadA+* isolates, 35 possessed alleles encoding intact peptide sub-variants that would potentially be recognised by 4CMenB induced antibody (NadA-1 and NadA-2/3). However, only three (0.56% overall, all cc11) were above the NadA PBT.

In 2014/15, 21.5% of MenB isolates (54/251) possessed *nadA* alleles, including all three cc1157 isolates, and a proportion of cc32 (22/23, 95.7%), cc213 (23/26, 88.5%), cc269 (1/60, 1.7%), and cc-unassigned (5/22, 22.7%) isolates. Half of the *nadA* (27/54) alleles encoded intact, peptide subvariants that would potentially be recognised by 4CMenB induced antibody, but only six isolates (2.4% overall, four cc32 and two cc-unassigned) were above the NadA PBT.

**PorA**

The PorA P1.4 subtype was identified in 20.0% (107/535) and 15.5% (39/251) of isolates in 2007/08 and 2014/15, respectively. This difference was primarily due to a lower prevalence of P1.4 among cc41/44 isolates (**Appendix, page 1**). Among the remaining isolates, PorA variable region 2 (VR2) was highly diverse in both years, with 49 distinct variants belonging to 15 families in 2007/08 and ten families in 2014/15. VR2 was deleted in one isolate (belonging to cc41/44) in 2007/2008.

NHBA

The vaccine-homologous NHBA peptide 2 was harboured by a quarter of MenB isolates for both years (Figure 3), but MATS-coverage was 3% lower (97% to 94%). Three other peptides (21, 17 and 18) accounted for 41.6% (223/535) of the remaining NHBA peptides in 2007/08 and 36.6% (92/251) in 2014/15. The lower MATS coverage by NHBA in 2014/15 was due to small reductions across several peptides, most notably, peptide 21.

Relative potency frequency and distribution

In 2007/08, 93 isolates (17.4%) had fHbp RP values ≤ LLOQ compared to 21 isolates (8.4%) in 2014/15. Overall, the distribution of fHbp variant 1 was similar between the two years, with most having RPs above the PBT (Figure 3). NHBA peptides, on the other hand, were more variable, with a high proportion of isolates falling within or below the 95% CI boundaries.

Age distribution

In 2007/08, MATS coverage increased with age, although the differences were not statistically significant (Table 3). The opposite was true for 2014/15 with the exception of those aged >5 years, where MATS coverage was the highest at 69.6%. When compared to 2007/08, MATS coverage and the number of antigens coverage is afforded by in 2014/15 were lower across the age-groups. In particular, 37.1% (24/70) of infant MenB isolates in 2014/15 were MATS-negative and a further 30.0% (26/70) were only covered by one vaccine antigen.

Regional Distribution

MATS coverage varied by region between 52-79% in 2007/08 and 48-80% in 2014/15. MATS coverage fell in 8/11 regions, with significant declines in the West Midlands and Yorkshire and Humber (Appendix, page 4). The increase in MATS-coverage in two regions was not statistically significant.

MATS Coverage and clinical characteristics

In 2014/15, 231/251 cases (92.0%) were from England. MATS positivity was associated with a lower prevalence of underlying comorbidities (P=0.002) and higher rates of intensive care admission and death, although these were not statistically significant (Appendix, page 5). In a logistic regression model, MATS positivity was associated with a 1.95-fold increased risk (95% CI, 1.02-3.76; p=0.017) of severe IMD (ICU admission and/or death), independent of age, gender, underlying co-morbidity or clinical presentation.

Discussion

This study provides the pre-vaccine baseline MATS coverage for MenB in England, Wales and Northern Ireland prior to 4CMenB (Bexsero®) introduction. MenB cases more than halved between 2007/08 and 2014/15, whilst MATS coverage declined from 73.1% to 65.7%, and the proportion of isolates covered by >1 antigen fell from 50.3% to 34.7%. In infants, the age-group targeted for vaccination, a third of isolates were MATS negative and a further third were only covered by one vaccine antigen. We found some evidence of more severe disease, in terms of lower comorbidity prevalence and higher rates of intensive care admissions and death, associated with MATS-positive isolates.

MATS coverage in 2014/15 was lower primarily because cases due to cc269, the most prevalent cc in 2007/08, declined, whilst the proportion of the less well-covered cc269 subpopulation ST-275 cluster increased, resulting in lower coverage attributable to NHBA. This change led to a higher proportion of isolates covered a single antigen (fHbp) only, which potentially could impact the protection offered by 4CMenB. In one study, using pooled infant immune sera, isolates with a single vaccine antigen were killed by strains with ≥2 antigens.4 At the same time, the cross-protection offered by vaccine-induced antibodies is lower in infants compared to older age-groups.4,13-15

MATS, however, provides a conservative estimate of protection offered by 4CMenB when compared to SBA. 5 There is, for example, some evidence of a synergistic effect, whereby antibodies that are not independently bactericidal, can augment the killing effect in SBA assays. 16 Antibodies against minor OMV components may also contribute to the killing.16Additionally, NadA coverage may be higher than predicted by MATS because *nadA* expression is repressed under *in vitro* growth conditions. 17

On the other hand, one third of 4CMenB-vaccinated adolescents in a recent US university outbreak had no bactericidal antibodies against the outbreak strain in an hSBA assay, despite this strain being predicted by MATS to be covered by fHbp and NHBA, although there have been no additional cases since the start of the vaccination campaign.18 Given the uncertainties surrounding both MATS and SBA for predicting clinical protection, the early UK data reporting high vaccine effectiveness and significant reductions in MenB cases among 4CMenB-eligible infants are reassuring.

Previous studies have shown that cc does not reliably predict MATS positivity or the killing in the SBA assay because of the dynamic antigenic diversity and/or expression of strains within ccs.6,21 The two main cc269 clusters, for example, exhibit different genotypic/phenotypic profiles with respect to the genes encoding 4CMenB antigens and MATS coverage.22 Furthermore, the diversification of isolates that cluster around ST-275 away from ST-269 underestimate the scale of both the ST-275 cluster, and the overall cc, making it difficult to accurately predict either cc or ST coverage. Recent genotype-phenotype modelling, however, has shown some promise, with one study estimating 66.1% MATS coverage for MenB isolates in the UK and Ireland during 2010-14.20

We assessed MATS coverage by region and found wide variation, even between neighbouring areas, and over time, similar to a recent Canadian study.23 This is important when interpreting coverage in countries with small numbers of MenB cases, especially if they undertake MATS assessment intermittently. MATS-positive strains were also associated with more severe disease. This is, perhaps, not surprising since the vaccine antigens are important virulence factors immunised infants could, therefore, potentially develop milder disease; this is being monitored after 4CMenB introduction. We did not find any association with clinical presentation, which suggests a more important role for host factors

The limitations of MATS for predicting killing of specific MenB strains are well-described.4-6 By using the same laboratory to perform MATS testing,6 we were able to compare regional and national variations in strain coverage over a seven-year interval, although we cannot comment on any trends between these two time-periods. Since MATS can only be performed on cultured isolates, we cannot predict the impact of 4CMenB on culture-negative, PCR-confirmed IMD cases, although MenB isolates are likely to provide good overall genotypic representation of invasive MenB strains.24

In England, a detailed multi-faceted plan is in place for enhanced IMD surveillance,25 which will provide invaluable data on the usefulness of MATS for monitoring vaccine impact and characterising the meningococci causing IMD in both vaccinated and unvaccinated cohorts.

Contributors

SRP, SNL and RB performed the literature search and wrote the first draft. SRP was responsible for the epidemiological surveillance data; LN, SS, MS, RDP,MG, MM,LS and MP ran and oversaw all MATS assays and collated the outputs for analysis. SRP performed the data analysis and prepared the figures. JL prepared genomic data and contributed to their analyses. SRP, JL, JF, SAC, SJG, RB, LS, MER and SNL contributed to the data interpretation. All authors commented on the drafts of the paper and agreed with the final draft of the manuscript.

**Conflicts of interest:**

 RB, JF, SAC, SS, LN, SJG perform contract research on behalf of Public Health England for GSK, Pfizer & Sanofi Pasteur. JF has also acted on behalf of PHE as a consultant and received travel assistance from GSK and Pfizer. JL reported contract research from GSK, during the conduct of the study.

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***Panel:* Research in context**

Evidence Before This Study

We searched PubMed with the terms “meningococcal B” and any combination of “vaccine”, “coverage”, “MATS” or “Meningococcal Antigen Typing System”. Publication dates and languages were not limited. Initial studies using MATS reported the assay to be reliable and reproducible, providing a conservative estimate of 4CMenB coverage. There is an association between the number of vaccine antigens predicted to be covered by MATS and the probability of being killed by immune serum in the serum bactericidal antibody (SBA) assay. Strains covered by two or more antigens have a 96% probability of being killed compared to ≥80%for strains with one antigen. In a large European survey of >1,000 clinical meningococcal group B (MenB) isolates from the 2007/08 epidemiological year, MATS predicted that 78% (95% CI, 63-90%) of all MenB strains would be killed by post-vaccination sera, with half of all strains and 64% of MATS-positive strains covered by more than one vaccine antigen. Other countries have since reported MATS coverage of their MenB isolates and, in a recent global review, MATS coverage ranged from 66% in Canada to 91% in the United States. We found one study from Canada reporting regional variation and trends in MATS coverage over time. There are no studies assessing MATS coverage with clinical disease,severity or outcome.

Added Value of this study

In England, Wales and Northern Ireland, MATS coverage of MenB isolates from patients with IMD fell from 73.1% in 2007/08 to 65.7% in 2014/15, and coverage by >1 antigen fell from 50.3% to 34.7%. These declines were mainly due to changes in circulating strains, particularly the ST-269 clonal complex. Regional MATS coverage varied between 48%-80% and was lower in 8 of 11 regions in 2014/15 versus 2007/08. Compared to older children and adults, MATS coverage was lower in infants, who are also less likely to benefit from the cross-protective effects of the vaccine. In England, MATS-positive MenB strains were more likely to cause IMD in healthy individuals and more severe disease, in terms of intensive care admission and death, than MATS-negative strains.

Implications of all the evidence

In the year preceding the introduction of 4CMenB, MATS predicted that two-thirds of MenB cases could be prevented by the vaccine. We observed a 7% lower MATS coverage between 2007/08 and 2014/15, and an increase in the proportion of isolates covered by one antigen only. We also assessed regional coverage, changes between two time periods and associations with clinical disease and outcomes. The UK will be the first country to assess the usefulness of MATS for monitoring vaccine impact and to characterise the meningococci causing IMD in both vaccinated and unvaccinated cohorts.

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