Author’s response to reviews

Title: Streptococcus pneumoniae and Haemophilus influenzae in paediatric meningitis patients at Goroka General Hospital, Papua New Guinea: serotype distribution and antimicrobial susceptibility in the pre-vaccine era

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Author’s response to reviews:

Reviewer reports

Reviewer #1:

The manuscript by Greenhill et al is well written and a necessary report for future surveillance after introduction of vaccination.

Methods:
which additional tests were performed in the CSF? Leucocytes? glucose? other? May be these data might be added in a table.

Cell counts, macroscopic examination, and Gram stains were conducted on CSF. We have added a supplementary table (Table S1) providing an overview of these WBC counts.

Results:

the authors might want to clarify whether the 1404 patients without bacterial isolate had other laboratory signs of meningitis/infection (like increased white blood cells, or changed glucose index in the CSF.

We have added a short paragraph to the results section addressing this issue; and provided Table S1.

Not all samples with 2 pathogens were excluded because of contamination. Why? Samples were excluded based on the conclusion that the organisms detected were likely contaminants. In two samples with multiple organisms detected, we concluded that those organisms were pathogens rather than contaminants, so we retained them in our analyses. No changes have been made to the manuscript in regards to this point.

The authors might want to expand on the rather high proportion of probable contaminants and possible pathogens.

We acknowledge that the high proportion of probable contaminants is an interesting aspect of the findings, and requires explanation. In the results section of the previously submitted manuscript we do state that there is a temporal cluster of S. aureus isolation, and the general absence of polymorphonucleocytes in CSF from which S. aureus was isolated. We believe that any further ‘expansion’ on this matter, and that of possible pathogens, is best suited to the discussion section. In the discussion section of the previously submitted manuscript we have addressed the issue of high S. aureus isolation rates. In the revised manuscript we have added an additional statement, as follows:

“Further investigation indicated that CSF collection methods were inadequate and likely to have contributed to high isolation rate of S. aureus in 2004-2005. Some of these isolates, and some of those in previous years (in which no more than 6 were isolated in any given year between 1997 and 2003) may have been the causative agent of meningitis. Of the 68 S. aureus isolates, 11 were observed to have elevated PMN counts. However, even in samples with high PMN counts we cannot discount the possibility of another undetected bacterial pathogen being the causative agent of meningitis. This cautious supposition is supported by the fact that elevated PMN counts were detected in some specimens from which no bacteria were isolated (Table S1 and Table S2)”

Aside from the S. aureus, the number of probable pathogens (8 isolates) is reasonable over almost 9 years of surveillance, and does not justify further explanation. Similarly, we isolated 32 likely contaminants over 9 years, in which we cultured almost 1900 CSF samples. A
contamination rate of <2% of all samples is consistent with other CSF culture studies. We have added a sentence to the discussion to address this point raised by the reviewer:

“Our isolation rate of other contaminates (aside from S. aureus) was <2%, which is consistent with other CSF culture studies (e.g. Dunbar et al [31]).”

The exclusion of probable contaminants and possible pathogens should be clarified in a better way.

We hope that through changes made throughout the revised manuscript we have now addressed this issue, include data in the supplementary tables.

Discussion:

The authors present data about meningitis in PNG between 1996 and 2005, the pre-vaccin era. By introducing data and results from later periods the discussion sometimes is a little confusing. May be the manuscript might gain clarity by focusing on the results of the study period. This also might shorten the manuscript.

We appreciate the reviewer’s feedback on this matter. In an attempt to improve clarity, we have moved the paragraphs of the discussion that pertain to more contemporary data and interpretations to be towards the end of the discussion. We believe this improves clarity, as the initial ~2/3 of the discussion now exclusively focuses on the findings of this study; without reference to recent data. The sections of the discussion that focus on more recent findings are relatively short; thus we do not feel that they detract from the overall manuscript now they have been relocated within the Discussion (in fact we believe these short sections are important and favour their retention in the manuscript).

The authors might want to explain why no MICs were performed (page 13 line 306) These isolates have since lost viability due to mechanical problems with the -80 C freezers at the PNGIMR (where the study was conducted). Thus, unfortunately we are unable to now conduct MIC tests on the isolates. We have added to the manuscript why we were unable to conduct MIC testing

Page 14 line 331 conducting or conducted?
Correction has been made.

Reviewer #2:

This is an impressive surveillance work from 1996 to 2005 continuing from the senior author's early work in Papua New Guinea. It provided useful information on vaccine serotype coverage and antimicrobial resistance of importance in S pneumoniae and H influenzae in children.
My comments are mainly to clarify some inconsistency:

(1). Line 40, please verify that it was 88.8% and not 91.5% stated in the text on line 157. The proportion on line 40 pertains to S. pneumoniae and H. influenzae type b. The proportion on line 157 pertains to S. pneumoniae and all H. influenzae.

(2). Please adopt consistent US spelling which is evident in the rest of the manuscript:

Line 46, "recognised"
Line 116, "paediatrician"
Line 319, "paediatric"
Line 326, "recognised"

We appreciate the reviewer drawing our attention to inconsistencies. We have opted to apply British English.

(3). Line 48, please spell EPI in full Ammended

(4). Line 124, please change to "well established in this setting" Ammended

(5). Line 152, please clarify timing of CSF collection in relation to antibiotic. This will impact on yield of CSF. If the information is not available, please state so in methods.

It is difficult to reliably get such information in our study site (due to the possibility of antibiotics having been administered prior to hospitalisation). Where possible CSFs are collected prior to administration of antibiotics in hospital, and this has been added to the revised manuscript. Additionally, we have briefly described the antimicrobial activity assay conducted in the Methods section, and added corresponding data to the results section.

(6). Lines 162-165, please clarify if there were any clinical symptoms and signs of infection. Was this CSF with S. aureus related to neurosurgery or with neurosurgical instrumentation such as external ventricular drains? The dismissal of a potential pathogen with very limited information (namely lack of PMN in CSF) is problematic.

All study participants had suspected meningitis, thus there were clinical signs and symptoms of infection.

It was stated in the original manuscript (lines 319-321 of original manuscript) S. aureus meningitis is typically associated “associated with pre-existing abnormalities of the central nervous system or recent surgery (which were not present in our patients)”. We have retained this sentence and expanded upon our discussion regarding S. aureus and its unlikely role in meningitis in this setting.

(7). There is no need to show X2 and degree of freedom; please state only P value: lines 175, 177, 181, 183, 247.
There is some value in presenting $\chi^2$ and df values; most notably it allows the reader to quickly assess the size of the groups. The authors defer this decision to the editor.

(8). Line 247, please clarify if the information on Hib isolation rate from CSF was from the present study. If so, it should be in the results before discussing in discussion section.

This data is from ref 19. We have reworded the relevant sentence in the hope that it improves clarity.

(We have relocated this paragraph to later in the manuscript, on the basis of comments from reviewer 1)

(9). Table 1, please clarify if it was 377 or 375 (mentioned in results section).

There were 375 samples that were positive, as stated in the results. However, from two samples multiple (two) pathogens were isolated, resulting in 377 pathogens (as listed in Table 1). Some clarification has been added to the text of the results section.

(10). Table 3, please clarify the median MIC for ceftriaxone. 124 isolates tested for ceftriaxone, 1 found to be intermediate (MIC=1) and none resistant (MIC >=2), hence odd to have median MIC of 1.

The median provided is for isolates with reduced sensitivity only (not the median of all isolates). So in the case of ceftriaxone we are stating the median of a sample size of 1.

(11) Table 4, please delete column 4 (1996-2005) as the table compared 1996-2000 with 2001-2005, hence this total information adds little.

The fourth column of Table 4 has been deleted.

Reviewer #3:

This is an interesting manuscript about the paediatric meningitis in Papua New Guinea.

My concerns are:

1. Page 6 2nd papa: Pneumococcal sensitivity to tetracycline and erythromycin is described. Neither of these antibiotics has good penetration into the CSF and would be considered inadequate treatment for meningitis. This information needs to be removed.

By including data for tetracycline and erythromycin we are not advocating their use in treatment of meningitis. We accept that we did not expressly state that to be the case in the previous manuscript, so have added a statement in the discussion for clarification. However, we have not removed the tetracycline and erythromycin data, due to its epidemiological relevance. In PNG
and other low-income countries routine culture and sensitivity is rarely conducted. Thus, we test a broad range of antimicrobial agents on the relatively small proportion of bacterial pathogens that are isolated in PNG; and in doing so we can obtain an insight into broader antimicrobial resistance in this setting. While it could be argued that these data are not central to the diagnosis and treatment of meningitis, if these data are not published in this manuscript they are likely to be lost to the scientific community. As an example of their relevance, we have added a reference (Unger et al, 2015) which has drawn on these data already. With the potential for the macrolide azithromycin to be used in malaria prophylaxis (Unger et al, 2015), having baseline data on erythromycin resistance is of great benefit. Similarly, we observed resistance to tetracycline in a small proportion of S. pneumoniae isolates in the current study, as was the case in a recent study conducted in a different setting in PNG (Manning et al, 2011: reference 24 of manuscript).

It is worth noting that even in high-income settings such as Australia where routine culture and sensitivity is conducted on a high proportion of suspected bacterial infections, S. pneumoniae isolates were being tested for susceptibility to erythromycin at the approximate time of our surveillance: http://www.agargroup.org/files/SPNE%2007%20report%20final.pdf

2. Page 9, Antimicrobial susceptibility> The authors describe reduced susceptibility to oxacillin in 17.2% and to penicillin in 21.5%. As oxacillin is not used for treatment but as a laboratory marker for penicillin resistance, could this discrepancy be explained.

There are numerous reasons that may contribute to this apparent discrepancy. First, I would like to bring to the reviewer’s attention that the oxacillin result was obtained by disk diffusion and penicillin results obtained by MIC. This was not clearly stated in the text of the results section, thus clarification has been added to the text. It now reads “Reduced susceptibility to oxacillin was observed in 34 (17.2%) S. pneumoniae isolates; resistance to other antibiotics was uncommon. Pneumococcal resistance to penicillin (MIC determined by E-test) was observed in 21.5% of isolates (Table 3).

Other factors may have also contributed to the discrepancy, not the least the differences in break points for pneumococcal isolates from CSF relative to other sites of the body. While there are differences in MIC break-points for penicillin by MIC for CSF/non-CSF isolates; this is not mirrored in zone diameters for oxacillin, with only one zone applied all isolates (CSF and non-CSF).

Other researchers have observed an imperfect correlation between oxacillin disk diffusion results and penicillin MIC results (e.g. Poulsen et al, 1996 APMIS 104:549-556). Finally, there are cellular mechanisms which are recognised that at least partially explain the imperfect correlation between oxacillin and penicillin resistance. One such mechanism is the presence of penicillin binding proteins in the pneumococcal cell wall. The presence of certain PBPs can dictate that an organism can appear resistant to one form of the B-lactam drug but less so to another form.

While the reasons behind potential discrepancies in oxacillin and penicillin resistance in vitro are of interest, we consider them to be beyond the scope of this study. Given the current length of the manuscript (as alluded to by reviewer 1) we believe it best to leave the level of detail outlined above out of the manuscript. We hope that the clarification included in the revised manuscript
(stating that penicillin resistance was determined by MIC) will suffice. However, if the reviewer or editor believes a further explanation should be included we would be prepared to do so. This is a good study and should be published when the above issues are explained.

------------------------Editorial Requests------------------------

Please note that all submissions to BMC Infectious Diseases must comply with our editorial policies. Please read the following information and revise your manuscript as necessary. If your manuscript does not adhere to our editorial requirements this will cause a delay whilst the issue is addressed. Failure to adhere to our policies may result in rejection of your manuscript.

Ethics:

If your study involves humans, human data or animals, then your article should contain an ethics statement which includes the name of the committee that approved your study.

If ethics was not required for your study, then this should be clearly stated and a rationale provided.

The manuscript states in the last paragraph of the Methods section “Ethics approval was granted by the PNG Medical Research Advisory Committee to conduct CSF bacterial culture and biochemistry as part of the acute flaccid paralysis surveillance.”

Consent:

If your article is a prospective study involving human participants then your article should include a statement detailing consent for participation.

If individual clinical data is presented in your article, then you must clarify whether consent for publication of these data was obtained.

Consent was not required by the ethics committee as this study was conducted as part of good clinical practice. We have added this detail to the Methods section.

Availability of supporting data:

BioMed Central strongly encourages all data sets on which the conclusions of the paper rely be either deposited in publicly available repositories (where available and appropriate) or presented in the main papers or additional supporting files, in machine-readable format whenever possible. Authors must include an Availability of Data and Materials section in their article detailing where the data supporting their findings can be found. The Accession Numbers of any nucleic
acid sequences, protein sequences or atomic coordinates cited in the manuscript must be provided and include the corresponding database name.

We appreciate the position taken by BioMed Central. We have discussed the possibility of depositing the entire dataset (with de-identified data) in a publically available repository with key stakeholders in Papua New Guinea. This has been met with some apprehension on this occasion, in part due to the complex multiple ownership of the data (PNG IMR where the bacteriology was conducted, and the hospital where the patients were admitted and outcomes were reported. At this stage it is our preference that the entire dataset is not deposited publicly. However, should any parties seek access to the data we would be willing to approach the relevant ethics review committees to enable sharing the data on an individual basis.

Authors Contributions:

Your 'Authors Contributions' section must detail the individual contribution for each individual author listed on your manuscript.

We have done this in a format consistent with other articles published in BMC Infect Dis.