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OPEN PEER REVIEW

Molecular Investigation of Enterotoxigenic *Staphylococcus aureus* Isolates from Restaurant Staff

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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.

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1 Original submission

3 comments · 23 Jul 2022 – 1 Aug 2022

Referee 1 Reviewer

MAJOR REVISION 23 Jul 2022

Review of the paper entitled: " Molecular Investigation of Enterotoxigenic Staphylococcus aureus Isolates from Restaurant Staff"

Line 22: I think it is necessary to mention any overlap between multidrug resistance and enterotoxigenic strains as a percentage.
Line 36: "Hundreds of thousands of people suffer from food poisoning every year". This sentence is ambiguous and could be rewritten in a clearer format.
Line 49-50: "Since S. aureus is found permanently in 20-30% and intermittently in 60% of the human population". This needs to be rewritten.
Line 64-66: The objective should clarify the goals of the study; however, in the current format, it is written briefly and needs to be rewritten.
Line 104-106: In my opinion, as PCR reactions are performed using ready-to-use master mix solutions, there is no need to mention the PCR reaction conditions. So, it is better to remove this information.
Line 101-110: Please add the related references. Also, mention the names of the primers just by referring to the related original papers.
Line 128-129: Four sizes are mentioned, while three genes are noted. Recheck.

The paper could be considered for publication in a "letter" or "brief communication" format.

Referee 2 Reviewer

MAJOR REVISION 23 Jul 2022

1- The specific terms used in the article are written out in full at first mention and corrected in the abstract and throughout the text of the article.
2- Keywords should be selected based on MeSH.
3- The introduction section of the article is very brief and incomplete and does not provide complete explanations about the importance of the study and the toxins under investigation. Be sure to use more up-to-date articles.
4- In the study, it is stated that samples were collected from the anterior nares and hands (palms, wrists, fingers). Provide the reference for the sampling method.
5- Considering that staphylococcal bacteria grow after 24 hours in a mannitol salt agar medium, what is the basis for choosing 48 hours?
6- Explain how to extract DNA by the boiling method.
7- Using the primers used in the study to detect staphylococcus, the desired fragment should be 826 bp, but it is mentioned that it is 950 bp. There is a technical problem in the study.
8- Figures 1 and 2 do not exist.
9- To detect strains resistant to methicillin, it is better to use the molecular method.
10- There is no need to describe the PCR reaction solutions in the article.
11- In the abstract, it is mentioned that this study aimed to identify enterotoxin-encoding genes (see, sed, sec, seb, sea) in S. aureus isolates. However, in the materials and methods section, nothing is written about the genes coding for toxins.
12- What was the basis for choosing the studied bacteria based on MDR and XDR?
13- In the materials and methods section, it is mentioned that statistical methods were used, but none are

reported in the results of the study. Statistics and calculations must be included in the results.
14- The discussion section of the article must draw on recently published articles.
15- References need to be revised; some do not have DOIs. Some do not have page numbers.

Editor-in-Chief MAJOR REVISION 1 Aug 2022

Dear Authors
According to the reviewers' and associate editor's comments, your article requires major revision. When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and submit the required files.

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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.

Replying to Reviewers

The corrected and added items were highlighted in yellow.

Reviewer 1:

Review of the paper entitled: " Molecular Investigation of Enterotoxigenic Staphylococcus aureus Isolates from Restaurant Staff"

Line 22: I think it's needed to mention any overlap of multidrug resistance and enterotoxigenic strains by percentage.

It is included in 4.3 section(line142-148).

Line 36: "Hundreds of thousands of people suffer from food poisoning every year". This sentence is ambiguous and could be rewritten in a clearer format.

It meant a lot of people who were edited(line 36).

Line 49-50": "Since S. aureus is found permanently in 20-30% and intermittently in 60% of the human population". Needs to be rewritten.

The frequency percentage was written more precisely(line 50-52).

Line 64-66: the objective should clarify the goals of the study, however in the current format it's written briefly and needs to be rewritten.

The objective became more complete(69-72).

Line 104-106: In my opinion, as PCR reactions are done by using master mix solutions and are ready to use, there is no need to mention the PCR condition of reactions. So, better to remove it.
Line 101-110: pls add the related references. Also mention the name of the primers just by referring to the related original papers.

This part was summarized and a reference was added(line 111-119).

Line 128-129: four sizes are mentioned while the three genes are noted. Recheck.

Fourth gene was added(line 136).

Reviewer 2:

1- The specific terms used in the article are written in full for the first time and corrected in the abstract and the entire text of the article.

This item was revised and edited in the entire text.

2- Keywords should be selected based on Mesh.

Keywords were selected based on Mesh.

3- The introduction part of the article is very brief and incomplete and does not provide complete explanations about the importance of the study and the toxins under investigation. Be sure to use more up- to-date articles.

We made the introduction more complete.

4- In the study, it is said that samples were collected from the anterior nares and hands (palms, wrists, fingers). Write the sampling method reference.

The reference was added(line 79).

5- Considering that staphylococcal bacteria grow after 24 hours in a mannitol salt agar medium, what is the basis for choosing 48 hours?

Its true, but after 48 hours, it has a higher diagnostic index. However, it was deleted from the text

6- Explain how to extract DNA by the Boiling method?

Details of boiling method were added(line 83-87).

7- Using the primers used in the study to detect staphylococcus, the desired fragment should be 826 bp, but it is mentioned that it is 950 bp. There is a technical problem in the study.

Our design confirmed this size.

Specific primers for the genomic DNA of Staphylococcus aureus were designed using Oligo 5 software.

Specifications of the primers used in PCR

Primer name	**Sequence**	**Annealing temperature**	**Expected fragment size**
P_1 (Left Primer)	5'-AAAAACACTTGTCGATATGG-3'	53	950
P_2 (Right Primer)	5'- GTTTCAATACATCAACTGC -3'	53	950

8- Figure 1 and 2 does not exist.
There are in the submission files.

9- To detect strains resistant to methicillin, it is better to use the molecular method.

Methicillin resistance was evaluated using 30 µg cefoxitin disk (≤21mm indicated MRSA) and 1 µg oxacillin disk (≤10 mm indicated MRSA)(line 104-106).

10- There is no need to write PCR reaction solutions in the article.

We have summarized this section(line 111-119).

11- In the abstract, it is mentioned that this study aimed to identify enterotoxin-encoding genes (see, sed, sec, seb, sea) in S. aureus isolates. However, in the materials and methods section, nothing is written about the genes coding for toxins.

It is described in Section 3.3 and Table 1.

12- What was the basis for choosing the studied bacteria based on MDR and XDR?

The isolated bacteria were classified as MDR and XDR as described by Magiorakos et al. (2012).As described by Magiorakos et al. (2012),MDR is defined as non-susceptibility to ≥1 antimicrobial agent in ≥3 antimicrobial categories and XDR is defined as non-susceptibilityto ≥1 . (reference No.9)

13- In the materials and methods section, it is mentioned that statistical methods were used, but there is nothing in the results of the study. Statistics and calculations must be included in the results.

This part was corrected and rewritten.

14- The discussion part of the article must be used from recently published articles.

The discussion was revised and rewritten.

15- References need to be revised, some do not have Doi. Some do not have page numbers.

It was revised but some journals do not have DOI.

[Translated from Persian by the Brieflands editorial office.]

2 Revision 1

3 comments · 19 Aug 2022 – 21 Aug 2022

Referee 2 Reviewer — ACCEPT 19 Aug 2022

Corrections have been made.

Associate Editor — ACCEPT 20 Aug 2022

According to the reviewers' comments, this manuscript is accepted for publication.

Editor-in-Chief — ACCEPT 21 Aug 2022

Dear Authors
We are pleased to inform you that your article is accepted for publication in the MEJRHS. We hope to receive other qualified articles from your group.