Author's response to reviews

Title: Analysis of S. epidermidis icaA and icaD genes by polymerase chain reaction and slime production: a case control study

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Author's response to reviews: see over
Dear Editor,

Many thanks for providing us an opportunity to revise the manuscript “Analysis of \textit{S. epidermidis} icaA and icaD genes by polymerase chain reaction and slime production: a case control study” (MS: 1720275908701412).

We appreciate very much your important decision and comments for the original manuscript. We have finished the revision according to your comments and now return the revised version (Analysis of \textit{S. epidermidis} icaA and icaD genes by polymerase chain reaction and slime production: a case control study _4th version) with the response to you. In this version, we have carefully addressed all points raised by you and make detailed revisions according to the suggestions. Our specific responses and the corresponding changes to each of the points are summarized on the following response sheet addressing to you and the changes in the revised manuscript (Analysis of \textit{S. epidermidis} icaA and icaD genes by polymerase chain reaction and slime production: a case control study _4th version) have been marked in RED font.

In summary, the manuscript has been thoroughly revised and the concerns raised by the reviewers have been addressed carefully on a point-by-point basis. We believe that the revision has significantly improved the quality of the manuscript and hope that it will be accepted for publication. Thank you for your considerations.
Sincerely yours,

Bao Liu, M.M.

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Response to the comments:

Note: the numbers of the line refer to the numbers of line in the 4th version.

Reviewer's report:

Authors corrects the manuscript

1. but references should be revised as there some references repeated 2 times and there is fault in writing some references

Re: Per your suggestion, I had revised the references and delete duplicate ones. Also, the citations in the next were updated accordingly. Thank you.

Reviewer: Tsai-Ling Lauderdale

Reviewer's report:

In general, the question posed by the authors is well defined, the methods are appropriate, and the data are OK for the most part. However, the writing is still not acceptable, which reduce the value of the manuscript. Some statements in Results and Discussion need to be clarified to improve coherency.

Major Compulsory Revisions

INTRODUCTION
2. L88 reference 7 (Gatermann SG et al., 2007) is on macrolide resistance, not investigation of the ica locus. Do the authors mean reference 17?

Re: In the line 85 “11” instead of “7”. In the line 336-338 “Arciola CR, Baldassarri L, Montanaro L: In catheter infections by Staphylococcus epidermidis the intercellular adhesion (ica) locus is a molecular marker of the virulent slime-producing strains. Journal of biomedical materials research 2002, 59(3):557-562.” was inserted. Thank you.

MATERIALS & METHODS

3. What is the length of study? At L90 is “12-month” and at L118 is “15 month

Re: In the line 87 “15” instead of “12”. Thank you.

4. L200: “PCR was performed on the 82 S. epidermidis isolates from infection associated with catheters, Typical results are shown in the Figure 2” Since not all 82 isolates were from catheter associated infections, this should be corrected. Also, change the second part to “Examples of PCR results are shown in Figure 2”.

Re: Per your suggestion, in the line 190 “from infection associated with catheter” was deleted. In the line 191 “2” instead of “4”. Thank you.

RESULTS
5. Again, PCR detection is not ‘expression”, so

- Table 1 footnote, change “expression” to “the presence”.
- L204. “and it was also demonstrated that icaD had higher expression rate than icaA…” Change “expression” to “positive”
- Also at L260-261 in Discussion, PCR positive does not mean “higher rate of expression”

Re: In the line 420 “the presence” instead of “expression”. In the line 194 and 250 “positive” instead of “expression”. Thank you.

6. L194-197: The sentence should be separated into two, one for Table 1 data and the other for Table 2 data. At L196-197, do the authors mean to say “All 15 slime producing S. epidermidis isolates were positive for both icaA and icaD (Table 2)”.

Re: Per your suggestion, I divided the sentence into two parts.

“Among the clinical isolates from blood of catheter of patients, 15 of the 22 S.epidermidis were found to be positive for both icaA and icaD, and 15 strains were found to be biofilm producers by the CRA method (Table 1), and 15 strains of S.epidermidis produced slime and were positive for icaA and icaD, two methods used for measuring in this study.” was moved from the line 187 to the line 195-198 and was replaced with “For the clinical isolates from catheter blood, 15 of 22 S.
epidermidis were found to be positive for both icaA and icaD, and 15 strains were found to be biofilm producers using the CRA method (Table 1). All 15 slime producing S. epidermidis isolates were positive for both icaA and icaD (Table 2)’’.

Thank you.

7. **L194-197 should also be moved to “PCR detection” section instead of under SEM.**

Re: Per your suggestion, “Among the clinical isolates from blood of catheter of patients, 15 of the 22 S. epidermidis were found to be positive for both icaA and icaD, and 15 strains were found to be biofilm producers by the CRA method (Table 1), and 15 strains of S. epidermidis produced slime and were positive for icaA and icaD, two methods used for measuring in this study.” was moved from the line 187 to the line 195-198 and was replaced with “For the clinical isolates from catheter blood, 15 of 22 S. epidermidis were found to be positive for both icaA and icaD, and 15 strains were found to be biofilm producers using the CRA method (Table 1). All 15 slime producing S. epidermidis isolates were positive for both icaA and icaD (Table 2)”.

Thank you.

**DISCUSSION**

8. **L227. Please explain how the finding from this study is similar to reference 3, which was a study on RsbU.**
Re: We deleted the sentence “This finding is similar to what have been reported by Knobloch JK et al” at L219. Thank you.

9. L 252-254. "In other respects involves the second stage of biofilm formation has demonstrates that cell aggregation and biofilm accumulation by some genes, ........”, what is this sentence trying to say?

Re: Slime accumulation was mediated by the chromosomal ica gene, which comprises four intercellular adhesion genes (icaA, icaB, icaC and icaD) and one regulator gene (icaR). To clarify, we have changed the sentence to “In other evidences, the biofilm accumulation is mediated by certain genes, such as icaA, icaB, icaC, icaD and icaR” at L242-243. Thank you.

10. L263. “The present study has shown the lack of icaC in some isolates”? How?

Also, “what does “mapped in the entire operon” refer to? Please rewrite to clarify.

Re: We meant that the reason for the absence of biofilm production, despite the presence of some ica operons, is the lack of positive icaC gene in some isolates. We have changed the sentence to “The present study has shown the reason for the absence of biofilm production in some isolates is the lack of icaC in some isolates, or only one promoter located at the upstream of the icaA and mapped in the entire operon” to “The present study has shown that the reason for the absence of biofilm production in some isolates, despite the presence of the ica operon, is the lack of
Minor issues not for publication but must be corrected

11. This manuscript should be carefully edited by a professional editor.

Re: The manuscript had been carefully edited by a professional editor who has a PhD and MD in USA and strong publication tracking records.

Below are some examples this reviewer found.

12. Keywords: “scanning electron microscopic” should be “scanning electron microscopy”

Re: Per your suggestion, in the line 33, 49, and 454 “scanning electron microscopy” instead of “scanning electron microscopic”. Thank you.

13. L87-88: “In the recent years, many studies to investigate the mechanisms of the ica locus is a marker of S. epidermidis obtained from nosocomial infections related to implanted medical devices” suggest changing to “In recent years, several studies have been conducted to investigate the ica locus as a marker of S. epidermidis obtained from nosocomial infections related to implanted medical devices”

Re: Per your suggestion, in the line 84-85 “many studies to investigate the
mechanisms of the ica locus is a marker of S. epidermidis obtained from nosocomial infections related to implanted medical devices” was replaced with “a study has been conducted to investigate the ica gene as a marker of the adhesive aptitude”. Thank you.

Re: Per your suggestion, in the line 150 “primer” was deleted. Thank you.

Re: Per your suggestion, in the line 176 and 212 “production” instead of “producing”. Thank you.

16. For S. epidermidis, there needs to be a space between the genus and species.
   “S.epidermidis” can still be found throughout the manuscript including the title.
Re: Per your suggestion, “S. epidermidis” instead of “S.epidermidis” in the whole manuscript. Thank you.

17. IRB approval was stated twice, once at L104-106 and again at L128-130.
Re: we agree with you. In the line 125 “Ethical approval for the study was obtained from the Central Research Ethics Committee in the Provincial Hospital. All enrolled
patients and volunteers provided written consent prior to the participation” was deleted. Thank you.

18. L211. “It has now be found” should be “It has now been found”
Re: Per your suggestion, in the line 203 “been” instead of “be”. Thank you.

19. L235-237 “In addition, we conjecture that the virulence factor of S. epidermidis will be weak” is stated twice.
Re: Per your suggestion, in the line 227 “In addition, we speculate that the virulence factor of S. epidermidis will be weak” was deleted. Thank you.

20. Line 246 “fetal infection”, “fetal” should be “fatal”
Re: Per your suggestion, in the line 236 “fatal” instead of “fetal”. Thank you.

21. Reference formats are inconsistent throughout. Some start with initials of first names of authors instead of last name, some list full first and last names.
   It is messy.
Re: The formats were corrected. Thank you.

22. Quality of written English: Needs some language corrections before being published
Re: The manuscript had been carefully edited by a professional editor who has a PhD
and MD in USA and strong publication tracking records.

23. Other revisions:

In the line 36 “production” was inserted.

In the line 37 “those” was inserted.

In the line 38 “both” was inserted.

In the line 42 “The co-expression of icaA and icaD was associated with enhanced slime production.” was deleted.

In the line 71 “which” was inserted.

In the line 76 “the” was inserted.

In the line 77 “of 55–77% and even 86%” was deleted. In the line 77-78 “to be 55–77%, or even 86%” was added.

In the line 83 “as” was inserted.

In the line 84 “The microbial production of biofilm absorbed onto the surface of
various biomaterials has been considered.” was deleted.

In the line 89 “analyzed” was inserted.

In the line 244 “due to their” was inserted. And “marker” was deleted.

In the line 245 “collected” instead of “come” and “patients with catheters associated” instead of “associated with catheters”.

In the line 249 “other studies” instead of “others result”.

In the line 252 “detection”