Author’s response to reviews

Title: Antimicrobial characteristics of Berberine against Prosthetic Joint Infection-related Staphylococcus aureus of Different Multi-locus Sequence Types.

Authors:

Jiaqi Tan (tan984572523@163.com)
Jin Wang (wangjin6hao@gmail.com)
Chuang Yang (arthroyang@163.com)
Chongzun Zhu (zhuchongzun998@126.com)
Geyong Guo (1013368129@qq.com)
Jin Tang (tangjin6ph@163.com)
Hao Shen (shenhao7212@sina.com)

Version: 3 Date: 20 Apr 2019

Author’s response to reviews:

Dear Dr. Liam Messin

Thank you very much for your edition of our manuscript BCAM-D-18-01496R2. We also are grateful for the reviewers’ detailed comments and suggestions, and we believe that their advice have greatly improved our manuscript. We have carefully considered those comments and made resubmission paper to address the reviewers’ concerns.

The following are our responses to the reviewers’ comments and original manuscript has been modified in line with some of reviewers’ advice and highlighted in yellow color.

Yours sincerely

Hao Shen
Editor

Question 1: Please represent authors' names using their initials, not their full name, in the Authors’ Contributions section. If there are any duplicated initials, please differentiate them to make it clear that the initials refer to separate authors.

Response: We have rewrote the author’s names in the ‘Author’s Contributions’ section.

Question 2: Please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. If Figure titles/legends are within the main text of the manuscript, please move them.

Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them

Response: We have added a 'Figure Legends' section after the References in the manuscript and removed the titles in the figure file.

Question 3: As berberine was not directly applied to patients in this study, we would ask that you amend your Conclusions to reflect this, as you cannot definitely conclude that this study reflects what would happen to patients.

Response: We have amended the Conclusion section accordingly.

Reviewer 1

Question 1: In order to be published to BMC complementary and Alternative medicine, this paper need to be accompanied by major revisions and entirely restructured, being focused on mechanistic insights.

Additional analysis on the NGS data for MLST check needs to be performed.

The main issue is related to the figure 3, additional information related to the altered transcriptomic pattern should be presented, the data need to have a biological significance using specific bioinformatics tools (KEEG, Reactome, Ingenuity…). Therefore the 'hub gene' investigation and 'Protein-Protein Interaction (PPI)' analysis resulted in insignificant housekeeping genes is mandatory to increase the value of the paper. Volcano plot gives only
information related to the number of altered transcript, no information related to the biological significance. Therefore gene ontology classification and some networks are mandatory.

The conclusion section needs to be sustained by a proposed mechanism of action for exerting antibacterial activity for S aureus. These should be sustained by in vitro data, to sustain the hypothesis related to PJI.

Response: On the background of our study, this paper aimed to investigate the antimicrobial ability of berberine against PJI related S. aureus in both planktonic and biofilm state which had never been done before, and the exact mechanism of berberine inhibiting S. aureus was not the goal of this paper but our next research program. We also did transcriptomic analysis to confirm the inhibiting property of berberine against tested S. aureus by showing berberine inducing disruption of major gene expression and especially the down-regulation of vital genes for bacteria survival. However, the exact mechanism of berberine inhibiting S. aureus was not sufficiently uncovered by our data in this study, and we were worried that merely showing the limited data of transcriptomic analysis and negative results of ‘hub gene’ and ‘protein-protein interaction (PPI)’ did not contribute to revealing the underlying mechanism. Therefore, we restricted ourselves from partially explaining the mechanism on insufficient data. And we have started a new program to investigate the exact mechanism by conducting both transcriptomic analysis and comparative genomic analysis between ST39 and ST239 on the assumption that genome structure differences might contribute to the distinct sensitivities of S. aureus to berberine.

Question 2: A flow chat with work flow for the transcriptome assembly and annotation for Staphyloccocus aureus will be very useful.

Response: We have created a workflow in the new figure 1 to show the transcriptome assembly and annotation for Staphyloccocus aureus.

Question 3: The data from figure 1, can be represented as % of control, in this case can be much easy demonstrated the antimicrobial activity of berberine..

Response: We reformulated figure to make it clearer in the first revision according to the editor’s advice. It’s true that ‘% of control’ would be demonstrated the antimicrobial activity of berberine, however, here we aimed to show the entire growth process as well as the details in growth kinetic of S. aureus treated in berberine. Therefore, we chose to show the results with a combination of five pictures.
Question 4: Additional information related to the berberine solution, please define GC for figure 2 and 3.

Response: We have defined GC as ‘Wells containing 1% DMSO and bacteria was the bacterial growth control (GC)’ in the new figure 3 and figure 4.

Reviewer 2

Question 1: The recommends have been essentially addressed. However, there are a few which have been once again highlighted for your action..

Response: We have rechecked our manuscript and made changes as necessary.