Author’s response to reviews

Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.

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Author’s response to reviews: see over
Dear reviewers and editor BMC Infectious Diseases,

Regarding the manuscript 8373319971270554, Neisseria meningitidis porA feta and fHbp gene distribution in Western Australia 2000 to 2011.

We make the following responses regarding reviewers comments:

Referee 4

Reviewer's report
Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.
Version: 3 Date: 17 September 2014
Reviewer: Rolando Pajon

Reviewer's report:
Minor Essential Revision
The manuscript is a trove of information. Very well done. The following points are minor tweaks that are required in my opinion.

Page 7: Please improve explanation on how many isolates were tested in respect to the TOTAL of cases reported for the whole period. It is not that all clear
Line inserted at introduction of the methods section.
Page 7: Data Bias. Please consider analyzing the data with and without 2010-2011 data. There are not representative of the trends that year and might skew the data if placed in the right bin.
Page 8 Line 15: (J42... etc.) Please clarify what is these. They are assigned codes, but the reader will not get to see that until after diving into the supplementary data.
Line added regarding isolates

Results:
Page 11: The population sample is biased. Look at the % of aboriginal in your sample compared to that in the general population. These needs to be better taken into account in all your analysis, or at least explained better if you did it.
Invasive meningococcal disease is known to have higher incidence in aboriginal people. Have added line at the end of “study population” showing the selected isolates are not biased for aboriginality to the total cases reported to the health department.
Page 11 Lines 14 to 18: The paragraph needs to be rewritten for clarity. And this comment is somehow connected to the one in page 7 (refer to the total of cases)
Paragraph rewritten
Tables 2-4 Need formatting or re-structuring. The p value should not be another row or at least a differentiated one.
Tables have been reformatted with p value column now more obvious.
The statistical models used are a major point that influences the data interpretation and your conclusions. In results you want to spend some more time explaining it, the coefficients and their meanings. There is nothing here about it. This part is essential for a reader to replicate your analysis.
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report
Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.
Version: 3 Date: 11 September 2014
Reviewer: Susanne Jacobsson
Reviewer's report:
Minor Essential Revisions
ABSTRACT
Page 3 row 7: F5-5 (N=8) should be (n=8).
Page 3 row 11: use one decimal 8.3%
BACKGROUND
Page 4 row 15: type the whole names for porA and fetA as you did for fHbp
I’m not sure there are any longer names for porA and fetA. Most papers only call them these names.
Page 4 row 21 and 22: Include date in the reference.
Included
Page 5 row 20: delete underscore
Changed
METHODS
In general use the symbol for degrees °, not 0
Changed
RESULTS
Page 11 row 3: use one decimal 4.6 years
Page 11 row 16: 87.1% P=0.521 should be p=0.521
Page 12 row 5: F5-5 (N=8) should be (n=8).
Changed all these
Discretionary Revisions
BACKGROUND
Page 4 delete row 12 and 13. ...for informing public health responses and is obtained by sequencing antigen encoding genes such as factor H binding....
Changed
Page 4 row 14: delete porB
Deleted
Page 6 row 5: regarding phrase “the small number of serogroup B strain tested” consider revise that. To my knowledge the vaccine is being used now days.
Changed
RESULTS
Page 11 row 16 and 17: hard to follow, consider revise.
Changed
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Referree 1;
Reviewer's report
Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.
Version: 3
Date: 10 September 2014
Reviewer: Georgina Tzanakaki

Reviewer's report:
Major Compulsory Revision

Comments to the authors:
Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.
Journal BMC Infect Dis
Manuscript ID:
By: Boan et al

Comments to the Editor
In this work, by Boan et al describes the epidemiology of the 3 antigen encoding genes in addition to FHbp for meningococcal typing.

General comments
The manuscript offers some interesting epidemiological data from Western Australia adding up to the existing knowledge. However, those data are not complete as the authors have already acknowledged in the discussion section. It is well known that porA, fetA and fHbp gene typing give additional epidemiological information of the circulating clones in a region/country. However, MLST typing is essential for more precise genetic information in order the authors to be able to compare the characteristics of the isolates with the worldwide meningococcal epidemiology. May the authors would like to consider carrying out MLST typing.

It is too labour and cost intensive for us to do MLST at the moment, and waiting for the opportunity and results would likely lead to several years delay in having the results in the public arena.

In order to conclude that “FHbp modular groups of the multicomponent 4CMenB vaccine make up 8.26 and 47.7% respectively of the examined serogroup B isolates” another methodological approach should be carried out. The genetic data presented have insufficient discriminatory power for vaccine coverage estimation. May the authors would consider of employing other methodologies such as MATS ELISA in order to enable them to draw such conclusions.

We don’t have ready access to MATS and again is too labour and cost intensive for us at the moment.

Therefore, the study, as it stands, does not provide full information for predicting the likelihood of meningococcal B vaccine efficacy in their region.

This is admitted.

Another point which is that the origin of the strains under study are not properly identified. Were there all from sporadic cases?

Another point which should be taken to the consideration is the annual incidence; it would be much interesting to show the annual IMD incidence amongst the aboriginal vs regional population rather than male vs female ratio or the age compared with the presence of the genes since the results have shown that none of the variables were independently associated with age/and or origin.

The incidence should also include the cases which were diagnosed by non cultural methods. Additionally, age vs serogroup should be shown in relation to
aboriginal/non aboriginal population.
Our feeling was the paper was already long and needed focus on the sequencing results and how they relate to other factors, rather than incidence data which has been reported by the Australian/West Australian public health authorities, for example through Communicable Diseases Intelligence Journal.

Specific comments
Methods
Line 19. The authors are advised to add the number of the non-culture IMD cases.
Have added further to the introduction of this section, and state 125 diagnosis by nucleic acid only.
RESULTS
1st paragraph, line 8, page 11: the serogroups annually should be presented in a table along with the annual incidence. It would be also interesting to add the aboriginal/non aboriginal origin
This line shows that throughout the period studied the ratio of serogroup B to C as the dominant types has been largely stable. Our focus is not on the incidence per se, and it can’t be really as we have not examined the complete set or even the same percentage of cases year to year, as specified in the methods. Incidence data would be more exactly reported related to all reported cases, the data for which is held and published by the public health authorities, not primarily by our group.
2nd paragraph, line 4 page 12: In order to facilitate the readers, another table could be made showing the porA, FHp fA results
We don’t feel this line is hard to follow, and as you suggest we already have quite a lot of tables.
Supplementary tables 5,6,7 are very detailed and not needed.
On the contrary other reviewers have asked specifically for the detail in these tables.
Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests

Referree 3:
Reviewer's report
Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.
Version: 3 Date: 11 September 2014
Reviewer: Maurizio Comanducci
Reviewer's report:
General comments
The paper by Peter Boan et al., “Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011” reports about genetic characterization of 128 of the 271 meningococcal strains which have been isolated between 2000 and 2011 in Western Australia, and stored at PMH. The focus of the paper is on the sequence variability of genes porA, fetA and fHbp, which code for proteins PorA, FetA and fHbp, respectively. PorA is the
immunodominant antigen in OMV-based anti-MenB vaccines (Frasch et al., Methods Mol Med. 2001;66:81-107). fHbp is the unique component of the Pfizer anti-MenB vaccine (ref. 6) and one of the multiple components of the Novartis vaccine 4CMenB (ref. 7). FetA is an important outer membrane protein and good vaccine candidate (ref. 10). Some association of PorA and fHbp variants with age and demographics are described in the paper, as well.

The subject of the present paper is quite interesting. The variability of the sub-capsular components of the anti-MenB vaccines is a major problem, because the immunities raised by those antigens are only partially cross-protective against meningococcal strains harbouring different variants of the same antigens. The immunogenicity and related features (efficacy and coverage) of all meningococcal vaccines should be tested by the unique correlate of protection available, which is the Serum Bactericidal Assay (SBA – Gotschlich EC et al., 1969. J Exp Med 129: 1367-1384). Surrogates of SBA have been set up (e.g. MATS), by the producers of the vaccines based on fHbp.

Concerns
1) Due to the fHbp expression level variability, any estimate of efficacy of both fHbp-based Pfizer and Novartis vaccines requires an account on the level of expression of fHbp. The susceptibility to killing of strains harbouring fHbp variants different than the vaccine variants are scarcely predictable by sequence homology. It is crucial to know the fHbp expression level in the target strain, which might be too low. In addition, in the case of the multi-component Novartis vaccine, the relative contribute of other vaccine components should be considered. Therefore, genetic data alone are of poor indicative value to predict any reliable vaccine efficacy. This is actually admitted by the authors as well, in the discussion section (pag 15, lines 15-20).

This is an acknowledged shortcoming of the study.
2) From a Molecular Epidemiology perspective, the lack of all strain genetic characterization (MLST) is a limitation of major impact. Incomplete strain classification, beside impeding the comparison with the worldwide meningococcal epidemiology (also this issue, like the one of point 1, is admitted by the authors, at the end of the discussion section) does not allow to assign strains to any previously described clone, and to fully compare the strain to each other.

Admittedly it would be preferable to perform MSLT but we do not have the capacity to do so, so the study is reviewed in light of this.
3) The lack of genetic characterization (MLST data) of the strains harbouring fHbp variant 2 and PorA P1.22,14-16, which have been postulated as associated with demographics and age, significantly impedes a more complete picture of possible associations. The clonal complexes of those strains could be added to the multivariate regression analysis, in order to evaluate other possible associations. Associations of single outer membrane protein variants with clonal complexes have been clearly demonstrated, and have become a solid reference for the meningococcal scientific community [Urwin R. et al., Infect Immun 2004; 72(10): 5955-62].

We admit the lack of MLST makes some associations uncertain.

Major Compulsory Revisions
1) Adding the MLST data corresponding to all strains. Unfortunately, as specified by the authors at the very end of the discussion, that will cost something, in terms of labour and time constraints. Though, genetic characterization is nowadays
expected to be included in high value Molecular Epidemiology papers. In order to do consistent comparisons, amount and quality of data should be comparable worldwide. MLST data are also important to confirm and corroborate the associations described in the paper, by having a more complete picture of all possible associations.

If/when MLST can be performed on our stored isolates it will be reported, but if done it will predictably be several years away. We feel the data even with it’s shortcomings should be available publicly.

2) Proposing the paper as a careful and detailed analysis of variability of three meningococcal genes, which code for vaccine candidates-components. I would avoid tentative predictions of vaccine efficacy using sequence data, only. Predictions of efficacy should be addressed by functional assays.

Your point is taken and we have acknowledged these shortcomings.

Discretionary Revisions

1) All through the paper, I would deal with translated protein sequences, instead of coding genes. It would be simpler, and maybe more interesting.

I agree with the authors it would have been more prudent to use published primers to amplify all genes of interest. Procedures and tools described in the original papers were able to successfully amplify-sequence target genes from any genotype.

We decided early in analysis to report according to genotype as others have also done, which we think is acceptable.

2) Page 12, line 2,3 – I suggest to specify how many of the 32 total translated fetA sequences (and of the three fHbp sequences) which were treated as “no results” contained stop codons, and how many were sequences with incomplete matches with the Neisseria database. Sequences with incomplete matches should be submitted to Neisseria database, to be given a new ID number.

Thank you for the clarification, in fact this should read that the sequences contained stop codons. This has been amended.

3) I think reference #30 would be more appropriate at the end of the period in line 6, page 6. Beside reference #30 in the new location, and in order to give an example of the results expected from (a surrogate of) a functional assay (MATS) I would also add reference Vogel U. et al, Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect Dis. 2013 May;13(5):416-25

Altered these references

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I do not think any company may gain or lose financially from the publication of this paper.
I have been a Novartis employee until two years ago. Novartis has possibly applied for patents relating to the content of the manuscript.
I can answer no to all other above.