OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jundishapur Journal of Microbiology
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Peer Review Report for "Molecular Detection of Genes Involved in Biofilm Formation in *Staphylococcus aureus* Strains Isolates: Evidence From Shahid Motahari Hospital in Tehran"

Author(s): Anis Mohammadi, Mehdi Goudarzi, Masoud Dadashi, Malihe Soltani, Hossein Goudarzi, Bahareh Hajikhani

Review Timeline:

Submit Date: 23 Feb 2020
Revised Date: 17 Sep 2020
Accepted Date: 17 Sep 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | Revision (0) — 1 May 2020

Dear AE,
Comments to this paper:
1. Please show the PCR products.
2. Explain about test for strains sorting in methods.
3. Results are very short.
Thanks.

Farhad Safarpoor Dehkordi: Reviewer | Revision (0) — 22 May 2020

Dear AE,
Manuscript Full Title: Molecular investigation of genes involved in biofilm formation in Staphylococcus aureus isolated from burn patients
Well, this is an original research work about the frequency of genes involved in biofilm formation in Staphylococcus aureus isolated from burn patients. The findings of this paper revealed that the resistance to erythromycin, clindamycin, ciprofloxacin, cephalexin, and

oxacillin was 77%, 79.5%, 62.6%, 75.9%, and 87.9% respectively. The percentage of biofilm genes were as follows: clfA 50.6%, clfB 54.2 %, bbp 13.2%, fnbA 42.1%, fnbB 12.1%, cna 74.7%, ebps 20.5%, mecA 87.9%.

However, this is an interesting work, but authors should address the following comments before its full acceptance.

1- Text is full of grammatical and typographical mistakes. Please use from a native English speaker person who is an expert in microbiology to revise your text.

2- My specific concern is about the method used for the detection of MRSA. Authors should use from the oxacillin and cefoxitin disk diffusion method or in molecular techniques, they should use from mecA (penicillins-encoding) and one of the genes encodes resistance against cephems. Thus, the method used in this part had so many defects!

3- Please add the full history of patients.

4- Please add the incidence of bacteria according to sex, age, and even type of burn wounds of patients. This is because of the data of this study are low and authors should increase the scientific base of their article using more data.

5- Add reference for bacterial identification.

6- Add reference for disk diffusion.

7- It is not clear which kinds of bacteria were examined. I mean MRSA or S. aureus? Authors should unique them in the text.

8- Which kinds of methods were used for DNA extraction?

9- How the quality and quantity of DNA were measured?

10- How the purity of extracted DNA was measured?

11- Add more details for statistical analysis in the method section.

12- Some parts of the text need additional gaps and some parts of the text need additional space between letters and words of the text.

13- What was the main reason for the selection of this kind of antibiotic agent?

14- What was the main reference for the Antimicrobial susceptibility testing? Please checked the CLSI another time. I think that some of the used disks have been deleted from the CLSI guidelines.

15- Authors used only the same control organism, Staphylococcus aureus ATCC25923, in the disk diffusion. They should use another un similar organism. For example Pseudomonas or Acinetobacter or …

16- Add the PCR program and volume of reactions into table 2.

17- What was the relationship between genes and the incidence of resistance? Please determine.

18- Pleas add the findings of statistical analysis into the result section.

19- What were the positive and negative controls in PCR?

20- Which concentration of bacteria was used for antibiotic susceptibility testing.

21- (Figure …) and (Table …) should change to (figure …) and (table …). Make it throughout the text.

22- The discussion section has some defects. Please complete the introduction part using an explanation about the characters of the topic. Additionally, add some explanation about the fact that why other surveys cannot solve the issue which caused that you and your colleague performed this research. Additionally, the novelty of work and also the application of the results obtained from this work. Some of your references were old. You should replace them with some novel references. Are there any surveys conducted on this field in

Iran and some neighboring countries? Please add them.
23- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their applications.
24- What are the main applications of your research in the field of microbiology based on the clinical view.
25- Please also unique the style of references throughout the reference lists.
Thanks.

Zohreh Mashak: Reviewer | **Revision** (0) 26 May 2020

Dear AE
Comments for the authors:
- Title is better change to “Molecular investigation of genes involved in biofilm Staphylococos aureus formation at Shahid Motahari Hospital in Tehran
- There are many grammatical mistakes in text.
- The inoculum size of bacteria for antibiotic resistance?
- What were the controls(positive and negative)in figs of electrophoresis?
- Describe statistical analysis.
- Describe the correlation between gens and resistances.
- Describe the DNA extraction method.
- Determine the control positive Staphylococous aureus (ATCC)for CLSI test.
- Correct similarity format of References.
My Decision: Minor revision
Thanks.

Zohreh Mashak: Associate Editor | **Revision** (0) 1 Jun 2020

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

REFEREE: EIC | **Revision** (0) 1 Jun 2020

Dear Authors,
Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments. Please answer point by point to reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers’ queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding.
When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made a point by point, and provide suitable

rebuttals for any comments not addressed. In addition, JJM INSTRUCTION attached to the mail, Pleases find it.

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Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor,

Thank you for your willingness to consider our initial manuscript "**Molecular investigation of genes involved in biofilm formation in *Staphylococcus aureus* isolated from burn patients"**. We have carefully read all comments and highly appreciate your valuable comments on our manuscript. Precise answer to all reviewers` comments is provided and all changes are highlighted with yellow. We hope this meets the established reputation for quality of your esteemed journal.

Yours sincerely,

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E-mail: b.hajikhani@gmail.com
Tel-fax: +98 21 23872556

Reviewer 1

1. **Comment:** Title is better change to “Molecular investigation of genes involved in biofilm Staphylococcus aureus formation at Shahid Motahari Hospital in Tehran

Response: Title was changed as requested by the reviewer

2. **Comment:** There are many grammatical mistakes in text.

Response: Dear reviewer, in revised version, manuscript was edited by a native speaker person.

3. **Comment:** The inoculum size of bacteria for antibiotic resistance?

Response: Dear reviewer, we suspended the organism in 2 ml of sterile saline and adjusted the turbidity of this suspension to a 0.5 McFarland standard (1.5×10^8 CFU/ml) by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. Related sentence was added to text.

4. **Comment:** What were the controls (positive and negative) in figs of electrophoresis?

Response: Dear reviewer, positive controls for adhesion and biofilm encoding gene were confirmed isolates which provided by Dr. Mehdi Goudarzi. Negative controls had all the PCR reagents with the exception of DNA template

5. **Comment:** Describe statistical analysis.

Response: We described statistical analysis as requested by the reviewer.

6. **Comment:** Describe correlation between gens and resistances.
   Response: Done and acknowledged.
7. **Comment:** Describe the DNA extraction method.

   Response: DNA extraction method was explained in Material and methods section.

8. **Comment:** Determine the control positive Staphylococcus aureus (ATCC) for CLSI test.

   Response: We greatly appreciate your thoughtful comment. It was added.

9. **Comment:** correct similarity format of References.

   Response: Done and acknowledged.

Reviewer 2

1. **Comment:** Please show the PCR products.

   Response: Dear reviewer based on your helpful comment, we showed all the PCR products in a Figure.

2. **Comment:** Explain about test for strains sorting in methods.

   Response: Dear reviewer we explain about saving of isolated and also transfer this part 2.1 section

3. **Comment:** Results are very short

   Response: We greatly appreciate your thoughtful comment; in revised version it was corrected.

Reviewer 3

1. **Comment:** Text is full of grammatical and typographical mistakes. Please use from a native English speaker person who is an expert in microbiology to revise your text.

   Response: Dear reviewer, in revised version, manuscript was edited by a native speaker person

2. **Comment:** My specific concern is about the method used for the detection of MRSA. Authors should use from the oxacillin and cefoxitin disk diffusion method or in molecular techniques, they should use from mecA (penicillins-encoding) and one of the genes encodes resistance against cephems. Thus, the method used in this part had so many defects!

Response: Dear reviewer, in present study, all *S. aureus* isolates were screened for methicillin resistance using cefoxitin (30 µg) disc diffusion test based on the clinical and laboratory standards institute (CLSI 2018) guideline (https://clsi.org/). In addition, the PCR method was applied for genotypic amplification of *mecA* gene.

3. **Comment:** Please add the full history of patients.

   Response: We greatly appreciate your thoughtful comment; I would like to inform you that due to the policies of the hospital authorities, unfortunately, we did not have access to this information.

4. **Comment:** Please add the incidence of bacteria according to sex, age, and even type of burn wounds of patients. This is because of the data of this study are low and authors should increase the scientific base of their article using more data.

   Response: In revised version these data were added.

5. **Comment:** Add reference for bacterial identification.

   Response: Done and acknowledged.

6. **Comment**: Add reference for disk diffusion.

   Response: Done and acknowledged.

7. **Comment**: It is not clear which kinds of bacteria were examined. I mean MRSA or S. aureus? Authors should unique them in the text.

   Response: Dear reviewer, in our study, of *S. aureus* isolates, 73 and 10 were MRSA and MSSA isolates. We unique them in the text

8. **Comment**: Which kinds of methods were used for DNA extraction?

   Response: DNA extraction method was explained in Material and methods section.

9. **Comment:** How the quality and quantity of DNA were measured?

   Response: The quality of DNA has adjusted approximately to 100ng/µl and calculating the A_{260}/A_{280} ratio which evaluated by a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

A_{260}/A_{230} absorbance ratio between 1.8 and 2.0 is an indicator of DNA purity to perform PCR technique. In revised version these data were added.

10. **Comment:** Add more details for statistical analysis in the method section.

    Response: Done and acknowledged.

11. **Comment**: Some parts of the text need additional gaps and some parts of the text need additional space between letters and words of the text.

    Response: We greatly appreciate your thoughtful comment; in revised version these were corrected.

12. **Comment:** What was the main reason for the selection of this kind of antibiotic agent?

    Response: A review of pharmacy records and prescription pattern showed that these antimicrobial agents were used too much.

13. **Comment**: What was the main reference for the Antimicrobial susceptibility testing? Please checked the CLSI another time. I think that some of the used disks have been deleted from the CLSI guidelines.

    Response: We used from CLSI version 2018 for interpretation of erythromycin, cefoxitin, and clindamycin (2 mcg) while EUCAST (2018) was used fro cephalexin, and ciprofloxacin

14. **Comment:** Authors used only the same control organism, Staphylococcus aureus ATCC25923, in the disk diffusion. They should use another un similar organism. For example Pseudomonas or Acinetobacter or

    Response: Dear reviewer as mentioned in manuscript, according to the CLSI guideline we used from reference strains *S. aureus* ATCC 25923, ATCC 43300, and ATCC 29213 and the use of un similar organisms is not recommended by CLSI.

15. **Comment**: Add the PCR program and volume of reactions into table 2.

    Response: We added PCR program in table 1 and volume of reactions were explaines in section “DNA isolation, screening for biofilm related genes”.

16. **Comment:** What was the relationship between genes and the incidence of resistance? Please determine. Pleas add the findings of statistical analysis into the result section.

Response: Dear reviewer we relationship between genes and the incidence of resistance in Results section.

17. **Comment**: What were the positive and negative controls in PCR?

    Response: Dear reviewer, positive controls for adhesion and biofilm encoding gene were confirmed isolates which provided by Dr. Mehdi Goudarzi. Negative controls had all the PCR reagents with the exception of DNA template

18. **Comment**: Which concentration of bacteria was used for antibiotic susceptibility testing.

    Response: Dear reviewer, we suspended the organism in 2 ml of sterile saline and adjusted the turbidity of this suspension to a 0.5 McFarland standard (1.5×10^{8} CFU/ml) by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. Related sentence was added to text.

19. **Comment**: (Figure …) and (Table …) should change to (figure …) and (table …). Make it throughout the text.

    Response: In revised version it was corrected

20. **Comment:** The introduction section has some defects. Please complete the introduction part using an explanation about the characters of the topic. Additionally, add some explanation about the fact that why other surveys cannot solve the issue which caused that you and your colleague performed this research. Additionally, the novelty of work and also the application of the results obtained from this work. Some of your references were old. You should replace them with some novel references. Are there any surveys conducted on this field in Iran and some neighbouring countries? Please add them.

    Response: Introduction was totally revised as requested by the reviewer. Old references were replaced with newer references. Related studies from Iran and neighbouring countries were added to text.

19. **Comment**: Please complete the conclusion with some more relevant information about the exact finding of your survey and also their applications.

    Response: Dear reviewer, conclusion was revised.

20. **Comment**: Please also unique the style of references throughout the reference lists.

Response: References were corrected based on the journal style both text and references section.

Associate Editor's Comments:

Comment: Title is better change to “Molecular investigation of genes involved in biofilm Staphylococcus aureus formation at Shahid Motahari Hospital in Tehran”

Response: Title was changed as requested by the reviewer

Comment: Correct grammatical mistakes in text. (Hot staining to Gram staining, Acinetobacter Pseudomonas to Acinetobacter ,Pseudomonas ;all of bacteria written to Italic form,;In Table 1: Secuence to Sequence ; ATCC25923 was used as a positive controlling factor;correct space between letters and words in “Storing the obtained strains “… transferred to -20'C. ….. storing in -70'C.and etc

Response: All the items were corrected.

Comment: It’s better add the incidence of bacteria according to sex, age and even type of burn wounds in patients.

Response: In revised version these data were added.

Comment: What was the main reason for the selection of this kind of antibiotic agent?

Response: A review of pharmacy records and prescription pattern showed that these antimicrobial agents were used too much.

Comment: The inoculum size of bacteria for antibiotic resistance?

Response: Dear reviewer, we suspended the organism in 2 ml of sterile saline and adjusted the turbidity of this suspension to a 0.5 McFarland standard (1.5×10^8 CFU/ml) by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. Related sentence was added to text.

Comment: Please correct the method detection of MRSA.(Authors should use from oxacillin and cefoxitin disk diffusion method or in molecular techniques, they should use from mecA (penicillins-encoding) and one of the genes encode resistance against cephems)

Response: Dear reviewer, in present study, all *S. aureus* isolates were screened for methicillin resistance using cefoxitin (30 µg) disc diffusion test based on the clinical and laboratory standards institute (CLSI 2018) guideline (https://clsi.org/). In addition, the PCR method was applied for genotypic amplification of *mecA* gene.

Comment: Correct in Table 2:for gen cna bP“744 Variable Montesinos “?

Response: Dear reviewer, Done and aknowledged

Comment: Determine statistical analysis in “Identification of biofilms encoding genes”

Response: We described statistical analysis as requested by the reviewer.

Comment: Describe the DNA extraction method. (Add the PCR program and volume of reactions into the table 2)

Response: DNA extraction method was explained in Material and methods section.

Comment: How the quality and quantity of DNA were measured? How the purity of extracted DNA was measured?

Response: The quality of DNA has adjusted approximately to 100ng/µl and calculating the A_{260}/A_{280} ratio which evaluated by a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). A_{260}/A_{230} absorbance ratio between 1.8 and 2.0 is an indicator of DNA purity to perform PCR technique. In revised version these data were added.

Comment: Why choose only a same control organism, Staphylococcus aureus ATCC25923 for all tests?

Response: Dear reviewer as mentioned in manuscript, according to the CLSI guideline we used from reference strains *S. aureus* ATCC 25923, ATCC 43300, and ATCC 29213 and the use of un similar organisms is not recommended by CLSI.

Comment: Determine the numbers of figs of electrophoresis

Response: Dear reviewer, we corrected figure and combined all in one figure.

Comment: Describe correlation between gens and resistances.

Response: Done and acknowledged.

Comment: Are there any survey conducted on this field in Iran and some neighbor countries? Please add them.

Response: Done and acknowledged.

Comment: add some explanation about the fact that why other surveys cannot solve the issue which caused that you and your colleague performed this research.

Response: The sentence was added to Introduction section.

Comment: Add reference for bacterial identification and for disk diffusion

Response: Done and acknowledged.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 14 Sep 2020

Dear Authors,
Please add DOI, Pmcid, or Pmid to every reference and reform them in the below format:
1) Reformat all references according to JJM format. Add DOI, PubMed ID, PMCID ID as follow (Use JJM Endnote style):
Youssef SS, Abbas EAE, Mostafa A, el Zanaty T, Seif SM. Association of IL28B polymorphism with fibrosis, liver inflammation, gender respective natural history of hepatitis C virus in Egyptian patients with genotype 4. J Interf Cytok Res. 2014;34(1):22–7. doi: 10.1089/jir.2013.0036. [PubMed: 23981065].
Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor,

Thank you for your willingness to consider our manuscript "**Molecular investigation of genes involved in biofilm formation in *Staphylococcus aureus* isolated from burn patients"**. We have carefully read your comment and corrected references style as requested by the EIC

Yours sincerely,
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Tel-fax: +98 21 23872556

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision** (2) 17 Sep 2020

Dear Authors
According to the associated editor report, your manuscript is accepted for publishing in JJM and sent to the publisher for the next process.
Note:
Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf.
Thanks.