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

Jundishapur Journal of Microbiology
Article DOI: 10.5812/jjm.98035
Published in: Jundishapur Journal of Microbiology: 13(2); e98035

Peer Review Report for "Construction of a Chimeric Plasmid Vector and Its Investigation for Usage as an Internal Control for Detection of Shiga Toxin-Producing *Escherichia coli* by Polymerase Chain Reaction and Real-Time PCR"

Author(s): Mahnaz Saremi, Masoud Alebouyeh, Parviz Owlia

Review Timeline:

Submit Date: 12 Sep 2019
Revised Date: 5 Mar 2020
Accepted Date: 7 Mar 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)

23 Jan 2020

Dear AE,
Manuscript Full Title: Construction of a Chimeric Plasmid Vector and its Investigation for Usage as an Internal Control for Detection of Shiga Toxin-Producing Escherichia coli (STEC) by Polymerase Chain Reaction and Real-time PCR
Well, this is an original research work about the Construction of a Chimeric Plasmid Vector and its Investigation for Usage as an Internal Control for Detection of Shiga Toxin-Producing Escherichia coli (STEC) by Polymerase Chain Reaction and Real-time PCR. It is an interesting work and can help us to know more about the topic. However, there were some points which should revise before its fully acceptance.
Please address all of the following comments:
1- Please correct ref no 17: 17.J. M. Molecular Detection of Foodborne Bacterial Pathogens. PCR Methods in Foods: Springer Science+Business Media, Inc.; 2006. p. 1-26 and 69-90.
2- Please correct ref no 13: 13.J. T. Validation of PCR assays for detection of Shiga toxin-producing E. coli O104:H4 and O121 in food; : Uppsala University School of Engineering;

2014.

3- Please correct ref no 3 and 4: 3.M.O. AMRaM. Bacterial Agents of Foodborne Illness: Escherichia Coli. Food Microbiology. Third Edition ed: The Royal Society of Chemistry, RSC Publishing.; 2008. p. 216-223. 4.D. L. Escherichia. Molecular Detection of Foodborne Pathogens: Taylor and Francis Group, LLC; 2010. p. 369-390

4- Please check all references another time.

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

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

7- Some topographical and grammatical mistakes were found in the text. Please read it another time.

8- How the quality and quantity of extracted RNA were measured?

9- How the quality and quantity of extracted cDNA were measured?

10- What were the positive and negative controls of both PCRs?

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

12- Please complete the introduction part using the explanation about the characters of the bacteria importance and their virulence factors.

13- 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.

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

15- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.

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

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

18- Please use from the following references about the importance of the STEC strains. It is essential:

- Ranjbar R, Safarpoor Dehkordi F, Sakhaei Shahreza MH, Rahimi E. Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing Escherichia coli strains isolated from raw milk and traditional dairy products. Antimicrob Resist Infect Control. 2018; 7: 53.

- Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E. (2017). Shiga (Vero)-toxin-producing Escherichia coli isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. Antimicrob. Res. Infect. Control. 6:4.

Hemmatinezhad, B., Khamesipour, F., Mohammadi, M., Safarpoor Dehkordi, F., & Mashak, Z. (2015). Microbiological investigation of O-serogroups, virulence factors, and antimicrobial resistance properties of shiga toxin-producing Escherichia coli isolated from ostrich, turkey, and quail meats. Journal of Food Safety, 35, 491-500.

- Momtaz, H., Safarpoor Dehkordi, F., Taktaz, T., Rezvani, A., & Yarali, S. (2012). Shiga toxin-producing Escherichia coli isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. The Scientific World Journal, 2012, 1-

- Momtaz, H., Dehkordi, F.S., Hosseini, M.J., Sarshar, M., & Heidari, M. (2013). Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia

coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathogens, 5, 39.
- Dehkordi, F.S., Yazdani, F., Mozafari, J., & Valizadeh, Y. (2014). Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes, 7, 217.
- Shahrani M, Dehkordi FS, Momtaz H. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol Res. 2014; 47:28.
- Ranjbar R, Seif A, Safarpoor Dehkordi F. Prevalence of Antibiotic Resistance and Distribution of Virulence Factors in the Shiga Toxigenic Escherichia coli Recovered from Hospital Food. Jundishapur J Microbiol. 2019;12(5):e82659.
Cheers

REFEREE: Associate Editor | **Revision** (0) 26 Jan 2020

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** (0) 14 Feb 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 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 point by point, and provide suitable rebuttals for any comments not addressed. In addition JJM INSTRUCTION attached to 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-in-Chief of Jundishapur Journal of Microbiology,

Thank you for considering our manuscript for publication in your valuable journal. All the reviewers' comments were responded and changes were done according to them. The changes are shown in yellow color within the revised file. Responses to the reviewers' comment are shown below. Thanks in advance.

Sincerely yours,
Masoud Alebouyeh

Reviewer 1:

1- Please correct ref no 17: 17.J. M. Molecular Detection of Foodborne Bacterial Pathogens. PCR Methods in Foods: Springer Science+Business Media, Inc.; 2006. p. 1-26 and 69-90.

Response 1: *The reference was corrected.*

2- Please correct ref no 13: 13.J. T. Validation of PCR assays for detection of Shiga toxin-producing E. coli O104:H4 and O121 in food; : Uppsala University School of Engineering; 2014.

Response 2: *The reference was corrected.*

3- Please correct ref no 3 and 4: 3.M.O. AMRaM. Bacterial Agents of Foodborne Illness: Escherichia Coli. Food Microbiology. Third Edition ed: The Royal Society of Chemistry, RSC Publishing.; 2008. p. 216-223. 4.D. L. Escherichia. Molecular Detection of Foodborne Pathogens: Taylor and Francis Group, LLC; 2010. p. 369-390

Response 3: *The reference was corrected.*

4- Please check all references another time.

Response 4: *All the reference were reviewed and revised.*

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

Response 5: *the words were corrected.*

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

Response 6: *No statistical analysis was done. The manuscript was revised and the "significant" term was changed in related sentences.*

7- Some topographical and grammatical mistakes were found in the text. Please read it another time.

Response 7: *The manuscript was revised totally and mistakes were corrected.*

8- How the quality and quantity of extracted RNA were measured?

9- How the quality and quantity of extracted cDNA were measured?

Response 8 & 9: *No RNA and cDNA were used in this study. Concentration and quality of the extracted plasmid and DNA samples were determined by NanoDrop 2000 (Thermo Scientific™, USA). The sentence was added to the revised text.*

10- What were the positive and negative controls of both PCRs?

Response 10: *No template sample served as negative control and DNA extract of a reference strain (E. coli O157:H7) served as positive control in all the assays. This note is highlighted in the methods section.*

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

Response 11: *Discussion section was revised. All the studies with similar subjects are reviewed and their results are compared with those we obtained.*

12- Please complete the introduction part using the explanation about the characters of the bacteria importance and their virulence factors.

Response 12: *A sentence was added at the end of first paragraph of the introduction section in this regard.*

13- 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.

Response 13: *The method that is generally used for detection of STEC in food and stool samples is magnetic separation of the strains based on the developed monoclonal antibodies against antigens related to serotypes that are mainly associated with hemorrhagic uremic syndrome. This method is expensive and need special equipment. In this study we tried to analyze validity of homemade molecular methods for direct detection STEC strains in stool samples of symptomatic patients, which don't need separation techniques. This note was described at last three paragraphs of the introduction section.*

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

15- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.

Response 14 & 15: *The following sentence was added at the start of discussion section. Infection with STEC is a worldwide public health concern. This bacterium accounts for 7%-47.7% of diarrhea cases among the patients at different age groups in Iran (15), (16).*

15. Miri ST, Dashti A, Mostaan S, Kazemi F, Bouzari S. Identification of different Escherichia coli pathotypes in north and north-west provinces of Iran. Iran J Microbiol. 9(1):33-37.

16. Momtaz H, Dehkordi FS, Hosseini MJ, Sarshar M, Heidari M. Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathog 5, 39 (2013). https://doi.org/10.1186/1757-4749-5-39.

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

Response 16: *The following sentence was added at the end of conclusion in discussion section. Rapid diagnosis of microbial agents in clinical samples could help clinicians to prescribe appropriate medication for better management of diseases. Results of this study confirmed application of PCR and real time PCR methods for detection of STEC in stool samples of patients with diarrhea. These methods could reduce turnaround time for laboratory report to 1-day, which is three-times faster than those attain by conventional methods.*

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

Response 17: *References were harmonized according to the journal style.*

18- Please use from the following references about the importance of the STEC strains. It is essential:

Ranjbar R, Safarpoor Dehkordi F, Sakhaei Shahreza MH, Rahimi E. Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing Escherichia coli strains isolated from raw milk and traditional dairy products. Antimicrob Resist Infect Control. 2018; 7: 53.

Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E. (2017). Shiga (Vero)-toxin producing Escherichia coli isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. Antimicrob. Res. Infect. Control. 6:4.

Hemmatinezhad, B., Khamesipour, F., Mohammadi, M., Safarpoor Dehkordi, F., & Mashak, Z. (2015). Microbiological investigation of O-serogroups, virulence factors and antimicrobial resistance properties of shiga toxin-producing Escherichia coli isolated from ostrich, turkey and quail meats. Journal of Food Safety, 35, 491-500.

Momtaz, H., Safarpoor Dehkordi, F., Taktaz, T., Rezvani, A., & Yarali, S. (2012). Shiga toxin-producing Escherichia coli isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. The Scientific World Journal, 2012, 1-

Momtaz, H., Dehkordi, F.S., Hosseini, M.J., Sarshar, M., & Heidari, M. (2013). Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathogens, 5, 39.
Dehkordi, F.S., Yazdani, F., Mozafari, J., & Valizadeh, Y. (2014). Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes, 7, 217.
Shahrani M, Dehkordi FS, Momtaz H. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol Res. 2014; 47:28.
Ranjbar R, Seif A, Safarpoor Dehkordi F. Prevalence of Antibiotic Resistance and Distribution of Virulence Factors in the Shiga Toxigenic Escherichia coli Recovered from Hospital Food. Jundishapur J Microbiol. 2019;12(5):e82659.

Response 18: *The following reference was added to the references of our manuscript based on its relatedness to topic of the study on clinical samples. Remainings articles are related to food and animals.*

Momtaz, H., Dehkordi, F.S., Hosseini, M.J., Sarshar, M., & Heidari, M. (2013). Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing *Escherichia coli* isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathogens, 5, 39.

Associate Editor 1:

1- Please complete the introduction part using the explanation about the characters of the bacteria importance and their virulence factors.

Response 1: *A sentence was added to the introduction section.*

2- What are the main applications of your research in the field of microbiology based on the clinical view? 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 obtained results.

Response 2: *The method that is generally used for detection of STEC in food and stool samples is magnetic separation of the strains based on the developed monoclonal antibodies against antigens related to serotypes that are mainly associated with hemorrhagic uremic syndrome. This method is expensive and need special equipment. In this study we tried to analyze validity of homemade molecular methods for direct detection STEC strains in stool samples of symptomatic patients, which don't need separation techniques. This note was described at last three paragraphs of the introduction section.*

3- There is no mention of statistical analysis.

Response 3: *No statistical analysis was done in this study. Percentage was simply calculated based on the formula presented at the methods section.*

4- How the quality and quantity of extracted RNA and extracted cDNA were measured?

Response 4: *No RNA and cDNA were used in this study. Concentration and quality of the extracted plasmid and DNA samples were determined by NanoDrop 2000 (Thermo Scientific™, USA). The sentence was added to the revised text.*

5- What were the positive and negative controls of both PCRs?

Response 5: *No template sample served as negative control and DNA extract of a reference strain (E. coli O157:H7) serves as positive control in all the assays. This note is highlighted in the methods section.*

6- Please complete the discussion with some literature-based surveys and interpretations of your obtained findings.
7- Are there any surveys conducted on this field in Iran and some neighbor countries? Please add them.
8- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.

Response 6, 7 & 8: *Two surveys were included at the start of discussion section as described above.*

9- Please unique the style of references throughout the reference lists.

Response 9: *Changes were done based on the journal guideline.*

10- Please use from the following references about the importance of the STEC strains:
Ranjbar R, Safarpoor Dehkordi F, Sakhaei Shahreza MH, Rahimi E. Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing Escherichia coli strains isolated from raw milk and traditional dairy products. Antimicrob Resist Infect Control. 2018; 7: 53.
Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E. (2017). Shiga (Vero)-toxin producing Escherichia coli isolated from the hospital foods; virulence factors, o-serogroups, and antimicrobial resistance properties. Antimicrob. Res. Infect. Control. 6:4.
Hemmatinezhad, B., Khamesipour, F., Mohammadi, M., Safarpoor Dehkordi, F., & Mashak, Z. (2015). Microbiological investigation of O-serogroups, virulence factors and antimicrobial resistance properties of shiga toxin-producing Escherichia coli isolated from ostrich, turkey and quail meats. Journal of Food Safety, 35, 491-500.
Momtaz, H., Safarpoor Dehkordi, F., Taktaz, T., Rezvani, A., & Yarali, S. (2012). Shiga toxin-producing Escherichia coli isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. The Scientific World Journal, 2012, 1-
Momtaz, H., Dehkordi, F.S., Hosseini, M.J., Sarshar, M., & Heidari, M. (2013). Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathogens, 5, 39.
Dehkordi, F.S., Yazdani, F., Mozafari, J., & Valizadeh, Y. (2014). Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes, 7, 217.

Shahrani M, Dehkordi FS, Momtaz H. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol Res. 2014; 47:28.
Ranjbar R, Seif A, Safarpoor Dehkordi F. Prevalence of Antibiotic Resistance and Distribution of Virulence Factors in the Shiga Toxigenic Escherichia coli Recovered from Hospital Food. Jundishapur J Microbiol. 2019;12(5):e82659.

Response 10: *One of the articles, which fit to subject of current study, was included. Remainings are related to food and animals.*

11- Some topographical and grammatical mistakes that were found in the text should be correct.

Response 11: *The manuscript was totally revised and syntax, topological, and grammatical mistakes were corrected.*

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)** 7 Mar 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. Pleases follow the below link for the process. http://single.demo.pub/en/knowledgebase/category/tree.html#orcid.html
Thanks.