Author’s response to reviews

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

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Author’s response to reviews:

Dear Dr. Liam Messin

Thank you very much for your edition of our manuscript BCAM-D-18-01496R1. 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: Figure 1 requires editing to make the data presented clearer. At the moment it is not clear the differences between conditions A - X are. The Absorbance axis could be adjusted to make it clearer which line is which. Also colour can be used as well.

Response: We have reformulated figure 1 to make it clearer.

Question 2: Additional information regarding the data analysis, particularly relating to NGS, should be included throughout the manuscript.

Response: As explained in the response to question 1 of reviewer 1, we found that the transcriptomic profile only was not adequate to explain the exact mechanism, and thus we are conducting a comparative genome-sequencing program of strain ST239 and strain ST39 in order to figure out the relation between the genomics structure of S. aureus and antimicrobial mechanism of berberine. Results will be presented in our next article.

Question 3: Please clarify the selection criteria for the gene panel used in the validation of qRT-PCR.

Response: The genes (icaA, icaR, fnbA, lrgA, lrgB, cidA, srrB, spa, nuc) are proved associated with the attachment, biofilm formation and toxicity of S. aureus which are very important parts of pathogenesis in PJI. Unlike house-keeping genes, these genes could be expressed in vast differences under various environments, so the determining the expression of these genes can fairly reflect the accuracy of RNA sequencing results. In addition, the expression of these genes reflects the stress response of S. aureus to berberine and shows the bacterial adaption to environment stress, which could be important for S. aureus resisting berberine.

Reviewer 1

Question 1: Figure 3, the altered gene expression level will be very usefully to be integrated in some software for gene ontology classification, emphasis important biological networks and the clinical relevance of this findings.

Response: Although we did RNA sequencing of S. aureus ST1792 treated with berberine and analyzed the antimicrobial mechanism of berberine against S. aureus, we found that transcriptomic profile only was not adequate to explain the exact mechanism. The ‘hub gene’ investigation and ‘Protein-Protein Interaction (PPI)’ analysis resulted in insignificant house-keeping genes (we can provide the results if needed). However, in our present data we found that
S. aureus ST239 (strain L, M, N) were particularly sensitive to berberine (MIC 64 μg/ml) while S. aureus ST39 (strain E) was most resistant (MIC 512μg/ml). Given this, we assume that the secret of distinct sensitivity of S. aureus to berberine may lie on the structural differences among the genomes of subtype strains. Therefore, we conducted comparative genome-sequencing of strain ST239 and strain ST39 to verify our assumption. Analyses are being done and further experiments investigating the exact mechanism of berberine inhibiting S. aureus are being performed by us at the moment.

Question 2: A new figure presenting the antimicrobial biological mechanism of action of berberine.

Response: We reviewed articles relating to the antimicrobial mechanism of berberine against S. aureus available online and found the exact mechanism is not clear at the moment. Our transcriptomic profile only was not adequate to explain the exact mechanism either. Therefore we are not able to draw a new figure presenting the antimicrobial biological mechanism of action of berberine.

Reviewer 2

Question 1: In the attached PDF file kindly provided by reviewer 2 and the reviewer asked that if ‘serotype’ was more appropriate to replace the term ‘subtype’ in the manuscript and as the MICs of berberine against S. aureus were relatively large, whether it’s proper to be used locally as an antibacterial agent.

Response: First of all, we are very grateful for your careful reading and tireless corrections of our manuscript, according to which we have revised the article.

As serotype is established according to the differences between the protein products produced by different bacterial strains which usually act as antigens within the host body, as in the case of staphylococcus. In our study, we identified the subtypes of S. aureus on the basis of Multi-locus sequence typing (MLST) result, which reveal the structural differences of 7 house-keeping genes. Therefore, subtype is more appropriate for our article.

It’s true that the MICs of berberine against S. aureus are relatively large comparing to widely used antimicrobials and it’s not effective to use berberine alone locally for infection control. That’s why we proposed in the article that berberine be administered locally as an ancillary agent combined with other antibiotics as studies have revealed that berberine can enhance the efficacy of antimicrobials and promote local host immunity against infection.
Question 2: The sample size collected might not justify the significance of Multi-locus Sequence Types used in the study. If there is a way to improve this by the Authors, it would strongly bring out the anti-infective characteristics of berberine.

Response: In our study, we focused on the inhibitory efficacy of berberine against S. aureus related to Prosthetic Joint Infection (PJI) which is a disastrous but uncommon complication (less than 1%) after total joint arthroplasty (TJA). Among the limited clinical cases we isolated 18 S. aureus in our hospital in recent years, which was an acceptable size of sample in respect of PJI. However, to strongly bring out the anti-infective characteristics of berberine against S. aureus, we have started a new program expanding our researching area from PJI to all orthopedic infection in our hospital, which has already isolated over 200 S. aureus strains. Further MLST identification of the bacteria and MIC testing of berberine are about to be performed.

Question 3: The Figures 1A -E were somehow difficult to appreciate. The Authors could have considered much more easy way of presenting this. Individual plots represented in one figure might help (as done in Fig 2). They could have also defined once again with note what the A to X represent.

Response: We have reformulated figure 1 to make it clearer.

Question 4: Again, Fig 3 is not clear and difficult to appreciate, particularly the B.

Response: Fig 3 was designed to show the overall changes in gene expression of ST1792 treated by berberine. B was a volcano plot widely used by researchers to present gene expression profile in transcriptomic sequencing programs.

Question 5: Finally, the study's attempt was recommendable however, the overall write-up was not comprehensive with limited discussions.

Response: We have expanded our discussion and polished our language.