OPEN PEER REVIEW

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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

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Peer Review Report for "Genetic Features of *bla*$_{ndm-1}$ and Characterization of the Corresponding Knockout Mutant of *Enterobacter cloacae* Produced by Red Homologous Recombination"

Author(s): Jing Yao, Shumin Liu, Na Du, Min Niu, Mengshuang Zhang, Ciyan Chen, Huanqin Li, Yan Du

Review Timeline:

Submit Date: 5 Feb 2020
Revised Date: 13 Apr 2020
Accepted Date: 15 Apr 2020

Revision (0)

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

Farhad Safarpoor Dehkordi: Reviewer | **Revision** (0) 16 Mar 2020

Dear AE,
Well, this is an original research work about the Genetic features of blaNDM-1 and characterization of the corresponding knockout mutant of Enterobacter cloacae produced by Red homologous recombination. Though, some point should address before its fully acceptance.
Please address all of the following comments:
1- Text is full from grammatical and topographical mistakes. Please use a native English speaker person which is an expert in microbiology to revise your text.
2- Authors studied only ten NDM isolates between June 2012 and January 2016! Number of isolates is too low.
3- Authors stated that The blaNDM-1 gene was located on plasmids in all isolates, with sizes ranging from∼33.3 to ∼ 50kb (6). How did you do that? Please add it with sufficient explanation.
4- Escherichia coli DH5α was used for cloning procedures.: According to which reference?
5- was used for the modification of pKD46. Should change to were used .....

6- E.cloacae should change to E. cloacae throughout the text.
7- Some parts of the text need gaps and some parts of the text need space between letters and words of the text.
8- How the quality and quantity of DNA were measured?
9- Figure caption should add below the figure.
10- Please add the name and concentration of antibiotic disks used for susceptibility test into the method.
11- What was the negative and positive controls in the PCR?
12- What was the main reason for selection of this kind of antibiotic agents?
13- What was the main reference for the Antimicrobial susceptibility testing? Please checked the CLSI another time. I think that some of the used disk have been deleted from the CLSI guidelines.
14- Which concentration of bacteria was used for antibiotic susceptibility testing.
15- What were the positive and negative controls of the PCR?
16- (Figure …) and (Table …) should change to (figure …) and (table …). Make it throughout the text.
17- I cannot find any statistical analysis of achieved data.
18- Please complete the discussion with some literature-based surveys and also explanation and interpretation of your obtained findings.
19- Please complete the introduction part using the explanation about the characters of the topic.
20- 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 application of the results obtained from this work.
21- Some of your references were old. You should replace them with some novel references.
22- Are there any survey conducted on this field in Iran and some neighbor 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 references lists.
26- Please add the main method used for isolation of bacteria. I mean NDM.
27- Please add the ethical considerations.
Regards
FSD

REFEREE: Reviewer | **Revision** (0) 17 Mar 2020

Dear AE,
By reading your manuscript, I found that
1. Some sentences have conceptual and grammatical problems, such as
The upstream and downstream structures of The upstream and downstream structures of the blaNDM-1 gene in E.cloacaeT1were obtained by primer walking. Primers were designed according to the sequencing results to carry out PCR mapping of the remaining nine

Enterobacter cloacae isolates(Table 1). All sequences were reconstructed with the use of DNAMAN Version 9, compared, and analyzed with BLAST.
In my opinion, if this reform of some sentences, this article is acceptable.
Thanks.

fereshteh yavari: Reviewer | **Revision** (0) 17 Mar 2020

Dear AE,
The review of the manuscript has led me to the conclusion that the paper needs major revision (especially in methods).
- What is the meaning of pKD46-Tetallows the expression of Gam, Beta, and Exo?
- The upstream and downstream structures of the upstream and downstream structures of the blaNDM-1 gene?
- Why were PCR-amplified kanamycin resistance fragments containing the homologous arms digested by DpnI?
- The explanations of Red homologous recombination, FRT recombination, VIKET-2 system are not sufficient.
- Gene or strain words must be used after names.
- Thanks for submitting their manuscript.
Thanks.

Zohreh Mashak: Associate Editor | **Revision** (0) 29 Mar 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) 31 Mar 2020

Dear Author,
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 editors,

First of all, thank you and review experts for your review comments. According to the opinions of you and experts, we have modified comments one by one as follows.

Reviewers' Comments:

Reviewer 1:

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

I have modified.

2- Authors studied only ten NDM isolates between June 2012 and January 2016! Number of isolates is too low.

In China, the *bla*$_{NDM-1}$ gene has been mostly detected in carbapenem-resistant *Acinetobacter* spp. But is less reported in *Enterobacteriaceae and* more rarely found in *E. cloacae.*

3- Authors stated that The blaNDM-1 gene was located on plasmids in all isolates, with sizes ranging from ~33.3 to ~ 50kb (6). How did you do that? Please add it with sufficient explanation.

The *bla*$_{NDM-1}$ gene was located on plasmids in all isolates, with sizes ranging from ~ 33.3 to ~ 50kb, which was confirmed by plasmid conjugation tests and Southern blot.

4- Escherichia coli DH5α was used for cloning procedures.: According to which reference?

References added.

Prasad UV, Vasu D, Gowtham RR, Pradeep CK, Swarupa V, Yeswanth S, et al. Cloning, Expression and Characterization of NAD Kinase from *Staphylococcus aureus* Involved in the Formation of NADP (H): A Key Molecule in the Maintaining of Redox Status and Biofilm Formation. *Advanced Biomedical Research*.2017;**6**:97.

5- was used for the modification of pKD46. Should change to were used …..

I have modified.

6- E.cloacae should change to E. cloacae throughout the text.

I have changed.

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

I have modified.

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

The OD260 and OD280 of DNA were determined by UV spectrophotometer. The ratio of OD260/OD280 was calculated. If the ratio was between 1.8 and 2.0, the purity Of DNA was high. DNA concentration: DNA(ug/ml)=50×OD260×dilution multiple

9- Figure caption should add below the figure.

I have modified.

10- Please add the name and concentration of antibiotic disks used for susceptibility test into the method.

We did not use antibiotic disks, only used imipenem, meropenem, and *ertapenem* E test gradient strips.

11- What was the negative and positive controls in the PCR?

The negative controls were blank control, and PCR amplification fragments were further confirmed by DNA sequencing.

12- What was the main reason for selection of this kind of antibiotic agents?

NDM-1belongs to carbapenemase.

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

The Minimal Inhibition Concentration (MIC) values were determined by the brothmicro dilution method using the VIKET-2 Compact system (bioMerieux, France). The values were confirmed using E test gradient strips (imipenem, meropenem, and ertapenem) (bioMerieux, France) on Mueller–Hinton plates. So results were interpreted according to the CLSI guideline.

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

0.5McF.

15- What were the positive and negative controls of the PCR?

The negative controls were blank control, and PCR amplification fragments were further confirmed by DNA sequencing

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

The format of other articles in the journal are Table and Figure

17- I cannot find any statistical analysis of achieved data.

I have added in text.

18- Please complete the discussion with some literature-based surveys and also explanation and interpretation of your obtained findings.

The corresponding part has been modified.

19- Please complete the introduction part using the explanation about the characters of the topic.

The corresponding part has been modified.

20- 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 application of the results obtained from this work.

I have modified.

21- Some of your references were old. You should replace them with some novel references.

I have modified.

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

I have added.

23- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their applications.

I have added.

24- What are the main applications of your research in the field of microbiology based on the clinical view.

I have added.

25- Please also unique the style of references throughout the references lists.

The format of references has been unified.

26- Please add the main method used for isolation of bacteria. I mean NDM.

This parts have been published in other journals.

27- Please add the ethical considerations.

No ethics involved.

Reviewer 2:

1-What is the meaning of pKD46-Tet allows the expression of Gam, Beta and Exo?

The plasmid pKD46 allows the expression of Gam, Beta and Exo following L-arabinose induction, leading the host bacteria to be suitable for homologous recombination.

2-The upstream and downstream structures of the upstream and downstream structures of the blaNDM-1 gene?

I have modified.

3-Why were PCR-amplified kanamycin resistance fragments containing the homologous arms digested by DpnI?

DpnI is a kind of restriction nuclease, which can specifically remove the methylated DNA strand. It is often used to remove template DNA after PCR.

4-The explanations of Red homologous recombination, FRT recombination, VIKET-2 system are not sufficient.

I have modified

5-Gene or strain words must be used after names.

I have modified

Reviewer 3:

1. Some sentences have conceptual and grammatical problems, such as The upstream and downstream structures of The upstream and downstream structures of the blaNDM-1 gene in E.cloacaeT1were obtained by primer walking. Primers were designed according to the sequencing results to carry out PCR mapping of the remaining nine Enterobacter cloacaeisolates(Table 1). All sequences were reconstructed with the use of DNAMAN Version 9, compared and analyzed with BLAST.

I have modified.

Associate Editor's Comments:

Associate Editor 1:

1-Text is full from grammatical and topographical mistakes and repeated sentences.

I have modified.

2- The number of isolates ; between June 2012 and January 2016;is too low(10).

In China, the *bla*$_{NDM-1}$ gene has been mostly detected in carbapenem-resistant *Acinetobacter* spp. But is less reported in *Enterobacteriaceae and* more rarely found in *E. cloacae.*

3- Authors stated that The blaNDM-1 gene was located on plasmids in all isolates, (with sizes ranging from∼33.3 to ∼ 50kb). How did you do that? Please add it with sufficient explanation.

The *bla*$_{NDM-1}$ gene was located on plasmids in all isolates, with sizes ranging from ~ 33.3 to ~ 50kb, which was confirmed by plasmid conjugation tests and Southern blot.

4- Escherichia coli DH5α was used for cloning procedures(reference?).

References added.

Prasad UV, Vasu D, Gowtham RR, Pradeep CK, Swarupa V, Yeswanth S, et al. Cloning, Expression and Characterization of NAD Kinase from *Staphylococcus aureus* Involved in the Formation of NADP (H): A Key Molecule in the Maintaining of Redox Status and Biofilm Formation. *Advanced Biomedical Research*.2017;**6**:97.

5- statistical analysis of data.

I have added

6- add the ethical considerations.

No ethics involved.

7-substitute new references and unique the style of references in references section.

I have modified

8- What were the positive and negative controls of the PCR?.

The negative controls were blank control, and PCR amplification fragments were further confirmed by DNA sequencing.

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

0.5McF.

10-for antimicrobial susceptibility test(reference?). Primers were designed according to the sequencing results to carry out PCR mapping of the remaining nine Enterobacter cloacaeisolates(Table 1). All sequences were reconstructed with the use of DNAMAN Version 9, compared and analyzed with BLAST

Antimicrobial susceptibility test were interpreted according to the CLSI guideline

11-Why were PCR-amplified kanamycin resistance fragments containing the homologous arms digested by DpnI?

DpnI is a kind of restriction nuclease, which can specifically remove the methylated DNA strand. It is often used to remove template DNA after PCR.

12-The explanations of Red homologous recombination, FRT recombination, VIKET-2 system are not sufficient.
I have modified.

In order to facilitate your review, the revised part has been annotated in blue font. In other places, I fully accept the revision opinions of you and the review experts. Thank you again and the reviewers for their valuable revision suggestions! At the same time, I hope that if you find any deficiencies during the review process, please let me know in time.
Thanks again!

Yours Sincerely,
Jing Yao
E-mail:454000846@qq.com

Corresponding author:Yan Du
E-mail: duyan_m@139.com

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)** 15 Apr 2020

Dear Authors,
According to the associated editor report, your manuscript is accepted for publishing in JJM and send 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.