Author’s response to reviews

Title: Population structure and transmission modes of indigenous typhoid in Taiwan

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Author’s response to reviews:

RE: MGNM-D-19-00151

Population structure and transmission modes of indigenous typhoid in Taiwan

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Dear Prof. Prashanth Suravajhala,

Thank you for kind consideration of our paper. The manuscript has been revised according to you and the reviewer’s suggestions. The major changes in the revised version and the responses to the comments are as the followings. The location of revision indicated in the responses were in accordance with the ‘clean version’ of manuscript.

Reviewer #1:

1. The authors mentioned that they have assembled the draft genome of each isolate with the same SPAdes. Do they make this available to the readers here?

Response: Yes, we have submitted the whole genome shotgun project to NCBI under the accession number PRJNA437172. The information was also provided in Methods of the Whole genome sequencing (WGS) section (page 14).

2. The authors wrote "The S. Typhi isolates used in the study were all clinical strains identified during 2001 and 2014 in Chang Gung Memorial Hospital (CGMH)". Does this mean they have already know the strains then what is the novelty of this study?
Response: Before this study, the only information available for the clinical isolates was the antibiograms determined by disc diffusion method. In this study, we performed the whole genome sequencing (WGS), determination of the pulsotypes and WGS-based genotypes. We also screened the resistance genes and plasmids, and did phylogenetic analysis to trace the source of the local typhoid strains. We believe the finding was novel in the epidemiology of typhoid fever in this island.

3. Isolation of the strains and WGS methods were not adequately described.
Response: We have revised the methods of strain isolation and WGS in page 14.

4. Is there any pic available for PFGE? If so that should be presented
Response: Yes, the PFGE dendrogram was presented as supplementary figure 1.

Reviewer #2: General Comments

1. I congratulate the authors for coming up with an interesting story on transmission modes of indigenous typhoid native to Taiwan. They not only augment and deliberate the pulsotypes but also discuss on the other strains through the whole genome sequencing (WGS) strategy. When I say "interesting" it is, but I think the manuscript can be improved in many other ways (Please see below)

Strengths: A detailed account of observations from PFGE/WGS studies

Limitations: lack of figures for PFGE and no good high resolution images.

Response: The figure of PFGE was presented as supplementary figure 1.

2. Introduction (Lines 16-57): The authors appear to have missed citing some of the latest references where virulotypes were screened using WGS. For example, Wong et al. 2019, Oo Km et al. 2019, Tanmoy et al. 2018 works could be a great inclusion.

Response: The studies conducted by Wong et al. 2019 and Tanmoy et al. 2018 were cited as reference #11, 12. Thanks for reminding.

3. They could also give a strong rationale why WGS is considered for such studies. For example, what ails WGS when compared to genoserotyping diagnostics using real time PCR? Why there is an inherent need to go for WGS for characterising the strains, thereby the transmission modes could be better inferred.

They could also subtly mention the phenotype predictions linking the virulence to WGS studies. A great deal of such studies are underway if NOT for Typhoid.
Response: We have modified the Background section by adding some lines to describe the disadvantage of currently used methods for typing S. Typhi in page 4 ‘S. Typhi is a genetically monomorphic pathogen and tracing its phylogeny has been challenging. PFGE was considered the golden standard for epidemiological surveillance and outbreaks investigation of a variety of Salmonella species. Unfortunately, PFGE was also described as unreliable, subjective method and hard to communicate between laboratories. In the last decade, SNP-based typing methods have successfully established phylogenetic markers for discriminating S. Typhi subtypes. However, the discrimination power remains not satisfactory.’

The potential advantage of WGS in linking the phenotype prediction and WGS data was also mentioned at page 5 ‘A variety of phenotypes of the S. Typhi strains, including the antibiotic resistance and virulence, can also potentially be explored by the WGS method.’.

4. Results:

Page 6:

The results largely weighed on the classification of isolates. One prominent thing that they discuss is on "resistome". They should complement these findings with genotype X environment interactions.

Response: The paragraph was to describe the resistance genes differentially carried by distinct genetic lineage of the S. Typhi strains. We believe the information provided in this paragraph, Figure 1 and Table 1 should be enough and clear to the readers.

5. Page 7: The so-called "only one SNP" identical to the core genome isolates is not seen in Figure 1. I wonder how/why do they show the SNP in this. There is no PFGE figure either to support their arguments

Response: With careful examination, one should see the tiny difference in the tree lengths between ST55 and other three strains, ST52, 53, and 54. We stated the ‘one SNP’ to be precise, however, this was not our main point. What we would like to say here is the four strains were of very closed genetic relatedness and should be belonged to the same strain. The PFGE figure is presented as supplementary figure 1 now.

6. Discussion:

Page 10: Lines 33-36. The authors haven't discussed what the SNPs are. If any SNVs have been inherent to the subpopulation of Taiwan.

Response: The numbers of SNPs were provided for readers to have an impression of how close or how far the genetic relatedness was between different strains. The exact locations or the involved alleles were not the focus of this study. To avoid misunderstanding, we decided to direct the readers to the figure 1 without providing the exact numbers of SNPs. The numbers of the SNPs were removed.
Page 11: Line 7: Please avoid starting the sentence with an abbreviation. Use "The...WGS..."
Response: corrected.

7. Lines 32-40: Whence mentioning the reason of certain strains not MDR, they could reason if the human microbiota and the food/dietary habits from the people have had any role to play in import/indigenous cases

Response: The reason was discussed at page 12, ‘The carriage of resistant genes in strains from different countries may result from a variety of environmental and host factors including use of antimicrobial agents, dietary habits and gut microbiota of the residences.’

8. Methods: I don't see any statistics in terms of coverage etc.

No tissue details for WGS are mentioned.

All the URLs, for example ITOL etc., must be supported by "last accessed dates"

Response: The methods of coverage calculation and WGS were revised at page 14. The ITOL was a tool for annotation of the phylogenetic trees. It was not affected by the date of access.

Thank you again for helpful comments on our manuscript. We are hopeful the revision is acceptable.

Best regards,

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