Author’s response to reviews

Title: Multilocus sequence types of clinical Burkholderia pseudomallei isolates from Peninsular Malaysia and their associations with disease outcomes

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Author’s response to reviews:

Date:

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Dear Editor,

MANUSCRIPT NO. INFD-D-17-00417: RESPONSE TO REVIEWERS’ COMMENTS AND SUBMISSION OF THE CORRECTED VERSION

The above matter is kindly referred to.

Thank you for your kind consideration. The authors would also like to thank the reviewers for their valuable comments. Below are the authors’ responses to the reviewers’ comments:

Reviewer 1 (Izabela Korona-Glowniak)

I have no objections to the quality of the manuscript: methods are appropriate, all parts of the manuscript (methods, results and discussion) are decent and well-described. It has been enjoyable paper to read.

☐ The authors thank the reviewer for her description and opinion for the article

1. Comment:
The strength of the study is deep molecular analysis suitable for such kind of epidemiological research. However, the authors should consider to calculate a diversity of MLST loci by Nei's index $= 1-\sum \text{(allele frequency)}^2$ (Malorny B, Junker E, Helmuth R. Multi-locus variable-number tandem repeat analysis for outbreak studies of Salmonella enterica serotype Enteritidis. BMC Microbiol 2008; 8:84. doi: 10.1186/1471-2180-8-84).

Response:

Thank you. The authors have referred to the most applicable evolutionary analyses used in similar literatures to compare their results with other previous results obtained by similar analyses and then find the genetic relatedness among them to predict their evolution. The genetic distance was best described by eBURST method that is similar to Nei’s index in constructing the evolutionary distance according to SLV’s and we compared our results with similar studies. In addition, we applied another evolutionary principle that measured the distance according to SNPs using UPGMA method.

2. Comment:

p.8, lines 203-205. This sentence is misleading and needs to be clarified. It is unclear if 59 Malaysian isolates were included to total amount of strains or not.

Response:

Thank you. The sentence was edited and clarified. The number 59 refers to the number of sequence types (STs) registered in the database, not the number of isolates.

3. Comment:

p.10, lines 249-252. I should be mentioned that data of this statement was not shown.

Response:

Thank you. The authors added (data not shown) to the end of the sentence.

4. Comment:

In my opinion, Table 2 should be included as a Supplemental file as raw data.

Response:

Thank you and the authors appreciate the reviewer’s opinion. The data in the table are important and needed to be included in the main manuscript, as done in similar literatures.

5. Comment
I am not sure if Figure 2 is necessary in the manuscript because a reader is capable to analyze Figure 3 (showing of diagram with total number of STs from Figs 1 and 2) according to explanation in the body text.

Response:

Thank you. The author’s point of view is to show the significant difference made by our study to the status of STs plotted on eBURST snapshot before and after the study to show how the relation between STs became after.

6. Comment:

p.25, lines 652-653. In Fig 3, it should be mentioned how many strains were included to analysis (83 strains?).

Response:

Thank you. Yes, the number is already mentioned (83) and refers to the number of strains.

7. Comment:

The authors should correct the format of references cited in the text and in the Reference list according to requirements of Journal: "All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. The reference numbers must be finalized and the reference list fully formatted before submission."

Response:

Thank you. All references were adjusted according to journal format.

Reviewer 2 (Derek S. Sarovich)

1. Comment:

Line 37: correlate with disease? Do you mean correlate with disease severity or disease presentation?

Response:

Thank you. The authors meant “Correlate with disease presentation and severity”.

2. Comment

Line 46: MLST phylogenetic tree? Do the author’s mean eburst?
Response:

Thank you. For eBURST we used population snapshot diagram, while phylogenetic tree was established using MEGA6 software.

3. Comment

Line 52: MLST database map?

Response:

Thank you. “MLST database map” refers to the distribution of STs on the map of Malaysia, before this study all STs were collected and identified from the Eastern part of Malaysia (Sarawak state), but this study has identified STs belong to Peninsular Malaysia (the Western part) obtained from clinical specimens.

4. Comment:

Line 71: The authors have ignored a key publication in this section. There is good evidence that certain B. pseudomallei genes contribute to different clinical presentations between Asia and Australia; in particular, the bimABm gene, which has been strongly associated with neurological melioidosis (Sarovich et al., 2014 PLoS ONE 9(3):e91682).

Response:

Thank you. The authors didn’t notice the article during literature search because we reviewed literatures to search for an association between specific multi-locus sequence type and clinical presentation, we didn’t search for genes outside the MLST-scheme. However, the article is of high value, so we added the information in the suggested place and we kindly used the reviewer’s words. The article was also added in the reference list.

5. Comment:

Line 96: missing reference

Response

Thank you. The missing reference was added.

6. Comment:

Line 107-108: What does "preserve the assumption of independence of observations" mean?

Response:
Thank you. The authors meant “To avoid the repetition of the same ST identified from repeated clinical specimens collected from the same patient in the same day”.

7. Comment
Line 132 to 137: This description should be in figure legends rather than methods

Response:
Thank you. From our point of view, this description can be used as legend if all population snapshots were viewed in a single figure. But since all were presented separately, it was written in the method. If the reviewer has further suggestion we’ll be so thankful.

8. Comment
Methods: The authors provide little to no description of how the clinical presentations were classified. For example, how was bacteremic vs non-bacteremic cases determined? Was this diagnosed with blood cultures or some other method?

Response:
Thank you. We added the relevant reference from which we categorize the clinical presentations and clinical definitions, i.e. Zueter et al. The epidemiology and clinical spectrum of melioidosis in a teaching hospital in a North-Eastern state of Malaysia: a fifteen year review. BMC Infectious Diseases (2016) 16:333

9. Comment:
Line 160-161: No mention is made as to when different statistical methods were used. i.e. Why did the authors use Fisher's exact over Pearson's Chi squared?

Response:
Thank you. Fisher's Exact test is a way to test the association between two categorical variables when you have small cell sizes (expected values less than 5). Chi-square test is used when the cell sizes are expected to be large. This interpretation for statistical test selection is well-known and the authors felt that it was not necessary to address it in the literature. But if the respected reviewer suggests to add it, we’ll appreciate that and will apply it.

10. Comment:
Line 178-184: The information contained in Table 1 and the associated explanation are unnecessary and should be removed. Reporting of the individual STs is sufficient. The authors report the ST analysis in unnecessary detail.

Response:
Thank you. We already built for the information in the table in the section of methods, and results were listed in the table and then were discussed in the desired section. We see that the usefulness of these details is to show how our STs diverse in spite of being taken from almost localized population. If the respected reviewer still suggests to omit this part, we’ll appreciate that and apply it.

11. Comment:

Line 271: "expressed" should be changed to "identified"

Response:

Thank you. The word was changed as suggested.

12. Comment:

The discussion is overly verbose and should be substantially shortened.

Response:

Thank you. The discussion was adjusted as recommended.

Reviewer 3: (Surasakdi Wongratanacheewin)

1. Comment:

The authors should compared those STs found with other regions in Southeast Asia and other countries instead of compare only in 83 isolates and gave some limited information.

Response:

Thank you. We already compared our STs obtained in this study with each other, then with local STs identified previously in Malaysia, and then with regional STs identified in endemic countries of Southeast Asia and China. The respected reviewer can refer to the last two paragraphs in the section (Phylogenetic analysis) in the Methodology. The reviewer may also have a look at figures 3 and 4 and their comments in the results and discussion.

2. Comment:

If authors can compare STs with environmental isolates to find out the source of infections, it will be novel and useful. As MLST has been used for long time with B. pseudomallei and the result found in this paper is similar to those found before. The MLST is not useful for finding the bacterial strains and clinical correlation but it is useful for relapse or recurrent infection. Therefore the finding in this paper is not novel.
Response:

Thank you. The authors have already compared our STs with environmental STs, the respected reviewer can refer to phylogenetic tree and population snapshots in the results and find their discussion. The source of infection is well-known to be environmental. In addition, some literature found genetic correlation with clinical presentation (Sarovich et al., 2014 PLoS ONE 9(3):e91682). Although our paper didn’t find that correlation but at least reported negative result and agreed with literature in the absence of MLST-disease correlation, but this time from Malaysia since almost all studies were done in Australia and Thailand. We kindly present this paper as firstly done in Malaysia to use MLST, and from that country we announced the negative correlation with disease. We identified 13 STs that are definitely novel (never published before).

3. Comment

The phylogenetic maps shown in this paper is descriptive and it gives no much new information.

Response:

Thank you. The phylogenetic maps described the genetic relatedness among STs and predicted their origin and helped to answered many questions regarding their evolutionary events.

The authors hope that all responses will satisfactorily meet the expectation of the reviewers and the editorial board.

Thank you.

Yours sincerely,

AbdelRahman M. Zueter