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Validation and use of a serum bactericidal antibody assay for *Neisseria meningitidis* serogroup X in a seroprevalence study in Niger, West Africa

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ABSTRACT

Invasive meningococcal disease (IMD) affects approximately 1.2 million people worldwide annually. Prevention of IMD is mostly provided through vaccination; however, no licensed vaccine is currently available to protect against meningococcal serogroup X associated infection. Limited data are available on the natural immunity to *Neisseria meningitidis* serogroup X within the African sub-Saharan meningitis belt.

The objective of the study was to provide an overview of natural immunity to serogroup X within a community in the African meningitis belt prior to the introduction of a pentavalent conjugate vaccine (NmCV-5). Prior to its introduction, a validated assay to assess vaccine efficacy was also required. This study therefore incorporated two objectives: a seroprevalence study to assess natural immunity in serum samples (n = 377) collected from Niger, West Africa in 2012, and the validation of a serogroup X serum bactericidal antibody (SBA) assay.

Seroprevalence data obtained found that natural immunity to *N. meningitidis* serogroup X were present in 52.3% of study participants. The highest putative protective titres (≥8) to serogroup X were seen in age group 5–14 years-old (73.9%) and lowest in ages < 1 year old (0%). The SBA assay was successfully validated for selectivity/specificity, precision/reproducibility, linearity, and stability. This study demonstrated the suitability of the serogroup X SBA assay in clinical trials for future meningococcal conjugate vaccines containing serogroup X polysaccharides.

1. Introduction

There are 1.2 million cases of invasive meningococcal disease (IMD) annually worldwide, resulting in ~ 335,000 deaths [1]. The most common causative serogroups of IMD are A, B, C, W, X and Y [2]. Vaccination prevents IMD worldwide, however, high incidence of IMD occurs within the African meningitis belt, a region which experiences annual seasonal outbreaks and recurring epidemics [3]. IMD here has previously reached 1,000 cases per 100,000, equating to 1% of the population [4]. Although historically serogroup A has been the predominant causative agent of IMD within the meningitis belt, other serogroups such as C, W and X have also been responsible for outbreaks. In 2018, serogroup C was responsible for 30.4% of cases, X: 19.1%, and W: 4.6% [5]. Cases of IMD caused by serogroup X continue to emerge in Africa, and more recently, in Azerbaijan, Kazakhstan, Poland and Turkey [2]. These cases are increasingly concerning as there is no currently licensed polysaccharide-based conjugate vaccine to protect against serogroup X.

Considering this, Serum Institute of India Pvt Ltd., in partnership with PATH, developed a pentavalent conjugate vaccine (NmCV-5) containing capsular polysaccharides of meningococcal serogroups: A, C, W, X and Y, conjugated to a protein carrier, tetanus toxoid.

Research into natural immunity to *N. meningitidis* serogroup X was investigated due to the paucity of previous seroprevalence studies. Using the serogroup X SBA assay, this study therefore assessed the protective immunity of a population in Niger, West Africa, part of the meningitis belt, to serogroup X prior to the introduction of NmCV-5. A protective SBA titre to

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serogroup X, based on previous literature relating to serogroup C, is defined as ≥8, when performing an SBA assay using rabbit complement [6].

The NmCV-5 vaccine completed a phase 1 trial (ClinicalTrials.gov Identifier: NCT02810340) in 2017 which explored its safety and efficacy [7], and a phase 2 trial (ClinicalTrials.gov Identifier: NCT03295318) was completed in 2018 on 376 Malian toddlers aged 12 to 16 months old [8]. The results obtained from the phase 2 trial were encouraging, with 99% of participants exhibiting a seroprotective titre of ≥128 for serogroups A, C, W, Y and X [8]. A further phase 2 trial (ClinicalTrials.gov Identifier: NCT03964012), involving 1800 participants aged 2–29 years, completed in 2021. Any phase 3 trials which aim to assess the immunogenicity of conjugate polysaccharide vaccines containing serogroup X will require a validated serogroup X SBA assay. This study therefore optimised and validated an SBA assay for *N. meningitidis* serogroup X. Following the assay validation, it may then be used in clinical trials to evaluate vaccines containing serogroup X polysaccharides, including the NmCV-5 vaccine.

2. Methods

2.1. Seroprevalence study sample selection

377 anonymised serum samples were selected from the previous MenAfriCar study (ClinicalTrials.gov Identifier: NCT01119482), collected in Niamey and Say, Niger in March 2012. All samples with sufficient residual volume were included. The age ranges for MenAfriCar participants were < 1, 1–4, 5–14, 15–29 and 30 + years old, specific ages were not available for each participant.

2.2. SBA assay validation sample selection

A range of serum samples with positive SBA titres to serogroup X (low, medium, high positive), and negative SBA titres (<4) were selected from the following sources:

- A previous PATH NmCV-5 phase 1 trial (ClinicalTrials.gov Identifier: NCT02810340). Serum samples were collected at the Center for Vaccine Development (CVD), University of Maryland, Baltimore, USA.
- A previous Public Health England (PHE) (now UK Health Security Agency - UKHSA), MenOccy phase 2 trial (ClinicalTrials.gov Identifier: NCT00962624). Serum samples were collected at the UKHSA laboratory, Manchester, UK.
- Non-pooled commercial sera (BioIVT – West Sussex, UK, TCS Biosciences, Buckingham, UK).

Ethical approval for use of serum samples in this study was provided by the following ethics committees: Republic of Niger - Ministere de la Sante Publique, University of Maryland, Baltimore, London School of Hygiene and Tropical Medicine, and the National Research Ethics Service, Wandsworth.

2.3. Serum bactericidal antibody assay

The serogroup X SBA assay was performed at the Vaccine Evaluation Unit, UKHSA, Manchester, UK, using strain BF2/97 with baby rabbit complement as previously published [9]. This method was followed for all assays of the seroprevalence study and the serogroup X SBA assay validation.

2.4. Serogroup X SBA assay validation

The serogroup X SBA assay was validated through evaluation of accuracy, selectivity/specificity, precision/reproducibility, linearity/recovery, and sample stability. Validation procedures performed followed the Food and Drug Administration's Bioanalytical Method Validation – Guidance for Industry [10]. Validation acceptance was defined as ≥ 85% of serum samples meeting individual parameter criteria.

Selectivity/Specificity: Inhibition utilised homologous (X) and heterologous (ACWY) antigens; *N. meningitidis* serogroup X (Serum Institute of India Pvt. Ltd.– Maharashtra, India) and A (13/262), C (07/318), W (01/428) and Y (01/426) polysaccharides (NIBSC – Hertfordshire, UK) at a concentration of 200 μg/mL. Serum samples were assayed neat, and 1:2, with a homologous and heterologous inhibitor on the same run. Specificity was considered acceptable if ≥ 85% of samples undergoing homologous inhibition resulted in a reduction of > 2 SBA titres. In addition, ≥ 85% of samples undergoing heterologous inhibition should not result in a reduction of > 2 SBA titres. Spiking was evaluated by spiking samples, known to be negative for *N. meningitidis* serogroup X antibody but positive to other meningococcal antibodies (e.g., *N. meningitidis* serogroup A, C, W or Y). A sample positive to serogroup X antibody was selected and spiked into six negative samples in three ratios (1:4, 1:16, 1:32) to cover a range of the assay. Spiking was accepted if ≥ 85% of negative samples exhibited a negative result, and ≥ 85% of the spiked samples (x dilution factor) resulted in an SBA titre within ± 1 SBA titre of the neat positive sample result.

Accuracy: There is currently no assigned international serum panel with known SBA titres for serogroup X strain BF2/97, therefore, a replicate spiking assay was conducted to assess accuracy. This was performed by spiking a positive sample in three ratios (1:4, 1:8, 1:16) into five replicates of human serum minus IgA/IgG/IgM (Sigma-Aldrich – Dorset, UK). Accuracy was accepted if ≥ 85% of negative samples exhibited a negative result, and ≥ 85% of the spiked samples (x dilution factor) resulted in an SBA titre within ± 1 SBA titre of the neat positive sample result. Additionally, ≥ 85% of spiked sample results should be within ± 1 SBA titre of the median SBA titre for that spiking ratio.

Precision/Reproducibility: This parameter was evaluated through intra-assay, inter-assay, inter-operator, and lower-end investigations. Five replicates of each sample were prepared and assayed until 5 results were obtained for each sample, except in inter-operator precision/reproducibility, where 3 results were obtained. Furthermore, as part of intra-assay investigations, microtitre plate location was investigated. All parameters were accepted if ≥ 85% of replicates were within 3 SBA titres.

Linearity/Recovery: Linearity and analyte recovery was evaluated by diluting serum samples positive to serogroup X antibody, 1:4 in human serum minus IgA/IgG/IgM. Neat positive and diluted samples were assayed at the appropriate starting dilution on the same run. Linearity was considered acceptable if ≥ 85% of diluted samples (x dilution factor) were within ± 1 SBA titre of the neat positive sample.

Stability: This parameter was assessed through freeze and thaw stability, short-term and long-term stability, and post-preparative stability. In freeze and thaw stability, sample aliquots were frozen for 12 to 24 h at −80 °C (-95 °C to −65 °C) and subjected to 1, 2, 3, and 4 freeze/thaw cycles and assayed on one run. For short-term stability, two sample aliquots of each sample were re-frozen for 12 to 24 h at −80 °C (-95 °C to −65 °C). One aliquot was then removed from storage and maintained at room temperature for 12 to 24 h prior to SBA assay. The second aliquot was removed from storage for a period of < 1 h. Both aliquots were then assayed on the same run. Long-term stability investigated sample stability over the duration of the study. All samples were assayed at the ref-

erence point (day 0), then aliquoted into multiple sterile microtubes. Aliquots were maintained at −80 °C (-95 °C to −65 °C) and assayed at intervals of 1, 6 and 12 months. In post-preparative stability, samples were prepared in the microtitre plate and left at room temperature for 6 h and < 1 h, all samples were then assayed on the same run. All stability investigations were considered acceptable if ≥ 85% of sample aliquots were within 3 SBA titres.

2.5. Statistical analysis

SBA titres for all samples (seroprevalence study and serogroup X SBA assay validation) were determined at the dilution at which ≥ 50% bacterial killing was observed, when compared with the colony forming units (CFUs) of the column 11 control well (viable CFU). Titres of <4 were assigned a value of 2 for computational purposes. Samples were required to pass assay acceptance criteria to be included in data analysis.

Statistical analysis was performed on results obtained for the seroprevalence study. Data on whether participants had received a meningitis vaccine in the last 6 months were collated from the MenAfriCar master database. Although data on the type of vaccine used was not recorded it is likely that participants received MenAfriVac (Serum Institute of India Pvt. Ltd.) vaccine in Niger. Vaccination status was displayed as either vaccinated, or unvaccinated. Stata (version 16.0) was used to perform logistic regression analyses to predict the likelihood of protective serogroup X SBA titres in unvaccinated participants versus previously vaccinated (with a vaccine not containing serogroup X polysaccharides) participants. Microsoft Excel (version number 2106) was used to perform reverse cumulative distribution analysis to provide percentage data in tables 1 and 2, and for illustration in Fig. 1.

3. Results

3.1. Seroprevalence study

Overall, 52.25% of participants (n = 377) possessed putative protective antibodies (SBA titre ≥8) to *N. meningitidis* serogroup X. The significantly highest prevalence of protective antibodies to serogroup X was 73.96%, observed in age group 5–14-years (71/96 participants within age demographic). This was closely followed by the 1–4- and 15–29-years age groups, possessing 52.94% (36/68 participants within age demographic) and 55.56% (55/99 participants within age demographic), respectively.

The lowest prevalence of putative protective antibodies to *N. meningitidis* serogroup X was seen in age group < 1 year, with 0% of participants exhibiting a SBA titre ≥8. Overall, 47.75% (180/377 participants across all age demographics) lacked any putative protective antibodies, exhibiting a SBA titre <8, to *N. meningitidis* serogroup X. Excluding the 1–4 and 5–14-year age group, a SBA titre to serogroup X of <4 was shown to be the most prevalent in each of the age groups, as displayed in Fig. 1. The SBA titres of all participants, in all age groups, excluding < 1 year, primarily fall into two categories: an SBA titre of <4, demonstrating the absence of protective antibodies, or an SBA titre ≥128, demonstrating a high level of putative protection against *N. meningitidis* serogroup X.

As displayed in table 2, an SBA titre ≥8 to serogroup X was observed to be more prevalent in participants who had received previous meningococcal vaccination, OR = 3.45 (p <.001, CI: 2.141, 5.565). Of 377 participants, 273 had received a previous meningococcal vaccine, whilst 104 had received no vaccination, or did not know their vaccination history. Of the 273 participants vaccinated, 166 (60.80%) possessed an SBA titre ≥8 to serogroup X. Of those participants not vaccinated (104), 31 (29.81%) participants possessed an SBA titre ≥8 to serogroup X. A notable cofounder is the higher prevalence of putative protective antibodies still observed in age groups 1–4, 5–14 and 15–29-years, 58.7%, 75.28% and 60%, respectively.

3.2. Serogroup X Serum bactericidal antibody assay validation

For selectivity/specificity, heterologous inhibition of samples with polysaccharide A (95%), C (100%), Y (100%) and W (100%) demonstrated a ≤ 2 SBA titre reduction when compared to the neat sample. In addition, 100% of homologous inhibited samples with X polysaccharide demonstrated a ≥ 2 SBA titre reduction when compared to the neat sample. For spiking, 94.44% of spiked aliquots (x dilution factor) were within ± 1 SBA titre of the neat positive sample. In accuracy, 88.88% of replicates (x dilution factor) were within ± 1 SBA titre of the neat positive sample. For precision/reproducibility, 100% of sample (n = 20) replicates in intra-assay reproducibility were within 3 SBA titres. In inter-assay reproducibility, 95% of sample (n = 20) replicates were within 3 SBA titres, and for inter-operator reproducibility, 90% of sample (n = 20) replicates were within 3 SBA titres. For lower-end precision/reproducibility, 87.5% of sample (n = 9) replicates were within 3 SBA titres. For linearity/recovery, 100% (n = 20) of 1:4 diluted samples (x dilution factor) obtained results within ± 1 SBA titre of the neat sample. For stability, 94.4% of sample (n = 20) aliquots in freeze and thaw stability were within 3 SBA titres. For short-term and long-term stability, 100% and 88.89% of sample (n = 20) replicates, respectively, were within 3 SBA titres. In post-preparative stability, 100% of sample (n = 20) aliquots were within 3 SBA titres of one another. Validation acceptance criteria for all

Table 1
Percentage of participants by SBA titre to *N. meningitidis* serogroup X, by age group. Number of participants tested, and in each category is highlighted in bold and italic.

Age Group	No. Tested	No. and percentage of participants with SBA titre (<4 to ≥128)						
		<4	**4**	**8**	**16**	**32**	**64**	**≥128**
<1 year		100.0%	0%	0%	0%	0%	0%	0%
	17	***17***	***0***	***0***	***0***	***0***	***0***	***0***
1–4 years		45.6%	1.5%	0%	2.9%	0%	0%	50.0%
	68	***31***	***1***	***0***	***2***	***0***	***0***	***34***
5–14 years		20.8%	5.2%	0%	4.2%	1.0%	1.0%	67.7%
	96	***20***	***5***	***0***	***4***	***1***	***1***	***65***
15–29 years		42.4%	2.0%	5.0%	1.0%	7.1%	1.0%	41.4%
	99	***42***	***2***	***5***	***1***	***7***	***1***	***41***
30 + years		55.7%	8.2%	8.2%	3.1%	3.1%	1.0%	20.6%
	97	***54***	***8***	***8***	***3***	***3***	***1***	***20***
Total participants:		43.5%	4.2%	3.4%	2.7%	2.9%	0.8%	42.4%
	377	***164***	***16***	***13***	***10***	***11***	***3***	***160***

Table 2
Number of participants by vaccination history status and SBA titre to *N. meningitidis* serogroup X, by age group. Percentages shown represent those in each category: vaccinated or unvaccinated, with an SBA titre ≥8.

Age group	No. Tested	No. Vaccinated	No. vaccinated, with SBA titre ≥8	No. Unvaccinated	No. unvaccinated, with SBA titre ≥8
< 1 year	17	0	0 *0%*	17	0 *0%*
1–4 years	68	46	27 *58.7%*	22	9 *40.9%*
5–14 years	96	89	67 *75.3%*	7	4 *57.1%*
15–29 years	99	80	48 *60%*	19	7 *36.8%*
30 + years	97	58	24 *41.4%*	39	11 *28.2%*
Total:	**377**	**273**	**166** ***60.8%***	**104**	**31** ***29.8%***

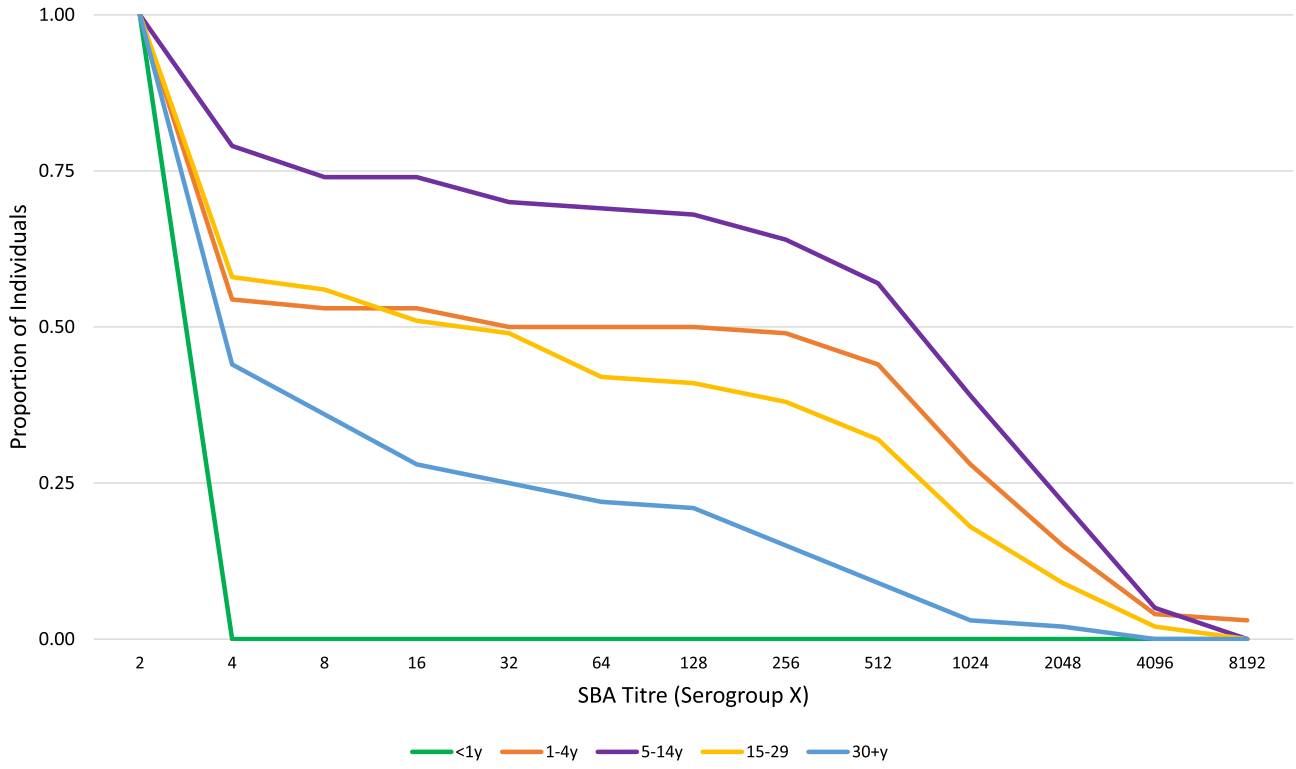

Fig. 1. Seroprevalence Study results – data displayed shows the proportion of participants in each age group, stratified by SBA titre (see key under chart) to *N. meningitidis* serogroup X. <1y = Under 1 year old, 1-4y = 1 to 4 years old, 5-14y = 5 to 14 years old, 15-29y = 15 to 29 years old and 30+y = over 30 years old (n = 377). Titres of <4 were assigned a value of 2 for computational purposes.

parameters were within the required ≥ 85% pass criteria, therefore, serogroup X SBA assay validation was successfully obtained for all parameters of the serogroup X SBA assay validation.

4. Discussion

Data showed that 52.25% of study participants within the communities of Niamey and Say, Niger possessed putative protective antibodies to *N. meningitidis* serogroup X, with a SBA titre ≥8. There are numerous reasons why over half of subjects demonstrated protective antibodies. One such explanation may be through natural immunity induced by asymptomatic carriage of *N. meningitidis* serogroup X within the nasopharynx. Specifically, a review conducted in 2019 exploring sixty-five studies with varying methodologies (swabbing of the posterior pharynx, oropharynx, pharynx and nasopharynx of participants) found that carriage of serogroup X within the meningitis belt was the highest in 5–17-year-olds, alongside serogroups W and Y [11]. The review findings support this study, as higher protective SBA titres were most prevalent in 5–14-year-olds. However, specific carriage in Africa in the review was generally low overall (usually < 0.5%), in comparison to a high incidence of IMD within the meningitis belt [11]. Nevertheless, carriage is known to be highly variable depending on individual strain characteristics. Carriage prevalence of *N. meningitidis* in Northern Ghana between 1988 and 2005 was reported as 7.3% (110/1497) and 6.1% (305/4999), of which 64% and 53%, respectively, were identified as specifically carrying *N. meningitidis* serogroup X [12].

Further data relating to serogroup X were gathered during an outbreak in Niger in January-June 2006, in which 51% of 1139 cases of IMD were caused by serogroup X [13]. Serogroup X related IMD were primarily seen in age groups 1–4 years (62.8 cases per 100,000) and 5–9 years (74.6 cases per 100,000), with the mean age of disease being 7.9 years [13]. As previously stated, sera for

this seroprevalence study were collected in Niger in 2012, approximately 6 years following the 2006 outbreak. This may account for the SBA titres ≥8 to serogroup X demonstrated here in participants aged 5–14 and 15–27. These are the groups which may have been most affected by the previous outbreak of serogroup X related IMD and would therefore demonstrate the highest putative SBA titres. This could indicate asymptomatic, or symptomatic, transmission of *N. meningitidis* serogroup X continuing within the community prior to and potentially after 2012.

Another study from 2003 explored further outbreaks of serogroup X related IMD between 1995 and 2000 in Niamey, Niger [14]. Serogroup X IMD was found to be non-existent in < 1-year olds, with cases increasing from > 1 year old [14]. This is surprising, as no protective titres to serogroup X were observed in the < 1-year-old age group in this seroprevalence study, making this age group potentially vulnerable to IMD. A study by Goldschneider *et al.* demonstrated that seroprotective titres against *N. meningitidis* are generally seen in < 3 months-old due to the presence of maternal immunoglobulins (IgG) against IMD [15]. What we may therefore be observing is the interim period, between the waning of maternal antibodies and the occurrence of serogroup X related IMD [11]. However, these assumptions are limited by a small sample size (17/377 participants < 1-year-old). Secondly, it was not possible to identify specific ages within the demographic.

A relationship between previous vaccination and SBA titre ≥8 to serogroup X was identified through logistic regression, OR = 3.45 (p <.001, CI: 2.141, 5.565). It is unknown from participant data available which vaccine had been administered, though it is believed to have been MenAfriVac. This association is unexpected, as any vaccine administered would not have contained polysaccharides for *N. meningitidis* serogroup X, and specifically, MenAfriVac contains only serogroup A. Any other vaccines given during serogroup C and W related IMD outbreaks would also not have contained polysaccharides for serogroup X. Interestingly, a genetic correlation between serogroup X and A has previously been identified [16]. The gene cluster, *xcbABC*, codes for the *xcbA* gene which is responsible for the synthesis of the capsular polysaccharide of serogroup X. The *xcbA* gene is 52% homologous to the *sacB* gene [16], which encodes the capsular polysaccharide of serogroup A [17]. It is therefore suspected that serogroup X may share capsular similarities to serogroup A, both being composed of homopolymers of amino sugars, α-D-ManNAc and α-D-GlcNAc [18]. This is interesting due to the previous prevalence of serogroup A related IMD, and consequent success of the MenAfriVac vaccine [18], within the meningitis belt. Significant protective SBA titres to serogroup A would therefore be seen within these communities, and with potential capsular similarities between serogroup A and X, this could explain SBA titres ≥8 to serogroup X in some study participants, even though they have not been vaccinated with serogroup X polysaccharides.

Similar findings were seen in a serogroup C seroprevalence study completed by Trotter *et al.* in 2003. The study discussed the theory of meningococcal antigens, as well as other non-pathogenic Neisseria species such as *N. lactamica*, which may elicit an antibody response, and provide protection across other serogroups of *N. meningitidis* [19]. Surface antigens such as outer membrane proteins (OMPs) have shown to be immunogenic. Specifically, subcapsular surface proteins, PorA, fHbp, NadA and NHBA, found in various meningococcal serogroups, have been shown to illicit an increase in SBA titre. Bexsero, a meningococcal group B vaccine, was licensed containing the above proteins [20], and cross-reactivity has been observed between all serogroups, including X [21]. It is therefore not unexpected that natural exposure to these subcapsular proteins, i.e., through asymptomatic or symptomatic exposure to other meningococcal serogroups, could result in an increase of SBA titre to serogroup X.

The introduction of glycoconjugate vaccines has been proven to disrupt and reduce the acquisition of carriage of *N. meningitidis* [22], therefore, the implementation of NmCV-5 would be crucial in preventing transmission and future outbreaks of serogroup X associated IMD. Put into context with a previous pre-vaccination seroprevalence study in the UK, which determined that 25–32% of adults over 25 years possessed an SBA titre ≥8 to serogroup C [19], 52.25% of participants in this study exhibiting protective titres to serogroup X is considered significant. This research however is limited in that sera were only available from Niger, with a sample size of 377. An area for further study could be to explore seroprevalence to serogroup X in a higher population, and in other communities within the meningitis belt with varying incidence of previous serogroup X IMD.

The serogroup X SBA was successfully validated for the following parameters: accuracy, selectivity and specificity, precision/reproducibility, linearity, and stability. All parameters achieved > 85% pass criteria.

Selectivity and specificity were successfully validated through inhibition, spiking, and accuracy. Results obtained for precision/reproducibility parameters overall demonstrate the capability of the serogroup X SBA assay to quantify a range of SBA titres accurately and consistently in human sera, across multiple assays, completed by multiple operators. During intra-assay precision/reproducibility, there was also no observed difference in SBA titre when serum samples were placed in a different position on the microtitre plate.

Linearity investigations utilised human serum minus IgA/IgG/IgM as the diluent for recovery. This parameter exceeded validation acceptance criteria with 100% of SBA titres within range, when multiplied by their dilution factor (1:4). In sample stability, all parameters obtained > 85% of the required acceptance criteria for validation. This confirms that the performance of the serogroup X SBA assay is not affected by any potential degradation that may arise in sera when samples are subjected to differing storage conditions.

5. Conclusion

In conclusion, seroprevalence data obtained shows that immunity to *N. meningitidis* serogroup X may be prevalent within Niger, West Africa, part of the sub-Saharan meningitis belt. Other communities within the meningitis belt may also demonstrate similar findings, though this could be an area for future research. The data collected by this study provides a naturally occurring baseline of bactericidal antibody to serogroup X. Conversely, this study also revealed that 47.7% of participants (n = 377) lacked any immunity to *N. meningitidis* serogroup X. Due to the potential presence of serogroup X within the Niger community, and almost half of study participants lacking any protective serum bactericidal antibodies to serogroup X, these data support the requirement of a licensed conjugate vaccine, NmCV-5.

Validation of the serogroup X SBA assay was successful for all parameters, and data obtained demonstrates suitability for use in future human clinical trials. Specifically, the validated assay will be used in upcoming phase 3 trials, providing data required to license the implementation of conjugate polysaccharide vaccines containing serogroup X into communities within the meningitis belt.

Declaration of Competing Interest

Authors S. Katz, K. Townsend-Payne, J. Louth and R. Borrow perform contract research on behalf of the UK Health Security Agency (UKHSA) for GSK plc, PATH, Pfizer and Sanofi Pasteur.

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