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OPEN PEER REVIEW

Study of Gorge Fisher Plumbing System Effects on Growth Inhibition and Amplification of *Legionella pneumophila* by Culture and PCR Based on *16sRNA* and *mip* Genes

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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

2 comments · 26 Feb 2022 – 5 Mar 2022

Referee 1 Reviewer

MAJOR REVISION 26 Feb 2022

1- The names of the bacteria should be written in italics throughout the text of the article. Write the names of the protozoa in italics.
2- What criteria were used to select the samples?
3- The statistics presented in the introduction are very old. Use new and up-to-date statistics. The statistics provided are for the years 2002 and 2006.
4- The following sentence has no reference. "More than 250 64 million people are infected with water-related diseases each year, leading to 5 to 10 million deaths 65 a year, well above the DALYs".
5- Persian words appear on Line 102. The article has grammatical problems that need a general review.
6- Write the purpose of the study at the end of the introduction.
7- Delete lines 131 and 132. The content is very basic. "An 131 autoclave (temperature 121 ° C, pressure 15 ppm and time 15 minutes) was used to sterilize the 132 components of the filtration system".
8- Remove lines 137 to 140 and write instead that it was grown in BCYE medium.
9- In the diagnosis of Legionella, it is said that the bacteria grown in the first days are not Legionella. Was a diagnostic test performed to detect the isolates grown in the first days?
10- The size given for the primers mentioned in the table is 260 bp, but the table lists 242 bp. Please check this item.
11- Write the manufacturer of the extraction kit.
12- Delete Figures 1, 2 and 3.
13- In the materials and methods, it is stated that the bacteria have a length of approximately 2-20 µm. By what technique was the size of the bacteria examined?
14- Regarding bacterial culture, it is said that the desired bacterium did not grow in blood agar medium, so the desired bacterium is gram-negative. On what basis did you come to this conclusion?
15- Figures 4 and 5 show the size of the marker in the figure.
16- In Figure 4, 8 samples are described in the caption but are not shown in the image. There is also no positive control in Figure 4.
17- Figure 5 shows the mip gene at 250 bp while the size of the gene is 384 bp.
18- Figure 6 does not exist and Figure 7 is written twice. Delete both figures.
19- Figures 10 and 11 should be fully described in their captions.

Editor-in-Chief

MAJOR REVISION 5 Mar 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.

> Reviewers' Comments:

Reviewer 1:

1- The names of the bacteria should be written in italics throughout the text of the article. Write the names of the protozoa in italics.

Revised

2- What criteria were used to select the samples?

The sample selection criteria were based on the claim of George Fisher that its pipes and fittings have anti-legionella properties. Also, the RayHo and metal piping system was used for comparison.

3- The statistics presented in the introduction are very old. Use new and up-to-date statistics. The statistics provided are for the years 2002 and 2006.

Revised

According to the Centers for Disease Control (CDC) in the environment Nosocomial incidence of legionellosis is between 25 to 45% and the rate of mortality due to the disease in cases Hospital 30% and in some sources more than 40% also has been reported. age, gender, smoking, consumption of Alcohol, underlying diseases such as chronic lung disease, insufficiency Cardiovascular, type 2 diabetes, inadequate antibiotic treatment, defect Immunity, long-term hospitalization and kidney transplantation are aggravating factors and disease risk factors are known (2).

Among these Causes, smoking and chronic lung disease are the most common. The World Health Organization report annually the Drinking Water Quality Index. The upper limit of this index for bearing the burden of diseases is equal to 10^{-6} determined for per person in year. Accordingly, the risk of infection and mortality from *Legionella* should be around 10^{-4} and 10^{-7}, respectively. *Legionella* reference values in the Drinking Water Quality Guide 0 CFU / 100ml or equivalent to 1 CFU / l. (2).

France, Italy and most European countries are at risk Water contamination by *Legionella* bacteria in the distribution network of 10,000 CFU / l determined and emphasized that if the pollution goes higher than this amount done Control measures are necessary. Review the results of studies

performed Shows that *Legionella* bacteria in hospital water sources, Hotels, swimming pools, other health facilities, temporary accommodation, Residential homes, workplaces (offices and factories), natural water resources and man-made can be present. (2).

Also, according to the World Health Organization, 2.4 million out of 4 billion cases of diarrhea are due to a lack of access to safe drinking water

According to 2018 study the subgroups analysis, the prevalence of *Legionella spp*. in hospital water, dental settings water and other water resources were 28.8%, 23.6% and 29.6% respectively. The most common species of *Legionella* was related to the *L. pneumophila* with a prevalence of 60.5% and the prevalence of all other species was 52.5%. Based on the location, the highest prevalence was reported in Isfahan with a rate of 55.7% followed by Tehran with the prevalence of 27.9% (10).

4- The following sentence has no reference. "More than 250 64 million people are infected with water-related diseases each year, leading to 5 to 10 million deaths 65 a year, well above the DALYs".

Revised

5- Line 102 Persian words are written. The article has grammatical problems that need a general review.

Revised

6- Write the purpose of the study at the end of the introduction.

Revised

This study aimed for investigating the effect of *Legionella* Stopper pipes and fittings of George Fisher Company in controlling *Legionella* growth in indoor water supply system.

7- Delete lines 131 and 132. The content is very basic. "An 131 autoclave (temperature 121 ° C, pressure 15 ppm and time 15 minutes) was used to sterilize the 132 components of the filtration system".

Revised

8- Remove from line 137 to 140 and write instead that it was grown in BCYE medium.

Revised

then was grown on BCYE medium

9- In the diagnosis of Legionella, it is said that the bacteria grown in the first days are not Legionella. Was a diagnostic test performed to detect the isolates grown in the first days?

Revised

The colonies that appear on the first and second days are not *Legionella (By Biochemical tests)*

10- The primers mentioned in the table are 260bp in terms of amplitude, which is written in table 242bp. Please check this item.

Revised

11- Write the manufacturer of the extraction kit.

Revised

Korean PROMEGA kit.

12- Delete Figures 1, 2 and 3.

Revised

13- In the materials and methods, it is said that a length of approximately 2-20 µm. By what technique was the size of the bacteria examined?

Revised

14- In the field of bacterial culture, it is said that the desired bacterium did not grow in blood agar medium, so the desired bacterium is gram-negative. On what basis did you come to this conclusion?

Revised

Therefore, it is a possible confirmation step for Legionella because Legionella is a fastidious bacterium that does not grow in blood agar medium.

15- Figure 4 and 5 show the size of the marker in the figure.

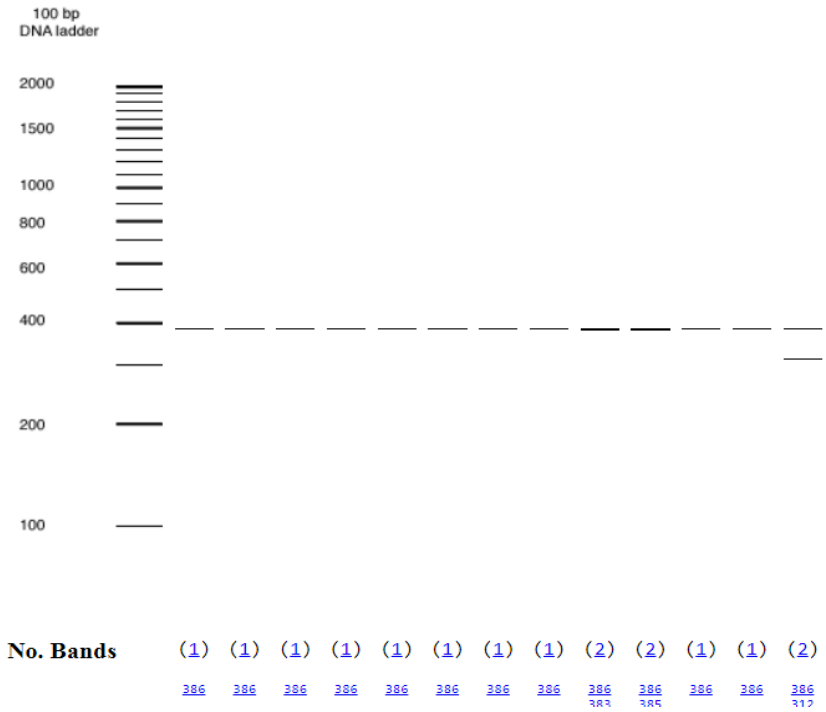
Revised

16- In Figure 4, 8 samples are described as subtitles, if not in the image. There is also no positive control in Figure 4.

Revised

17- Figure 5 shows the mip gene at 250 bp while the size of the gene is 384 bp.

Revised 386 bp



18- Figure 6 does not exist and Figure 7 is written twice. delete both shapes.

Revised

19- Figures 10 and 11 should be fully described with subtitles.

Revised

Reviewer 2:

Reviewer's comments:

1. "60% of Taleghani hospital samples were positive". Usually the sentences donot start with numbers or percent, if so, it should be in this format" Sixty percent"

Revised

2. "PCR results based on 16sRNA from George Fisher system were 35.2%, from Ray Ho piping 45.4% and from Taleghani hospital 54.5% positive", Should be rewritten.

Revised

3. “George Fisher's Legionella stopper pipes and fittings have a good ability to control the growth Legion density in the indoor water supply system and can be considered as a suitable option for use in indoor plumbing and water supply” . rewrite it.

Revised

4. Line 116-118 “All three centers were located close to each other and in area one of Tehran 117 Municipality, and due to the lack of distance in the geographical location, the chemical quality of 118 water entering the urban network in both centers was no different”. Not clear, It needs to be rewritten.

Revised

the goals of the study are not clear, it needs to mention them clearly at the end of introduction

Revised

. Also, as I found after reading half of the paper, the aim of the study was to investigate the prevalence of legionella contamination in the water filtering system. So in my opinion, the “inhibition effect” is not a meaningful word in the title. There is too much information in the introduction that is unrelated to the purpose of the paper.

Revised

A quick glance revealed that several figures don't add any value/don't provide any additional information to the manuscript, such as electrophoresis figures or blasting results. Hence, they should be removed, and their results bring in a sentence inside the text.

2 Revision 1

1 comment · 7 May 2022

Editor-in-Chief

ACCEPT 7 May 2022

Dear Authors
We are pleased to inform you that your article has been accepted for publication in the MEJRHS. We hope to receive other high-quality articles from your group.