Author’s response to reviews

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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Author’s response to reviews: see over
Reply to the reviewers’ comments

Dr Catherine Olino
BMC Pediatrics
Journal Editorial Office BioMed Central

Dear Dr Olino,

RE: MS: 1670154452176925 - Using a practical molecular capsular serotype prediction strategy to investigate *Streptococcus pneumoniae* serotype distribution and antimicrobial resistance in Chinese local hospitalized children

Thank you for the opportunity to revise our manuscript. We are pleased to address all the four reviewers’ comments point-by-point as below.

Referee 1:
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
Version: 2
Date: 26 August 2015
Reviewer: Hans-Christian Slotved
Reviewer's report:
Major Compulsory Revisions

Question 1. In this study, the authors mention three different types of data. 1. They have developed a new approach for serotype identification, 2. They describe serotype distribution and antibiotic susceptibility based on 193 isolates, 3. The collected isolates are mainly from children with different levels of pneumonia. I think the authors need to focus much more on the molecular identification approach, a this study uses isolates (both carrier and IPD isolates) from sick children with different levels of pneumonia, bacteraemia and meningitis, which I believe are not representative for the region (Supplementary Table S3). I think the authors need to reduce the focus on the serotype distribution in the manuscript, and focus more on the molecular typing strategy. Furthermore, I think the authors need to add information on how representative the isolates are to the region, if they intend to compare these isolates. I
therefore recommend that the manuscript (particularly the discussion) is reduced with more focus on the molecular approach.

Answer: We appreciate the reviewer’s comments for improving our manuscript on this practical, simple strategy suitable for routine *Streptococcus pneumoniae* serotype prediction, which is the main thrust of our study. Though China ranks in the top ten countries with high prevalence of pneumococcal disease, there is limited serotype surveillance data, mainly due to low *S. pneumoniae* serotype identification capacity. We agree with the reviewer’s comments on the need to focus more on the new serotyping prediction strategy. As such, we have increased the content focusing on the strategy in the manuscript, including addition of a workflow algorithm (Figure 1) for improved clarity on our new strategy to facilitate easier understanding. We also significantly reduced the serotype distribution content in the discussion section. However, considering that BMC Pediatrics is a clinical journal, and to take care of other reviewers’ concerns and interest in the serotype distribution (see reviewer 3 comments below) and antibiotic susceptibility data (see reviewer 2 & 3 comments below) of our study population, we still kept some relevant parts after significantly cutting the discussion. Furthermore, given the limited epidemiological data on *S. pneumoniae* serotype distribution and antibiotic susceptibility in China, we think it is important to briefly report on this for both our local and international colleagues for future comparisons. We also think that our isolates are a reasonable representation of our community as our hospital is the biggest in the Bao’an District (~ 6 million populations) in Shenzhen managing most children in the district (also see below answer for Question 3). However, we cannot extrapolate our findings to the rest of China given the huge population diversity of this country.

Question 2. Regarding the susceptibility data, you also have to specify the limitation of the collected isolates, see comment 1.

Answer: We have specified the limitation of the collected isolates in the last paragraph of the discussion.

Question 3. The collected material is mainly from children with pneumonia. Why do the authors believe that these data can be used to describe the pneumococcal serotype distribution in the region?

Answer: Bao’an Maternal and Child Health Care Hospital is an affiliated hospital of Jinan University, and is responsible for the health care of women and children in the whole Bao’an district which include ten towns. We agree with the reviewer’s observation that the isolates
studied were mainly from children with pneumonia, and hence cannot generalise the serotype distribution results to the general population in the region, only to hospitalized children in the region. We have specified this as a limitation of the study in the last paragraph of the discussion section (lines 410-420).

**Question 4.** In this study, the authors base their serotype identification solely on molecular techniques. This means that they show that the isolates possess the capsular genes. However, by using only molecular techniques, the numbers of NT isolates will be very low when compared to a phenotypical method. I acknowledge that the authors state that their presented method is only to be used for predicting the serotype distribution. However, I think the authors need to add a section in the discussion, where they discuss the differences between a molecular method and a phenotypical method, particularly in relation to possible isolates, which do not express the capsular gene (often referred to as NT isolates or unknown serotypes). Remember that PCV vaccines only protect against pneumococcal isolates that express their capsules!

**Answer:** We fully agree with the reviewer’s comment and have added the relevant discussion highlighting the importance of conventional serotyping and/or its alternative (lines 361-369), in addition to the new Figure 1, to highlight the point.

**Question 5.** How would the authors handle a PCV-13 vaccinated child, who a year after vaccination was brought to the hospital with an IPD due to pneumococcal infection, the authors tested the isolates with their molecular techniques and the test results showed serotype 19F? Would this be enough information to go to the Vaccine Company and claim that their vaccine does not protect against 19F, or would an additional test be required to show if the capsular gene was expressed?

**Answer:** Similar to question 4 above. Molecular techniques are good serotype predicting assays but are not serotyping per se, as “serotype” is a phenotypic-based definition. So as it stands, even if the molecular assay showed serotype 19F, this cannot be considered sufficient evidence to argue that the vaccine does not protect against 19F. Instead, extra testing, including Quellung serotyping or to show capsular gene expression, would be required as evidence. We believe conventional serotyping or its validated alternative is the gold standard for serotype determination rather than any molecular assays. So, similar to question 4 above, we have added the relevant comments in the discussion, highlighting that serotyping is a phenotypic rather than a genotypic-based definition (lines 366-369). On another note, serotyping data does not necessarily provide evidence to claim vaccine “failure” as other factors such as vaccine injection time, personal immune response, and many other factors, affect vaccine effectiveness.
Question 6. My English is not sufficient to evaluate the quality of the overall written English; however, there are several examples where the sentences do not make sense! One example is on page 6 line 109 to 111. I therefore suggest a thorough editing of the language.

Answer: The manuscript has been extensively edited for better English quality. The sentence of “All the children with pneumonia were satisfied the WHO case definitions for non-severe pneumonia or severe pneumonia (include very severe pneumonia).” was revised as “All the children with pneumonia satisfied the World Health Organization (WHO) standard definition for pneumonia, including classification as non-severe, severe and very severe pneumonia.”

Minor Essential Revisions

Question 7. Page 5, line 78: what does “first-tier” mean?

Answer: In China, “first-tier big city” refers typically to the biggest conventional cities in China, and is usually used to refer to the so-called “The Big 4”, namely Beijing, Shanghai, Guangzhou, and Shenzhen. We have however deleted the phrase “first-tier big city” and replaced it with a more appropriate one- “One of the biggest cities in China… (line 80)

Question 8. Page 11, line 224: what does sequetyped mean?

Answer: Sequetyped means \textit{cpsB} sequence-based molecular typing according to the original JCM publication by Leung \textit{et al.} (ref 13). Use of this term is helpful in avoiding confusion with the term “sequence type (ST)”, which is commonly used for MLST, as pointed out by another reviewer (3).

Discretionary Revisions

Question 9. On page 12 line 226, the authors mention the possibility of new serotypes. What do the authors find that defines a new pneumococcal serotype?

Answer: Sorry use of the term “sequetype” rather than “serotype” is correct there. We also have revised the sentences to clarify this.

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

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
Referee 2:
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
Version: 2
Date: 28 September 2015
Reviewer: Allan Truant
Reviewer's report:
Major Revisions

Question 1: The authors in several areas of the manuscript have cited the Clinical and
Laboratory Standards Institute (CLSI), for example in lines 180, and 273. The authors also
mention "new susceptibility breakpoints for S. pneumoniae in the United States" in line 365.
There appears to be no citation of the version/source used within the manuscript or the
references. The current version of the pertinent CLSI standard is CLSI M100-S25 (January,
2015), volume 35, No. 3 which replaced M100-S24. The authors should specify which
version was used, and should preferably use the most current version of the CLSI standard.
Alternatively, the authors could use the version which was in place during the time of the
study period and then discuss changes made since that time and what the differences might be
scientifically and clinically.

Answer: We adopted CLSI M100-S25 version of the antibiotic susceptibility breakpoints for
S. pneumoniae as criteria for determining drug resistance, and have specified this in the
manuscript and references (lines 211-214). To our knowledge, there is apparently no
difference on criteria for antibiotic susceptibility breakpoints for S. pneumoniae since 2008,
and have thus not discussed this in the manuscript.

Minor Revisions
Question 2: Line 50, systematic to replace systemic

Answer: Sorry for this clumsy mistake which we have corrected.

Question 3: Line 80, capsular to replace capsule

Answer: This has been fixed.

Question 4: Line 109, delete "were"
**Answer: This has been fixed.**

**Question 5:** Line 180, CLSI is the Clinical and Laboratory Standards Institute

**Answer:** We have revised this to include the full name.

**Question 6:** Lines 209-211 needs to be re-written/re-phrased for clarity

**Answer:** Thanks, we have rewritten the sentence (see lines 241-243).

**Question 7:** Line 309, typeable

**Answer:** We have revised.

**Question 8:** Line 320, share the same sequence.....

**Answer:** We have revised.

**Question 9:** Line 371, replace administrated with treated (or similar term)

**Answer:** Thanks, we have fixed this.

**Question 10:** Lines 382, 384, should be "et al."

**Answer:** Thanks, we have fixed this.

**Question 11:** Line 421, should be, Centers for Disease Control and Prevention

**Answer:** Thanks, we have revised.

**Question 12:** Line 423, see CLSI above

**Answer:** Thanks, we have revised.

**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.
Referee 3:
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
Version: 2
Date: 2 October 2015
Reviewer: Paul Turner
Reviewer's report:
This article describes a combination of cpsB sequencing and multiplex PCR-based serotyping of disease-associated isolated 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).

Question 1: 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.

Answer: We understand the reviewer’s concern which stems from our failure to fully describe how sputum was collected to lessen the chances of contamination. Thus we have edited our manuscript to incorporate this in the Methods (lines 124-131).
Question 2: 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.

Answer: We have avoided the use of “sequence types (ST)” in the revised manuscript and resorted to using “sequetyping” or “sequetype” instead.

Question 3: 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).

Answer: The entire section and Table S1 were edited, including deletion of the word “voucher”. Furthermore, we have added two workflows (Supplementary Figure S1 and Figure 1) to bring further clarity from a different angle for the *cpsB* sequetyping reference building up and how to use it for serotype prediction.

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 sputa collected were done in a way which minimised pharyngeal contamination or if sputa were included only on the basis of some sort of microscopic quality criteria.

Question 4: Why beta-lactam drugs are not mentioned in the MDR patterns?

Answer: As supplementary Table S5 shows, all Beta-lactam resistant isolates were either serotypes 19F or 23F and had very high rates of erythromycin resistance. Because the Beta-lactam drugs (penicillin and ceftriaxone) are important choices for children, and also given that in this study non-meningitis isolates had low rates of resistance to parenteral penicillin (1.0%) and ceftriaxone (5.2%), which is especially relevant for clinical antibiotic selection, we decided to discuss the two drugs independently for oral and meningitis situation rather than focus much on the antibiotic resistant patterns. For the penicillin, ceftriaxone parenteral resistant non-meningitis isolates, the multidrug resistance patterns were; erythromycin +
clindamycin + sulfamethoxazole-trimethoprim + tetracycline + chloramphenicol + penicillin + ceftriaxone (n=1); erythromycin + clindamycin + sulfamethoxazole-trimethoprim + tetracycline + penicillin + ceftriaxone (n=1); erythromycin + clindamycin + sulfamethoxazole-trimethoprim + tetracycline + ceftriaxone (n=6); erythromycin + clindamycin + sulfamethoxazole-trimethoprim + ceftriaxone (n=2).

**Question 5:** Correlation of serotype and clinical presentation. I think this section is not very useful …..unless the authors can somehow confirm that the sputa collected were done in a way which minimised pharyngeal contamination or if sputa were included only on the basis of some sort of microscopic quality criteria.

**Answer:** We have deleted this section and summarised it in a single sentence in the results section (see lines 300-302). We have also clarified that sputum was collected in a way that minimised pharyngeal contamination (see lines 124-131). Isolates were obtained from sputum specimens of good quality and considered potentially causative based on predominance on the Gram stain or on the culture plate over other organisms. In addition, the quality of the sputum specimens was monitored to ensure that only those with squamous epithelial cell numbers of <10 per 10× objective microscopic were cultured as suggested (Patrick R, Murray. Manual of Clinical microbiology 9th Edition: Specimen collection, Transport, and Processing: Bacteriology. American Society for Microbiology. 2007; 319-320.).

**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.

**Question 6:** 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.

**Answer:** To address reviewer 1’s comments, we have shortened the serotype distribution discussion but tried to include reviewer 3’s comments in as “A further 15 (7.8%) of the 193 isolates belonged to serogroup 15 (2 serotype 15F/15A isolates; 13 serotype 15B/15C isolates), which is quite similar to the pre-PVC7 period proportion in Hong Kong for serogroup 15 (5.7%) [29].”

**Question 7:** The differences in invasiveness between serotypes are well described….. This part of the discussion should be modified to reflect this.

**Answer:** Thanks, we have added this part in the discussion (lines 384-391).

**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.

**Question 8:** “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.

**Answer:** We have revised the sentence as “We showcased the utility of this new serotyping strategy by identifying serotypes of 193 *S. pneumoniae* isolates from children. This strategy enables most routine laboratories equipped with PCR to predict the majority of pneumococcal
serotypes without the need for an expensive set of serological reagents in China.” We have also included some discussion on affordability and costs of molecular assays for developing countries and China (lines 338-344).

**Question 9:** 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.

**Answer:** We have included further discussion on this (lines 338-344), but avoided making the discussion too long. But we give further details on this as below:

The reagents used for PCR are commonly available in laboratories and relatively inexpensive; sequencing is convenient in most cities in China. Commercial sequencing costs is ¥ 16 yuan (≈ $ 2.5 dollar, ¥1yuan= $ 0.1567 dollar) for each sample. Therefore, the PCR and sequencing costs of serotyping based on deduction from sequencing and mPCR results is affordable, compared with serotyping using antisera. In fact, only 59.5% of the isolates in our study needed to be confirmed by mPCR without updating the cpsB database with our cpsB local data. After we update our cpsB database (as Supplementary Table S2), the proportion of isolates requiring further mPCR testing would be reduced further. Except for 3.6% of the isolates, most of them could be identified in the first three mPCRs in this study. When isolating DNA and performing mPCR, the isolates can be handled in batch, which reduces the hands-on time per isolate, and the amount of work per isolate is very reasonable. In our laboratory, these sequencing and mPCRs are performed when a sufficient number of samples have been collected for a run, which makes the cost very reasonable and acceptable.

**Minor Essential Revisions**

**Introduction**

**Question 10:** 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.

**Answer:** Thanks, and we agree with the suggestion and have added the sentence “On the other hand, latex agglutination is a simple and efficient alternative method to Quellung
reaction serotyping, but still needs further work to improve its capacity to detect colonizing pneumococcal strains at low density [7].” in the introduction section (lines 87-89).

Methods
Question 11: The methodology for sequence alignment should be specified (line 129).

Answer: “sequence alignment” means the sequences were aligned using either the Basic Local Alignment Search tool (BLAST) in PUBMED (http://blast.ncbi.nlm.nih.gov/Blast.cgi) or ClustalW (we have added a supplementary Figure S1). We have clarified this in the manuscript (lines 147-152). Based on the alignment, each GenBank sequence was given a unique sequertype name.

Results
Question 12: The footnotes to Tables 2 and 4 do not seem necessary.

Answer: Thanks, we have deleted them.

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
Referee 4:

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

Version: 2
Date: 21 October 2015
Reviewer: David Bean

Reviewer's report:
The manuscript builds on the “sequetyping” approach to determination of pneumococcal capsular type described by Leung et al (2012). This provides a simple method to predict serotype compared to traditional phenotypic tests and current multiplex PCR based molecular methods. Overall, I think this manuscript is a good addition to the data in this field and warrants publication.

I have no major compulsory revisions.

While the science is sound, the manuscript would benefit from some editorial attention before publication. This is fairly extensive, and I have marked up areas on a hard copy – I have no intention of transcribing them all here. I would expect there to be significant corrections before final publication.

Some examples of minor essential revisions (discretionary where indicated):

Question 1: Line 78: The term “first-tier big city” is not one I was aware of previously – perhaps omit/change?

Answer: We have extensively revised the manuscript for English consistency. “first-tier big city” means they are typically the biggest convention cities in the country. China’s first-tier cities usually refer to Beijing, Shanghai, Guangzhou, and Shenzhen. We have however deleted the phrase “first-tier big city” and replaced it with a more appropriate one- “one of the biggest cities in China” (line 80).

Question 2: Line 96: “We hope…” better replaced with “The aim…” (or similar)

Answer: Thanks, we have revised this.

Question 3: Line 109: Omit “were”
Answer: Thanks, we have omitted it.

Question 4: Line 110/199: “severe pneumonia (include very severe pneumonia)”

Answer: We revised the sentence as “severe and very severe pneumonia”.

Question 5: Line 112: “similar” should probably be “identical”.

Answer: Thanks, we have fixed this as suggested.

Question 6: Line 118/213: “Isolates culture”

Answer: Sorry, no found “Isolates culture” in line 213.
We have revised line 118 “Isolates culture and DNA preparation” as “DNA extraction from bacterial isolates”.

Question 7: Line 206: Perhaps include “To our knowledge, it is the most…”

Answer: Thanks, we revised the sentence as suggested.

Question 8: Line 226: It certainly represents a new ST, but far less likely to be a new serovar. The latter is a phenotypic description.

Answer: We agree and have revised the sentence.

Question 9: Line 239: mPCR – introduce the acronym in line 235.

Answer: Thanks, we have fixed this in line 99.

The above list is far from exhaustive.

Question 10: The paragraph beginning on line 213 is very important – yet very cumbersome to read. This should be better articulated. Perhaps augmented by a table? It also presents some epidemiological data – and while this is certainly worthy of note, it does distract from what is the main message in the manuscript.

Answer: We have generated a new Figure 1 to make the methodology clearer. We also have rewritten the paragraph (lines 245 to 263).
**Question 11:** Consequently I would urge the authors to consider omitting tables 3 and 4. The former, for example, is also covered in a paragraph (lines 283-289) which could be summarised in a single sentence.

**Answer:** Thanks, we omitted Tables 3 and 4, changed them to Supplementary Table S4 and S5 respectively. We summarised them in serotype distribution and antimicrobial susceptibility respectively, in the results section.

**Question 12:** The authors often make the comment that the technique is designed for “resource-poor” regions. Such regions are unlikely to have the luxury of their own sequencing machine. I don’t think this is the best application of the technology. Rather it simplifies serotype designation, removes the need for costly antibody libraries and potentially hastens the procedure.

**Answer:** We agree with the reviewer’s comments and have revised the wording appropriately. However, in China, “resource-poor” regions which don’t own sequencing machine, can send DNA samples for sequencing to companies such as Shanghai bio Engineering Technology Service Co., Ltd, which is convenient and cheap.

**Other comments:**

**Question 13:** Supplementary Table S3: “We can’t decide it is 15F or 15A in the study, so the ST name is a temporary name and may change after decide the actual serotype.” This comment is should not be included - clarify the situation.

**Answer:** We have revised the wording and deleted the comment.

**Question 14:** Supplementary Table S1: Gender is better expressed as male/female. Also this is ordered curiously – looks like it is roughly based on serotype – perhaps make this the first column and correct anomalies (such as 6B before 6A and put “unknown” last”.

**Answer:** We have revised the table (S1) and expressed gender as male/female.

**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.
Thank you for taking the time to consider this manuscript for publication.

Yours sincerely,

Professor Qiyi Zeng,

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