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

Authors:

Shusheng Zhou (zhouss108@163.com)
Xiaoguang Chao (chaoxg1001@gmail.com)
Mingming Fei (feimm202@gmail.com)
Yuanyuan Dai (daiyy118@163.com)
Bao Liu (az306w@gmail.com)

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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 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 S. epidermidis icaA and icaD genes by polymerase chain reaction and slime production: a case control study _3rd 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 S. epidermidis icaA and icaD genes by polymerase chain reaction and slime production: a case control study _3rd 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.

Department of Laboratory

Affiliated Provincial Hospital of Anhui Medical University
Response to the comments:

Note: the numbers of the line refer to the numbers of line in the revised version.

1st Reviewer's report:

Major Compulsory Revisions

1. The question posed by the authors is not well defined and the writing is confusing.

Re: we have revised the study questions and had the manuscript edited by professionals. Thank you.

① In the line 104, “The isolates were identified were performed by classical microbiological methods.” was replaced with “All S. epidermidis isolates were characterized using vitek 2 compact (bioMe´rieux, France)”;

② As your suggestion, in the line 98 “This study analyzed 82 S. epidermidis isolates collected from May 2009 to July 2010” was inserted. Thank you.

③ In the line 82-85 “Already in 1972, the “slime” production of S. epidermidis was noted as an important factor in the pathogenesis of infections. The biofilm formation enables attachment and persistence of the bacteria on foreign materials. Slime is an accumulation of embedded
microbes in a polysaccharide matrix on various biosurfaces” was replaced with “In early study, the “slime” production by *S. epidermidis* was found to be significantly associated with clinical infections [9]. Coagulase-negative staphylococci, particularly *S. epidermidis*, are the important cause of infections associated with foreign materials. The microbial production of biofilm absorbed onto the surface of various biomaterials has been considered.”

Thank you.

2. **Better literature review is needed to support their study rationale and conclusions.**

Re: Thanks you. As your suggestion, some new references were added.


Thank you.
3. The study design and objectives need to be better defined.

Re: The study design and objectives in line 118-130 were rewritten for clarity.

In the line 118-130 “This study was prospective. Eighty-two strains of S.epidermidis were collected by sterile swabs. Only cases with bacteraemia from catheter-associated infections, as confirmed by positive blood cultures and clinical evidence of catheter-associated infections were included in the study. Cases without neutropenia and cases with bacteraemia caused by mixed isolates were excluded in order to avoid the contamination of S.aureus and confusion about the true pathogen” was replaced with “This study was a prospective design. Eighty-two strains of S.epidermidis were collected over a 15 month period. Eighty-two S. epidermidis isolates were collected using sterile swabs from patients’ central venous catheter blood, and from the nasal vestibules of non-medical volunteers, ICU hospital staff, and patients. A total of 70 volunteers and 69 staff (doctor and nurse) who haven’t had any diseases were included. Among them, the volunteers were 1st year medical students from Anhui Medical University. Only cases with bacteraemia from catheter-associated infections, confirmed by positive blood culture and clinical evidence of catheter-associated infections, were included in the study. Cases without neutropenia and cases with bacteraemia from mixed pathogens were excluded in order to avoid the contamination of S.aureus and confusion about the true pathogen. The study was conducted in the ICU of the Affiliated Provincial Hospital, Anhui Medical University, Hefei, China. The unit has the capacity to provide care for 54 patients. 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”.

In the 27-28 lines “We aimed to define the nature of S. epidermidis isolates by studying the icaA and icaD and slime in relation to clinical and microbiological features” was replaced with “We aimed to study the effects of icaA and icaD genes on the slime formation of Staphylococcus epidermidis associated with catheter-associated infections.”

Thank you.

4. The authors also made several suggestions on the meaning of their results in the Discussion section without supporting data (similar to those cited above).

Re: We made several changes in the discussion section, particularly on the suggestions made from this study.

In the line 227-228 “suggests the possibility that some environmental conditions, such as body fluid and no-body fluid or the presence of accessory genes, can influence the phenotypic behavior in the Congo red agar plate” was replaced with “is similar to what have been reported by Knobloch JK et al [3].” thank you.

In the line 269-270 “The co-expression of icaA and icaD can be considered as important markers for the clinically significant slime-positive phenotype” was replaced with “, and another study demonstrated that co-expression of icaA and icaD was associated with enhanced slime production [30]”
In the line 280 there is a “suggest” in “This finding suggests the possibility that co-expression of mecA and icaD is associated with enhanced clinical isolates that produce an extracellular matrix called slime and may make them more resistant to antibiotics”. In general, we think that MecA gene expression level may correlate with the level of Staphylococcus resistance, and a high rate multi-drug resistance (MDR) at the same time. In our study, all isolated S.epidermidis slime-positive strains also showed that icaD gene had a higher rate of expression (in the 207-210 lines). As a result, we think that the co-expression of mecA and icaD is associated with enhanced clinical isolates that produce an extracellular matrix called slime, which may make them more resistant to antibiotics. In the line 280 “and” instead of “that”. In the line 284 “and in guiding clinicians to therapeutic measures.” was deleted.

Thank you.

5. Speciation of CNS can be method dependent. How was identification of S. epidermidis made in this study?

Re: As your suggestion, in the 104 line “All isolates were characterized by classic microbiological methods” was replaced with “All S. epidermidis isolates were characterized using vitek 2 compact (bioMe´rieux, France)” Thank you.

6. All the references need to be rechecked for citing accuracy and relevance. Some of the
references cited are either misplaced or not appropriate. For example,

- Reference 1 is on coagulase-negative staphylococci (CNS) bacteremia in a newborn ICU and was published in 2002. It is not a good reference to show that “CNS are the most frequent cause of nosocomial bloodstream infections” as the authors stated in the first sentence of their manuscript.

- Reference 4 is on activation mechanisms of biofilm formation in S. epidermidis published in 2001. How does that relate to the authors’ statement in the first paragraph that “S. epidermidis is isolated with increasing frequency as the causative pathogen of nosocomial sepsis, and accounts for approximately 30% of all nosocomial bloodstream infections”? I assume the authors meant reference 3.

Re: we double checked all references cited in the manuscript and made the corrections Thank you.


Thank you.

7. Several sentences in the manuscript are identical to those found in published articles. A few examples of this inappropriate use are below (and this reviewer only checked a few selected sentences).

- First and second sentences in paragraph 2 “Already in 1972, the “slime” production of CNS was noted as an important factor in the pathogenesis of infections. The biofilm formation enables attachment and persistence of the bacteria on foreign materials.” are found in the “virulence factors” section of reference 3.

- Another sentence in the same paragraph “Bacterial adhesion with biomaterials has been suggested as playing a crucial role in the induction of severe nosocomial infections” is identical to a sentence in Chaieb K, et al., J Hosp Infect. 2005;61:225-30.

The conversion of the CNS from symbiont to human pathogen has been a direct reflection of the use of indwelling medical devices” is from Rogers KL et al., 2009;23:73-98. Infectious Disease Clinics of North America.

Re: We made the corrections. Thank you.

① in the line 82-83 “Already in 1972, the “slime” production of S. epidermidis was noted as an important factor in the pathogenesis of infections” was replaced with “In early study, the “slime” production by S. epidermidis was found to be significantly associated with clinical infections”;

② In the line 83-85 “The biofilm formation enables attachment and persistence of the bacteria on foreign materials. Slime is an accumulation of embedded microbes in a polysaccharide matrix on various biosurfaces” was replaced with “Coagulase-negative staphylococci, particularly S epidermidis, are the important cause of infections associated with foreign materials. The microbial production of biofilm absorbed onto the surface of various biomaterials has been considered” ;

③ inline 85-86 “Bacterial adhesion with biomaterials has been suggested as playing a crucial role in the induction of severe nosocomial infections” was replaced with “Bacterial adhesion has been considered the leading cause of severe nosocomial infections related to implanted medical devices” ;
In the line 191-192 “Slime is an biomass of exopolysaccharide material that clings on Culture medium surfaces, germinal slime is viscoelastic in consistency and binds with communal bacteria” was replaced with “Slime is a biomass of exopolysaccharide that adheres to culture medium surfaces. It appears as clear mucus.” in the line 192 “(Scale bar _2 µm)” was added.

In the line 213-214 “The conversion of the CNS from symbiont to human pathogen has been a direct reflection of the use of indwelling medical devices” was replaced with “The major virulence factor is that they can form biofilm on polymeric surfaces and adherence to catheters and other artificial materials during the early phase of biofilm development”.

Thank you.

8. The Groups in Tables 1 & 2 need to be defined. I assume these correspond to Group 1, blood isolates obtained from patient IV line; Group 2, nasal isolates of healthy volunteers; Group 3, nasal isolates of the same patients in Group 1; Group 4, nasal isolates of ICU staff. This should also be explained in text.

Re: Yes, I agree with you. in the line 419-420 and 433-434 “Group 1, blood isolates obtained from patients’ IV line; Group 2, nasal isolates of healthy volunteers; Group 3, nasal isolates of the same patients in Group 1; Group 4, nasal isolates of ICU staff.” were inserted. In the line 436-437 “There was no statistical difference among other three groups (P>0.05).” was inserted. Thank you.
9. When were the isolates recovered? What was the S. epidermidis recovery rate? i.e, how many staff and volunteers were surveyed? How were they chosen? What was the time frame compared to the blood isolates?

Re: The S.epidermidis were stored in trypticase soy broth (TSB), to which 15% glycerol was added at -20°C about 1-15 month period. When we started the study, the isolates were recovered. The S. epidermidis recovery rate reached 100% according to our calculation. In the line 113-115 “for 1 to 15 months long. The isolates were recovered when the study was initiated. The S.epidermidis recovery rate reached 100%.” was added.

A total of 70 healthy volunteers and 69 staff (doctor and nurse) were enrolled during May 2009 and July 2010. All volunteers were freshmen of Anhui medical University. The meaning was expressed in the line 121-123 (from “A total of 70 ---” to“--- Anhui medical University”).

The time frame compared to the blood isolates was 15 month in the study.

Thank you.

10. What % of the blood isolate patients were surveyed for nasal carriage and what % were positive? Did the authors compare the nasal isolates with the blood isolates from the same patient?

Re: Sixty five blood isolate patients were surveyed for nasal carriage and 33.9% (22/65) of them were positive. In the line182-183 “Sixty five patients who provided the blood isolate were surveyed
for nasal carriage and 33.85% (22/65) of them were positive.” was inserted.

Yes, we compared the nasal isolates with the blood isolates from the same patient between group 1 and group 3.

Thank you.

11. Although slime production was found in lower % of nasal isolates, a good % of the nasal isolates were slime producers and carried icaA and icaD genes. How do the authors explain this?

Re: We think there are three potential reasons ① small number of cases in the study; ② we did not investigate the regulator gene (such as icaR), which could be engineered to inhibit icaA/D expression, in this study; ③ lack of the icaC in some isolates. We think additional study is needed to test these hypotheses. Thank you.

12. Although icaD was found in more isolates, it does not mean it is “more expressed” (page 11).

Re: Yes, I agreed with you. In the line 260-263 “In addition, our investigation demonstrated that icaD is more expressed than the icaA in all isolated S.epidermdis, but slime production occurred only when both icaD and icaA were expressed. The reasons for the absence of slime production in some isolates despite the presence of the entire icaD remain unclear studies [24,25] have shown the lack of transcription of the icaC in some isolates, or only one promoter located at the upstream of
the *icaA* and mapped in the entire operon.” was replaced with “In addition, our investigation demonstrated that *icaD* has a higher rate of expression, but slime production dependeds on the presence of both *icaD* and *icaA*. The present study has shown the lack of *icaC* in some isolates [28, 29], or only one promoter located at the upstream of the *icaA* and mapped in the entire operon.”

Thank you

13. Page 12, the sentence “This finding suggests the possibility that co-expression of *mecA* and *icaAD* is associated with enhanced clinical isolates that produce an extracellular matrix called slime that may make them more resistant to antibiotics” is another example of the confusion of this manuscript. Enhanced what? Other than the presence of *mecA*, there were no data showing that slime producers were more resistant to antibiotics in this study.

Re: Co-expression of *mecA* and *icaD* may enhance the ability of clinical isolates to produce slime. At the same time, in general, we think that the level of *MecA* gene expression may correlate with the level of *Staphylococcus* resistance, and the rate of multi-drug resistance (MDR). As a result, we think it is the co-expression of *mecA* and *icaD*, *rather than slime production*, that makes clinical isolates more resistant to antibiotics. In 280 line “and” instead of “that”. In the line 284 “and in guiding clinicians to therapeutic measures.” was deleted.

14. What was the ratio of *mecA*-positive isolates in *icaA/D*-positive group vs. *icaA/D*-negative groups?
Re: The ratio of mecA-positive isolates in icaA/D-positive group vs. icaA/D-negative groups in the study was shown in following table. Thank you.

<table>
<thead>
<tr>
<th>Groups</th>
<th>strains(n)</th>
<th>mecA-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>icaA positive</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>16(72.72%)</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>8(38.09%)</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>8(42.11%)</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>11(55.00%)</td>
</tr>
</tbody>
</table>

15. This reviewer does not dispute that S. epidermidis are significant nosocomial pathogens but how do the data from this study “support that S. epidermidis are significant nosocomial pathogens in the ICU” as the authors stated in the last paragraph of their manuscript?

Re: We rewrote the last paragraph of the manuscript. In the line 291-296 “these data support that S.epidermidis are significant nosocomial pathogens in the ICU. In addition, the presence and expression of icaA/D can clarify the different adhesion mechanisms in the pathogenesis of infections associated with medical devices, and assist in the development of new preventive and therapeutic measures to eradicate biofilm in hospitals.” was replaced with “these data suggested that ica is typically associated with biofilm production of S. epidermidis in ICU. Both icaA and icaD can support the adhesion mechanisms of S. epidermidis involved in the infections associated with medical devices. Co-expression of mecA and icaD is associated with enhanced clinical isolates that produce slime and may be more resistant to antibiotics. This study may help the development of
rapid diagnosis approach for slime producing and methicillin resistant strains in hospitals.” Thank you.

Minor Compulsory Revisions

16. **S.epidermidis** should be **S. epidermidis** and should be italicized.

    Re: As your suggestion, each “S.epidermidis” in the manuscript was italicized. Thank you.

2nd Reviewer's report:

Major Compulsory Revisions

In general:

17. **English language should be revised.**

    Re: the language of the manuscript was edited and revised by a native speaker scientist (a M.D. and PH.D. in USA). Thank you.

18. **Staph. epidermidis & Staph. aureus** should be written in **Italic.**

    Re: as your suggestion, each *S. epidermidis* in the manuscript was italicized. Thank you.

A. Abstract:

Methods:

19. **Congo red test agar: should be Congo red agar test.**

    Re: in the line 32-33 “*Congo red agar test*” instead of “Congo red test agar”. Thank you.
20. Slime was examined and …… By what? If authors means that slime was examined by Scanning electron microscope they should write that slime was examined by scanning electron microscope.

Re: Yes, I agree with your. In the line 34 “scanning electron microscope” was inserted. Thank you.

Results:

21. S. epidermidis were received it is better to say (were collected).

Re: Yes, I agree with your. In the line 36 “collected” instead of “received”. Thank you.

22. (This study has assisted in the development of new preventive and therapeutic measures to eradicate) Authors write this statement in conclusion, but they did not make any studies on how to prevent slime formation they can say rapid diagnosis for slime producing and methicillin resistant strains.

Re: Yes, I agreed with your. In the line 295-296 “This study has assisted in the development of new preventive and therapeutic measures to eradicate” was replaced with “This study may help the development of rapid diagnosis approach for slime producing and methicillin resistant strains in hospitals.” Thank you.

23. Bloodstream: should be Blood stream

Re: Yes, I agreed with you. In the line 49 “Blood stream” instead of “Bloodstream”.

Study design:
24. Authors said that 82 strains were collected by sterile swabs but in Abstract they said that 60 strains were collected from nasal vestibules and 22 blood samples were collected. How did you collect these samples?

Re: We made the corrections. In the line 141 “by sterile swabs” was deleted. At the same time, in the line 118-130 “Study design” was rewritten. Thank you.

Scanning electron micrograph:

25. procedure for sample preparation for scanning electron microscope examination should be written with a reference.


26. How can electron microscope show types of slimes? Figures contains images for slime producing strains and slime non producing strains (no typing) (types should be omitted)

Re: Yes, I agreed with you. In the line 141 “the types of” was deleted. Thank you.

PCR:

27. icaA band should be at 198 bp or 188 bp. Please check?

Re: We corrected the mistake. In the line 157 and 202 “188bp” instead of “198bp”.
28. Authors said that PCR was done for 62 strains but in table 2 they did PCR for all isolates (82).

Re: Sorry, here is a mistake because of our carelessness. In the line 200 “82” instead of “62”.

29. Authors gave a comment only on group 1 but what about other groups? They did not write what groups are referring to below tables.

Re: Sorry, here is a mistake because of our carelessness. in the line 419-420 and 433-434 “Group 1, blood isolates obtained from patients’ IV line; Group 2, nasal isolates of healthy volunteers; Group 3, nasal isolates of the same patients in Group 1; Group 4, nasal isolates of ICU staff.” was inserted.

Thank you.

Discussion:

30. They compared their results to others without any comment. They write references only without showing the differences between their results and results for others.

Re: We added the following comments to our study results in comparison to others.

1) in the line 227-228 “suggests the possibility that some environmental conditions, such as body fluid and no-body fluid or the presence of accessory genes, can influence the phenotypic behavior in the Congo red agar plate.” was replaced with “is similar to what have been reported by Knobloch JK et al [3]” was inserted;

2) In the line 280-281 “The results of our study are similar to Cafiso V’s study, which suggested
that the *ica* and *mecA* could be considered as important virulence markers for clinical *staphylococcal isolates*” was inserted. Thank you.

3rd Reviewer's report:

In this manuscript, the authors described that *S.epidermidis* bacteria were significant nosocomial pathogens, and *icaA/D* could clarify the different adhesion mechanisms in the pathogenesis of infections associated with medical devices.

This manuscript is interesting.

In sum, the questions posed by the authors were well defined.

The methods appropriate and well described.

Data sounds convincing.

In my view, this manuscript met the relevant standards for publication after some revisions and editing.

Minor revisions

31. In the “Bacterial strains”, the author states “Twenty-two *S.epidermidis* studied were isolated from patient blood taken from intravascular catheters. ---”. Patients or patient? The period of selection? The patients are Consecutive cases? The statement is too simple.

Re: Yes, these consecutive cases were collected from May 2009 to July 2010 (over a 15-month period) in this study.
In the line 98 “Twenty-two S.epidermidis studied were isolated from patient blood taken from intravascular catheters” was replaced with “This study analyzed 82 S. epidermidis isolates collected from May 2009 to July 2010.”

In line 99 “patients” instead of “patient”. Thank you.

32. The discussion looks not very good; you’d better highlight the novelty points and the importance of your finding in this work, rather than describe the results. I suggest that you should rewrite the “discussion”, and move the data description portions into the “Results” section.

Re: We revised the discussion section according to your comments.

① in the line 212-214 “The conversion of the CNS from symbiont to human pathogen has been a direct reflection of the use of indwelling medical devices” was replaced with “The major virulence factor is that they can form biofilm on polymeric surfaces and adherence to catheters and other artificial materials during the early phase of biofilm development”;

② In the line 215-218 “In particular, the current study demonstrated that S. epidermidis should be recognized as a major cause of catheter infections. This study investigating the biofilm formation showed that slime accumulation is mediated by the chromosomal ica gene, which comprises four intercellular adhesion genes (icaA, icaB, icaC and icaD) and one regulator gene (icaR) [11]” was inserted;
In the 236-239 line “In addition, we speculate that the virulence factor of *S. epidermidis* will be weak. We believe that a future study of the presence and expression of genes (such as the *icaA* or *icaD* after passage may help clarify the relevance of slime, virulence factor of *S. epidermidis*, and *ica*.” was inserted;

In the 249-252 line “*Coagulase-negative staphylococci*, particularly *S. epidermidis*, are the major causes of infections associated with catheters and other artificial materials. Usually considered to be of low virulence, *S. epidermidis* is now recognized as a potential pathogen because of its ability to produce biofilm and adhere to the walls of artificial materials” was inserted;

in the 269-270 line “The co-expression of *icaA* and *icaD* can be considered as important markers for the clinically significant slime-positive phenotype.” was replaced with “., and another study demonstrated that co-expression of *icaA* and *icaD* was associated with enhanced slime production”;

In the 280-282 line “The results of our study are similar to Cafiso V’s study, which suggested that the *ica* and *mecA* could be considered as important virulence markers for clinical *staphylococcal isolates*” was inserted. Thank you.

33. Limitations of the work stated are too general. The limitation is suitable to many papers
other than this paper. The authors should state some specials related to this study.

Re: Thanks, we have added additional comments to the study limitation according to your suggestion.

in the line 286-289 “Limitations of this study included small numbers of cases and lack of multiple study sites” was replaced with “Limitations of this study included small numbers of cases, single study site. A future study about the mechanism of the ica with prospective design and more numbers of cases will enhance our understanding of the pathogenesis of CVC-related BSI and provide useful information for the development of more effective therapeutic measures to eradicate biofilm in hospitals”. Thank you.

34. The authors are non-English speaking, so some of their grammar can be polished, but this criticism will not be held against them, in a major way for the Scientific Content. The other reviewer(s) may hold a different opinion. The authors should get help with their grammar/syntax.

Re: the language of the manuscript was edited and revised by a native speaker scientist (A MD and PHD in USA). Thank you.

35. The other revision:

In the line 46-47 “This study has assisted in the development of new preventive and therapeutic measures to eradicate slime.” was replaced with “This study result could be useful for the development of rapid diagnosis for slime producing and methicillin resistant S.epidermidis strains”