Jundishapur Journal of Microbiology
Volume 17 · Issue 7 · e147096

OPEN PEER REVIEW

Induction of Viable but Non-culturable (VBNC) State in *Shigella flexneri* Under Osmotic and Nutritional Stresses at Low Temperature

Ali Nouri Baghi, Mohammad Roayaei Ardakani, Elham Rezatofighi

SUBMITTED	REVISED	ACCEPTED	DOI
16 Apr 2024	31 Jul 2024	3 Aug 2024	10.5812/jjm-147096

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Referee 1, Ramin Abiri 2
2 **Revision 1** 5
Authors' reply to reviewers 6
Reviewer and editor comments 10

1 Original submission

2 comments · 9 Jun 2024 – 10 Jun 2024

Referee 1 Reviewer

MAJOR REVISION 9 Jun 2024

For the following reasons:
1- The genes considered for real-time are not explained in the introduction and methods sections.
2- Most of the references are dated before 2019.
3- Real-time results are not shown as curves, and there is no interpretation.

Ramin Abiri Reviewer

MAJOR REVISION 10 Jun 2024

Dear Editor-in-Chief
A manuscript entitled " Study of the Possibility of Creating Viable But Non-Culturable (VBNC) State Shigella flexneri and Investigating The Expression Of Some Genes (IpaD & IpaH) in this StateSubject" is submitted for publication in JJM.
1) Is the article within the scope of the journal?
Y
2) Does the article have novelty?
Y
Title
3) Does the title reflect the content appropriately?
N. The assessment of pathogenicity in animal models is not mentioned.
4) Is there any abbreviation in the title?
Y
Abstract
5) Is there a structured Abstract?
Y, but it is not informative.
6) Are Keywords (3-10) selected from MESH?
y
Introduction
7) Clarity of the objectives/hypotheses? (Are the objectives clearly stated?)
N. We know that VBNC is important and that Sh, flexneri is important, but why is the production of VBNC important? Why was the expression of the two genes assessed? Why was an animal model used?
8) Does Introduction include: “What we know,” “What we don’t know” and “Aims”?
N. We know what we know, but the focus on what we don't know is not enough.
Materials And Methods
9) Clearly describe the selection of participants (patients or laboratory animals, including controls)?
y
10) Study Design Described Sufficiently?
y
11) Adequacy of the sample studied?
N
12) Subjects Representative of Target Population?
N
13) Provided sample size calculation or justification?

A manuscript entitled " Study of the Possibility of Creating Viable But Non-Culturable (VBNC) State Shigella flexneri and Investigating The Expression Of Some Genes (IpaD & IpaH) in this StateSubject" is submitted for publication in JJM.

1) Is the article within the scope of the journal?

Y

2) Does the article have novelty?

Y

Title

3) Does the title reflect the content appropriately?

N. The assessment of pathogenicity in animal models is not mentioned.

4) Is there any abbreviation in the title?

Y

Abstract

5) Is there a structured Abstract?

Y, but it is not informative.

6) Are Keywords (3-10) selected from MESH?

y

Introduction

7) Clarity of the objectives/hypotheses? (Are the objectives clearly stated?)

N. We know that VBNC is important and that Sh, flexneri is important, but why is the production of VBNC important? Why was the expression of the two genes assessed? Why was an animal model used?

8) Does Introduction include: “What we know,” “What we don’t know” and “Aims”?

N. We know what we know, but the focus on what we don't know is not enough.

Materials And Methods

9) Clearly describe the selection of participants (patients or laboratory animals, including controls)?

y

10) Study Design Described Sufficiently?

y

11) Adequacy of the sample studied?

N

12) Subjects Representative of Target Population?

N

13) Provided sample size calculation or justification?

N

14) Control Subjects Appropriate?

Y

15) Power of study declared?

N

16) Reported actual P values for primary analyses?

N

17) Blinding of Investigators (if applicable)?

N

18) Blinding of subjects (if applicable)?

N

19) Identify the methods and apparatus (give the manufacturer’s name and address in parentheses)?

N

20) Identify procedures in sufficient detail to allow other workers to reproduce the results?

Y. Actually, there is too much detail!

21) Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration?

y

22) Inclusion/Exclusion Criteria Explained?

No Need

23) Appropriate Statistical Analysis?

N

Results

24) Appropriateness, clarity, and adequacy of the Tables and Figures?

Y/N

25) Present results in logical sequence in the text, tables, and illustrations, giving the main or most important findings?

Y

26) Duplicate data in graphs and tables?

N

27) Use technical terms as non-technical in statistics, such as “random” (which implies a randomizing device), “normal,” “significant,” “correlations,” and “sample.”?

N

Discussion

28) Conclusion justified & substantiated?

N

29) Analysis supports Conclusion?

N

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewers' Comments:

Dear Reviewers

Many thanks for your efforts and good comments. We hope that the level of the article has improved with this revision.

Reviewer 1:
Dear Editor-in-Chief
A manuscript entitled:" Study of the Possibility of Creating Viable But Non-Culturable (VBNC) State Shigella flexneri and Investigating The Expression Of Some Genes (IpaD & IpaH) in this StateSubject" is submitted for publication in JJM.
1) Is the article within the scope of the journal?
Y
2) Does the article have novelty?
Y
Title
3) Does the title reflect the content appropriately?
N. The assessment of the pathogenicity in animal models is not mentioned

The title of the manuscript was completely changed. We hope that it now reflect all aspects of research.

4) Is there any abbreviation in the title?
Y
Abstract
5) Is there a structured Abstract?
Y, but it is not informative.

The structure of abstract was completely changed. We think it is now informative.
6) Are Keywords (3-10) selected from MESH?
y
Introduction
7) Clarity of the objectives/hypotheses? (Are the objectives clearly stated?)
N. we know that VBNC is important, Sh, flexneri is important, but why the production of VBNC is important? why the expression of the two genes were assessed? Why animal model was used?

The interpretation of these subjects were added to the articles and highlighted with green color. In summary, many foods are stored for a long time in the refrigerator (4 °C). Contamination of these foods to VBNC form of pathogens can lead to problems in consumers. Concerns have been raised about the possibility of regeneration and pathogenicity of VBNC bacteria. Therefore, we want to know is it possible for S. flexneri.

8) Does Introduction include: “What we know,” “What we don’t know” and “Aims”?
N. We know what we know but the focus on what we don't know is not enough

The introduction was completely revised. We think it is much better now.
Materials And Methods

9) Clearly describe the selection of participants (patients or laboratory animals, including controls) ?
y
10) Study Design Described Sufficiently?
y
11) Adequacy of the sample studied?
N

Due to financial constraints, we only studied *Shigella flexneri*, but each test was repeated six times
12) Subjects Representative of Target Population?
N

It is not applicable for our study.
13) Provided sample size calculation or justification?
N

It is not applicable for our study.
14) Control Subjects Appropriate?
Y
15) Power of study declared?
N

It is not applicable for our study.
16) Reported actual P values for primary analyses?
N

It is not applicable for our study.
17) Blinding of Investigators (if applicable)?
N

It is not applicable for our study.
18) Blinding of subjects (if applicable)?
N

It is not applicable for our study.
19) Identify the methods apparatus (give the manufacturer's name and address in parentheses)?
N

It was added to the article where is applicable and showed with green.
20) Identify procedures in sufficient detail to allow other workers to reproduce the results?
Y. Actually the details are extra much!

It was summarized.
21) Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration?
y
22) Inclusion/Exclusion Criteria Explained?
No Need

23) Appropriate Statistical Analysis?
N

It was added where applicable.
Results
24) Appropriateness, clarity, and adequacy of the Tables and Figures?
Y/N

The figures and tables were revised.
25) Present results in logical sequence in the text, tables, and illustrations, giving the main or most important findings?
Y
26) Duplicate data in graphs and tables?
N
27) Use technical terms as non-technical in statistics, such as “random” (which implies a randomizing device), “normal,” “significant,” “correlations,” and “sample.”?
N
Discussion
28) Conclusion justified & substantiated?
N

Discussion and conclusion were completely revised. We think it is much better, now.
29) Analysis supports Conclusion?
N

Conclusion were completely revised.
Reviewer 2:
For the following reasons:
1- The genes considered for real-time are not explained in the introduction section and methods.

The genes were explained in the introduction.
2- Most of the references are dated before 2019.

Some new references were added.
3- Real-time results are not shown as curves and there is no interpretation.

We do reverse transcriptase PCR not real time. We only investigated the presence of mRNA but not level of expression. Unfortunately, we do not have the facilities to do real time.

Associate Editor's Comments:

Associate Editor 1:
Dear authors thank you for submitting your valuable manuscript for publication in The Journal of Microbiology. However, there are some points that need consideration.
1- The English writing needs a complete revision, for example upper case letters are in the middle of sentences, cultivability is used instead of cultivable, culture is used instead of grown or growth.

The article completely was revised and edited.

2- Why the samples were incubated in 4C.Is there any reference for it.

This was one of the aims of the study. We want to investigate the possibility of S. flexneri entering to the VBNC state in refrigerator temperature.

3- Animal experiments are not presented in the title or the abstract .

Animal experiment was deleted because it need more assessments and we have time limitation and could not complete it.

4- Please consider the reviewer's commend for your revision.

Reviewer 1

All of them were considered in revised format.

2 Revision 1

0 comments

No public comments in this round.