



**SDI Review Form 1.6**

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| Journal Name:            | <a href="#">British Microbiology Research Journal</a>                                                                      |
| Manuscript Number:       | 2013_BMRJ_6433                                                                                                             |
| Title of the Manuscript: | <b>Prevalence and Molecular Diversity of <i>Legionella pneumophila</i> in Domestic Hot Water Systems of Private Houses</b> |
| Type of the Article      | <b>Research Paper</b>                                                                                                      |

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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**PART 1: Review Comments**

|                                     | <b>Reviewer's comment</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b>Author's comment</b> (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
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| <b>Compulsory</b> REVISION comments | <p>My comments to the authors:<br/>The objective of this work is to determine the presence and diversity of <i>Legionella</i> spp. in domestic water systems in the city of Aqaba. It uses an adequate sampling size and a well established analysis methodology with slight modifications. What is more, the results and conclusions can be very useful for the region.</p> <p>Based on the above mentioned reasons, I would like to recommend this paper to be published in British Microbiology Research Journal after major revision.</p> <p>-I think the material and methods are well explained, but I would like to know if the slight modifications introduced, like the amount of water sampled (250mL instead of 2L) or the filter pore size (0.45µm instead of 0.20µm) could explain the low number of positives found (8.5% instead 30.5% [14])?</p> <p>-In my opinion, the amount of positives obtained is not enough to conclude that the genotype heterogeneity in serogroup 1 is higher than in the other serogroups.</p> <p>Anyway, in the sentence "We can also suppose that this high tolerance of <i>L. pneumophila</i> serogroup 1 isolates to elevated temperature within the water heating system is in relation with the genetic diversity among the isolates and may result in enhancing their capacity towards colonizing these water systems." (lines 260-263) it seems</p> | <p>The modification using slightly bigger filter pore size (0.45µm instead of 0.20µm) were also used in previous studies for example:<br/><b>Wójcik-Fatla A, Stojek NM, Dutkiewicz J. Efficacy of the detection of <i>Legionella</i> in hot and cold water samples by culture and PCR. I. Standardization of methods. Ann Agric Environ Med. 2012;19(2):289-93.</b></p> <p>And did not result in lowering the number of positive samples, concerning the less amount of collected water sample (250mL instead of 2L), this may explain the low number of positives found and this should be viewed with concern during our future surveillance studies.</p> <p>In this manuscript It is important to point out that, one of the limitations in this study, was the small number of the total <i>L. pneumophila</i> isolates belonging to serogroup 2-14, which necessitates continuous monitoring through surveillance studies besides trying to put more effort in properly identifying the <i>L. pneumophila</i> 2-14 serogroups, observe their genetic heterogeneity using various DNA typing methods and compare it with those of serogroup 1 in future work.</p> <p>"We can also suppose that this high tolerance of <i>L. pneumophila</i> serogroup 1 isolates to elevated temperature within the water heating system is</p> |



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|  | <p>that the authors suggest that the diversity is the reason of the colonization, and in my point of view it is the opposite. A better adaptation to a niche increases the diversity of that strain in that place.</p> | <p>in relation with the genetic diversity among the isolates and may result in enhancing their capacity towards colonizing these water systems.” (Lines 260-263). In this statement we the Authors agree with reviewer that A better adaptation to a niche increases the diversity of that strain in that place. And the statement was rephrased and highlighted in yellow inside the manuscript</p> |
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| <p><b><u>Minor</u></b> REVISION comments</p> | <p>-In table 1. It is possible to indicate the data of each sample? Are there replicates and/or areas with samples taken in different months? Seeing the evolution of the distribution of each genotype/serogroup in the different areas could be interesting.</p> <p>-In the last paragraph (lines 267-271) I would recommend you not to use the word “eradication” in a global sense, because <i>Legionella pneumophila</i> in the natural water systems rarely causes disease, it is when the artificial conditions of human water systems, and a high growth of these strains can affect to susceptible people. So, I recommend using “control Legionella proliferation in human water systems” or something similar.</p> | <p>Additional data for sample collection was added to table 1 as advised by the reviewer and highlighted in yellow.</p> <p>As mentioned in the materials and methods section, about 200 samples were collected over a period of eight months starting from May and till the end of December 2012, and as mentioned in the results and discussion section “in summer (from May to September), during this period of the year the water heating systems in Aqaba city are usually turned off, and thus the water temperature inside the heater storage tank ranges between 25 to 30°C. “in winter (mainly November and December), a period during which water heating systems are usually turned on with variable heat set points usually between 55°C to 60°C to prevent scalding. <b>So yes there were replicates of areas with water samples taken in different months</b>, and this also could be observe from the table that areas with isolates collected at temperature ranges of 25 to 30°C and 55°C to 60°C indicates that it was taken in different months.</p> <p>“Seeing the evolution of the distribution of each genotype/serogroup in the different areas could be interesting” the authors completely agree with the reviewer point of view but, in this study our main goal was to determine the presence and diversity of <i>Legionella spp.</i> in domestic water systems in the city of Aqaba in relation to the temperature inside the water heating tank. So we</p> |
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|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | <p>could through similar surveillance studies and continuous monitoring in the future try to determine the evolution of the distribution of each genotype/serogroup in the different areas.</p> <p>The word "Control" was used instead of the word "eradicate" and highlighted in yellow inside the manuscript</p>                                                   |
| <u>Optional/General</u> comments | <p>I am not a native English speaker but in spite of finding the text, in general, clear and well written, I strongly recommend that many corrections should be done by a native English speaker.</p> <p>For example:</p> <ul style="list-style-type: none"> <li>-In the abstract it is written "were Legionella species other than pneumophila". It must say "were <i>Legionella</i> species other than <i>L. pneumophila</i>".</li> <li>- "30 C and 35 C" (33)</li> <li>- Legionellae spp. (143)</li> <li>- "<b>Legionella pneumophila</b> serogroup 1 and five were <b>Legionella pneumophila</b> serogroup 2-14." (151-152).</li> </ul> <p>Genus is only written in full when it is used the first time.</p> <ul style="list-style-type: none"> <li>- "10 out of the 17" (157)</li> <li>- <i>L. pneumophila</i> is the correct name of the specie and not "<i>L. pneumophila</i>" (lines 198, 257, 259 and others)</li> <li>- diveristy (262).</li> </ul> | <p>All the Optional/General corrections suggested by the reviewer were made inside the manuscript and highlighted in yellow as advised, and the manuscript was sent to Dr Eric R. Dabbs, (A British national and a dear colleague) working at the School of Molecular and Cell Biology, University of the Witwatersrand, South Africa, for English proofreading.</p> |