**Author’s response to reviews**

**Title:** Streptococcal toxic shock syndrome caused by the dissemination of an invasive emm3/ST15 strain of Streptococcus pyogenes

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**Author’s response to reviews:**
October 10, 2017

Yi-Tsung Lin, MD, PhD

Editor, BMC Infectious Diseases case report,

RE1: INFD-D-17-01180

Streptococcal toxic shock syndrome caused by the global dissemination of an invasive emm3/ST15 strain of Streptococcus pyogenes

Dear Dr. Lin,

We wish to resubmit our manuscript entitled “Streptococcal toxic shock syndrome caused by the global dissemination of an invasive emm3/ST15 strain of Streptococcus pyogenes” (RE1: INFD-D-17-01180) for publication as a Case Report in BMC Infectious Diseases.

The manuscript had been revised in accordance with the suggestions made by the Reviewers and the additional editor. All revisions are highlighted to the manuscript are highlighted in yellow. We appreciate the helpful suggestions offered by the reviewers.

The manuscript has been edited by English language editing service ENAGO, please find the attached certificate of editing.

I trust that you will now find the manuscript suitable for publication.

I look forward to your final decision.

Sincerely,

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From editorial

Where a mandatory Declarations section is not relevant to your study design or article type, please write "Not applicable" in these sections.

For the 'Availability of data and materials' section, please provide information about where the data supporting your findings can be found. We encourage authors to deposit their datasets in publicly available repositories (where available and appropriate), or to be presented within the manuscript and/or additional supporting files.

Reply to query:

Additional information has been included in the revised manuscript.

From reviewers

Chuan Chiang-Ni (Reviewer 1): This report described a case, the patient infected by invasive emm3/ST15 GAS strain. In addition to describe results from different medical examinations, authors claimed that the isolated strain is more similar to invasive GAS isolates from the UK but not other emm3-type isolates in Japan.

The conclusions of this report are "this case was caused by the invasive emm3/ST15 GAS strain" and "the genetic background of this invasive strain is more similar to invasive strains isolated from the UK but not isolates from Japan". The first conclusion can be achieved easily by typing this isolate by regular emm- and MLST-typing methods. Nonetheless, the results presented in this article cannot support the second conclusion. Is ST15 the frequently isolated type in Japan? If the isolate found in this report and other invasive isolates found in Japan were ST15, other analytical methods (e.g. cgMSLT used in this study) should be utilized to distinguish these isolates. In this study, the phylogenetic analysis was done. Nonetheless, was any clinical isolate other than SSI-1 from Japan included in this analysis? If the isolate found in this study is different from other invasive isolates in Japan, the phylogenetic tree should show the difference.

Reply to query:

We discussed the increased prevalence of emm3 GAS isolates in Japan (line 114) as follows; “Intriguingly, the molecular epidemiology of GAS in Japan suggests that the frequency of emm3 GAS has been recently increasing (2–6.9% in 2010–2012), but is not as dominant as emm1 (> 60%) 1.”

Thus far, emm3 GAS is not the predominant type in Japan. However, the molecular epidemiology, such as MLST, of emm3 GAS has not been well investigated. Accordingly, whole genome sequences of other Japanese emm3 GAS strains have not been yet been deposited in
public databases. This study presents the first complete genome sequence of Japanese emm3 GAS strain.

Other comments:

Figure 2: the ST type of selected strains should be indicated.

Reply to query:

Because ST406 (highlighted in green) strains are closely associated with ST15 strains, the former phylogenetic tree was not appropriate for this report. ST15 strains appear to be discriminated as two clusters by cgMLST. Both are highlighted as ST15 in red.

The format of references should be carefully checked and modified (e.g. group "a" "streptococcus").

Reply to query:

As the reviewer suggested, all references have been checked and revised.

Claire Turner (Reviewer 2): The authors have described a severe case of S. pyogenes infection caused by a genotype emm3 ST15 isolate. I think this is an interesting case report demonstrating the severity of disease that this genotype can cause. I do have a few minor comments that I feel need to be addressed.

1. I am not sure I agree with the reference that emm3/ST15 is the iGAS strain. I think certainly emm3/ST15 has the potential to cause iGAS but also causes non-invasive infections as well (as shown in your Figure 2). I think the title of the manuscript should be changed from 'the invasive emm3/ST15 strain' to 'an invasive emm3/ST15 strain' as that is what is being described in the paper.

Reply to query 1:

We discussed the invasive potential and the frequency by citing the following: “the most prevalent emm types found to cause iGAS disease worldwide, particularly emm1 and emm3 2,3.” in the background section.

As the reviewer suggested, the title has been revised.
2. Relating to my first point, the first line in the discussion is misleading. I think this needs to be changed from 'is known to be' to 'has the potential to be'. Not all isolates of emm3/ST15 have high capsule production - does KS030 have a mucoid morphology? High levels of capsule production in emm3 strains are often related to mutations in the regulatory system CovRS or rocA. Do the authors know if KS030 carries mutations in these regulators?

Reply to query 2:

As the reviewer suggested, the sentence was revised.

Whole genome information of all tested GAS strains is summarized in Additional file 1. Each carried a typical 1-bp deletion in the rocA gene, leading to hypervirulence. Some of those strains showed additional mutation in covR and covS.

3. The last line of the discussion - again I am not sure you can define emm3/ST15 as an iGAS strain. It is the global dissemination of emm3/ST15 with the potential to cause invasive disease.

Reply to query 3:

As the reviewer suggested, the sentence was revised.

4. Lines 98- When the authors refer to 'all known GAS strains' do they mean all emm3 GAS strains that have been genome sequenced?

Reply to query:

As the reviewer suggested, the sentence was revised as “all publicly available genome sequences of GAS strains.”

5. Line 99- Can the authors please clarify what they mean by the core genome of MLST? I don't quite understand how the genomic comparison and extraction of SNVs has been performed here.

Reply to query 5:

I apologize the short description of the methods. The description of comparative genome analysis was expanded in the revision.
6. Figure 2 - Can the authors please provide more information here as to the source of the isolates included in the phylogenetic tree? There are no references to any publications.

Reply to query 6:

Additional information of all publicly available genome sequences of GAS strains, including ST15, ST315, and ST406, are summarized in Additional file 1.

7. Line 72 - was this fever in the brother associated with pharyngitis?

Reply to query:

Yes, it was. The sentence has been revised as “his brother developed a fever with pharyngitis, which was diagnosed as a GAS infection”.