OPEN PEER REVIEW

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Peer Review Report for "Genomic Characterization of Multidrug-Resistant Carbapenemase-Producing *Enterobacter cloacae* ECL189, Co-producing KPC-2, NDM-1, TEM-1, TEM-95, and SHV-66"

Author(s): Na Du, Shumin Liu, Jing Yao, Kai Yang, Yun Lin, Min Niu, Qingwen Zhang, Zhiping Yin, Huanqin Li, Yan Du

Review Timeline:

Submit Date: 30 May 2020
Revised Date: 6 Feb 2021
Accepted Date: 8 Feb 2021

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) 26 Sep 2020

Dear AE,
The authors' manuscript description the report of valuable sequence base data of Genomic characterization of multidrug-resistant E.cloacae strain ECL189 plasmids.
1- In results at Genetic characteristics of plasmids, it mentioned that the pECL189-1 harbored a wide array of resistance genes, including aadA1, TEM-1, folP, APH (3')-Ia, SHV-66, TEM-95, and KPC-2. The question is; were the sequences of these genes the same as previous reports?
2- In results at Genetic characteristics of plasmids, it mentioned that: numerous mobile elements were in the pECL189-1, including transposons (tn21, tn3) and insertion sequence (IS1, IS26).
Would you please align your mobile elements against previous reports of these sequences? Would you please mention the observed copy number of mobile elements (tn21, tn3, IS1, and IS26) in your report?
3- In other parts of "Genetic characteristics of plasmids" at line 8, it mentioned that: It contained lots of conjugal transfer proteins, such as traJ, traG, traF, traO, and traA. Did you

mean: It contained lots of conjugal transfer proteins coding sequences, such as traJ, traG, traF, traO, and traA?

4- At line 6 of part 4.3 results it mentioned: The C+C content of them were 50.35% and 51.89%.

Did you mean: The G+C contents of them were 50.35% and 51.89%.

5- The name of enzymes in the first appearance in the text should bring complete.

6- If you submitted the sequences in Gene Bank, would you please mention your sequences ID.

7- Would you please correct your spelling and add commas in the right positions.

Thanks.

Farhad Safarpoor Dehkordi: Reviewer | **Revision (0)** 22 Oct 2020

Dear AE,

Title: Genomic characterization of multidrug-resistant carbapenemasep roducing Enterobacter cloacae ECL189, co-producing KPC-2, NDM-1, TEM-1, TEM-95 and SHV-66

Manuscript ID: 105761

Comments to authors

- Well, this is original research and full-length paper about the genetic characters of the carbapenemasep producing Enterobacter cloacae ECL189, co-producing KPC-2, NDM-1, TEM-1, TEM-95, and SHV-66. Authors described that ECL189 was resistant to 21 of 23 tested antibiotics, but its transconjugant was resistant to 8 of 18 tested antibiotics. The genome of ECL189 consists of a 5,026,406 bp chromosome and four circular plasmids. In total, 26 resistance genes and 58 resistance proteins were identified. In addition, 77 determinants associated with bacterial virulence were identified. A large number of resistance and virulence genes were located on the plasmids. The results of the whole-genome sequence were consistent with amplifying the β-lactamase genes. MLST analysis revealed that this strain belonged to ST74.

- The findings of this study further support the resistance, virulence, and transmission mechanism of carbapenem-resistant E. cloacae. Resistance and virulence genes were spread among bacteria by the horizontal transfer of plasmids, which should attract more attention from the relevant departments.

- However, the main findings of this study were interesting but there were so many defects in the text of the manuscript which should address before its full acceptance. Thus, I potentially invite authors to address the following comments one by one:

My decision: Major revision

Comments:

1. All parts of the manuscript need re-checked by a native English speaker. There were several grammatical and typographical mistakes in the text.

2. Background is too short and cannot support the main idea of the work. Please introduce the main idea and purpose with sufficient supporting data.

3. Ethical consideration is essential for this study.

4. Please add the method of sampling

5. Please add the method of determination of the sample size.

6. Please add the method used for the isolation and identification of bacteria.

7. Please add the main reason for the selection of Cefotaxime, Ampicillin/Sulbactam,

Piperacillin/Tazobactam, Cefoperazone / Sulbactam, and Fosfomycin antibiotics.
8. Please add the method of statistical analysis of data.
9. Please add the results of the statistical analysis into all attached tables.
10. Please add the positive and negative controls of the PCR.
11. Please noted that the scientific names of bacteria and also the name of genes
12. Discussion is so poor exactly like the background section. should write in italic. Authors should compare their obtained findings with those of other investigations completely. Additionally, self-interpretation of your achieved data is required.
After these changes, you should submit the article another time to the journal and then I will assess the other aspects of your paper. It is essential for publication.
Cheers
FSD

Zohreh Mashak: Reviewer | **Revision** (0) 27 Nov 2020

Dear AE,
- There were several grammatical and typographical mistakes in the text. Rewrite scientific names spelled of bacteria and also the name of genes
- Ethical consideration is essential for this study.
- Mention: method of sampling; sample size; the method used for isolation and identification of bacteria.
- explain the main reason for the selection of different antibiotics.
- Add statistical analysis of data.
- add the positive and negative controls of the PCR.
Decision:
Major revision
Thanks.

Zohreh Mashak: Associate Editor | **Revision** (0) 27 Nov 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) 24 Dec 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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Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html

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.

Reply to Reviewers

Reviewer 1:

1. In results at Genetic characteristics of plasmids, it mentioned that the pECL189-1 harbored a wide array of resistance genes, including aadA1, TEM-1, folP, APH (3')-Ia, SHV-66, TEM-95, and KPC-2. The question is; were the sequences of these genes the same as previous reports?

Answer: The sequences of these genes were the same as previous reports. The author compared these gene sequences on the NCBI BLAST website, and the Nucleotide identity was 100%.

2. In results at Genetic characteristics of plasmids, it mentioned that: numerous mobile elements were in the pECL189-1, including transposons (tn21, tn3) and insertion sequence (IS1, IS26).

Would you please align your mobile elements against previous reports of these sequences?

Would you please mention the observed copy number of mobile elements (tn21, tn3, IS1, and IS26) in your report?

Answer: The sequences of mobile elements were the same as previous reports. The author compared these gene sequences on the NCBI BLAST website, and the Nucleotide identity was 98-100%. The size of mobile elements was modified in the article. The copy number of mobile elements was not counted, please understand.

3. In other parts of "Genetic characteristics of plasmids" at line 8, it mentioned that: It contained lots of conjugal transfer proteins, such as traJ, traG, traF, traO, and traA. Did you mean: It contained lots of conjugal transfer proteins coding sequences, such as traJ, traG, traF, traO, and traA?

Answer: Yes. It was modified in the article.

4. At line 6 of part 4.3 results it mentioned: The C+C content of them were 50.35% and 51.89%.

Did you mean: The G+C contents of them were 50.35% and 51.89%.

Answer: Yes. It was modified in the article.

5. The name of enzymes in the first appearance in the text should bring complete.

Answer: It was modified in the article.

6. If you submitted the sequences in Gene Bank, would you please mention your sequences ID.

Answer: We have been mentioned the Nucleotide sequence accession numbers at the end of the article.

7. Would you please correct your spelling and add commas in the right positions.

Answer: It was modified in the article.

Reviewer 2:

1. All parts of the manuscript need re-checked by a native English speaker. There were several grammatical and topographical mistakes in the text.

Answer: It was modified appropriately.

2.Background is too short and cannot support the main idea of the work. Please introduce the main idea and purpose with sufficient supporting data.

Answer: It was modified in the article.

3. Ethical consideration is essential for this study.

Answer: The strain in this study was isolated from clinical specimens and we were not tested on animals and humans, so ethics is not involved.

4. Please add the method of sampling

Answer: Sampling is not involved in this study.

5. Please add the method of determination of the sample size.

Answer: Only one strain was included in this study.

6. Please add the method used for isolation and identification of bacteria.

Answer: Isolate was identified by Vitek-2 automated identification system (bioMerieux, France). It was modified in the article.

7.Please add the main reason for selection of Cefotaxime, Ampicillin/Sulbactam, Piperacillin/Tazobactam, Cefoperazone / Sulbactam and Fosfomycin antibiotics.

Answer: Ampicillin/Sulbactam, Piperacillin/Tazobactam, Cefoperazone / Sulbactam may be effective against resistant bacteria, and fosfomycin is one of the combination drugs that can be used in the treatment of carbapenem resistant bacteria. We provide as many antibiotic susceptibility results to the clinic as possible, so that they can make more treatment plans.

8. Please add the method of statistical analysis of data.

Answer: Statistical analysis of data is not involved in this study.

9. Please add the results of statistical analysis into all attached tables.

Answer: statistical analysis of data is not involved in this study.

10. Please add the positive and negative controls of the PCR.

Answer: It was modified in the article.

11. Please noted that the scientific names of bacteria and also the name of genes.

Answer: It was modified in the article.

12. Discussion is so poor exactly like the background section. should write as italic. Authors should compare their obtained findings with those of other investigation completely. Additionally, self-interpretation of your achieved data is required.

Answer: We compare our obtained findings with those of other investigation without the antibiotic susceptibility results, but the part was involved in our own thinking.

Reviewer 3:

1.There were several grammatical and topographical mistakes in the text. Rewrite scientific names spelled of bacteria and also the name of genes.

Answer: It was modified in the article.

2.Ethical consideration is essential for this study.

Answer: The strain in this study was isolated from clinical specimens and we were not tested on animals and humans, so ethics is not involved.

3.Mention: method of sampling;sample size; method used for isolation and identification of bacteria.

Answer: Sampling is not involved in this study. Only one strain was included in this study. The identification method has been added.

4.explain main reason for selection of different antibiotics.

Answer: We provide as many antibiotic susceptibility results to the clinic as possible, so that they can make more treatment plans.

5.Add statistical analysis of data.

Answer: Statistical analysis of data is not involved in this study.

6.Add the positive and negative controls of the PCR.

Answer: It was modified in the article.

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: Reviewer | **Revision** (1) — 9 Jan 2021

Dear AE,
Comments for the authors:
- The genes should be further explained in the background.
- PCR program and primers should be mentioned in Conjugation experiments.
- The reasons for E-test and MIC are not mentioned.
- Describe genome extraction protocol by boiling.
- K-B method is not explained in the methods but it is in the results section.
- This article needs a lot of revision.
Thanks

REFEREE: Reviewer | **Revision** (1) — 23 Jan 2021

Dear AE,
Some changes in the text are suggested, which could see in the attached file.
In the results, it mentioned The C+C content. Would you please explain the meaning of the C+C content?
Thanks.

Zohreh Mashak: Associate Editor | **Revision** (1) — 1 Feb 2021

Dear EIC
The reviewer(s) have recommended publication, but also suggest some minor revisions to the manuscript. Therefore, I invite the author to respond to the reviewer(s)' comments and revise his manuscript.
Kind regards,

REFEREE: EIC | **Revision** (1) — 1 Feb 2021

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.

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. Pleases follow the below link for the process. https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html

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.

Reply to Reviewers 2

Reviewer 1:

1.The genes should be further explained in the background.

Answer: It was modified in the article.

2.PCR program and primers should be mentioned in Conjugation experiments.

Answer: It was modified in the article. PCR program and primers were according to references 9.

3.The reasons for E-test and MIC are not mentioned?

Answer: VITEK2 system (MIC) is a routine antimicrobial susceptibility testing method in our laboratory, but there are some limitations. Therefore, we use E-test method to confirm the sensitivity of the ECL189 isolate to carbapenem antibiotics.

4.Describe genome extraction protocol by boiling.

Answer: Boiling method was performed as previously reported according to references 8(DNA templates for PCR process were generated by suspending 5 colonies of an overnight culture of isolates growing on Luria Bertani agar in 500 lL of DNase- and RNase-free water. The suspension was boiled at 100 ℃ for 10 min in thermal block, then centrifuged at 19,000g for 5 min. An aliquot of 1 lL of the supernatant was used as DNA template for PCR).

5.K-B method is not explained in the methods but it is in the results section.

Answer: K-B method was mentioned in 3.2 of the article.

Reviewer 2:

1.In the results, it mentioned the C+C content. Would you please explain the meaning of the C+C content?

Answer: The value of guanine (G) + cytosine (C) in the bacterial genome is very stable and is not affected by the factors such as bacterial age and mutation. (G+C) mol% is an important genetic index for bacterial identification.

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) 8 Feb 2021

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. For more details:
https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html
Thanks.