Reviewer's report

Title: Using a practical molecular capsular serotype prediction strategy to investigate Streptococcus pneumoniae serotype distribution and antimicrobial resistance in Chinese local hospitalized children

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Reviewer: Paul Turner

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This article describes a combination of cpsB sequencing and multiplex PCR-based serotyping of disease-associated isolates from Chinese children. It combines and develops two existing assays: sequetyping (described by Leung et al) and the CDC multiplex PCR assay (described by Pai et al).

Comments regarding the manuscript:

Major Essential Revisions

Methods

A high proportion of isolates were sputum of young children – i.e. likely to represent carriage (there was no mention of how sputum was collected in such young children). This is alluded to in the discussion. It has no real bearing on the description of the serotyping methodology but significantly impacts on what the actual serotype results mean. Thus, I think this should be mentioned up front.

Using the term “sequence type” / ST is quite confusing as this is standard nomenclature in the internationally used multi-locus sequence type (MLST) genotyping scheme. I would strongly suggest finding a different term / initials.

I am not sure what is meant by the word “voucher” in line 133. In fact I had a little trouble deciphering this entire section and Table S1 – I wonder if there is a way to improve clarity (perhaps a flow diagram of the process would help).

Results

Resistance section. I would count erythromycin and clindamycin as one drug for resistance calculation given their similarity (and shared resistance mechanisms). I am not sure why beta-lactam drugs are not mentioned in the MDR patterns (the definition in the methods seems to imply that they should be)?

Correlation of serotype and clinical presentation. I think this section is not very useful given that many of the sputum isolates are likely to represent carriage. It is probably only worth highlighting the serotype data for invasive infection in this context unless the authors can somehow confirm that the spuata collected were done in a way which minimised pharyngeal contamination or if spuata were included only on the basis of some sort of microscopic quality criteria.

Discussion
Paragraph regarding the high prevalence of serogroup 15 isolates (lines 345-356). Given the comments above regarding serotypes from sputum in young children, couldn’t this be explained by colonisation (i.e. detection of colonising serogroup 15 in sputum contaminated by OP/NP secretions). If so, then 7.8% is not so different from the pre-PCV7 period result of 5.7% from Hong Kong.

The differences in invasiveness between serotypes are well described (e.g. Brueggemann AB, Griffiths DT, Meats E, Peto T, Crook DW, Spratt BG. Clonal relationships between invasive and carriage Streptococcus pneumoniae and serotype- and clone-specific differences in invasive disease potential. J Infect Dis 2003; 187:1424-32.; Smith T, Lehmann D, Montgomery J, Gratten M, Riley ID, Alpers MP. Acquisition and invasiveness of different serotypes of Streptococcus pneumoniae in young children. Epidemiol Infect 1993; 111:27-39.). This part of the discussion should be modified to reflect this.

Conclusions
We showcased the utility of our new serotyping strategy by identifying serotypes of 193 S. pneumoniae isolates and are confident that it provides an opportunity for most routine laboratories with PCR to serotype the majority of pneumococcal strains without the need for an expensive set of serological reagents in China.
- Is this really true? Need to sequence and then PCR over half to confirm – this would be beyond most non-specialist laboratories I can think of. Also, the method could still not resolve some serotypes fully. I think this statement should be tempered.
- It would be helpful to include a mention of the time and costs to perform this method – given the need for both sequencing and PCR it may not be cost/time effective compared with Quellung or latex agglutination, especially if used in a low throughput setting.

Minor Essential Revisions
Introduction
Whilst molecular methods are becoming increasingly used for pneumococcal typing (particularly direct from specimens or from non-viable pneumococci), phenotypic methods still have a role for serotyping viable pneumococci. In particular, latex agglutination has shown to be extremely cheap and accurate for high throughput serotyping projects (see Turner P, Turner C, Jankhot A, Phakaudom K, Nosten F, Goldblatt D. Field Evaluation of Culture plus Latex Sweep Serotyping for Detection of Multiple Pneumococcal Serotype Colonisation in Infants and Young Children. PLoS ONE 2013; 8:e67933.). I think the introduction could be a bit more balanced in this respect.

Methods
The methodology for sequence alignment should be specified (line 129).

Results
The footnotes to Tables 2 and 4 do not seem necessary.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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