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

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.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	The main concluding observation of the paper is that the temperature of the water tanks may be one of the most important factors that affect the genotypic behaviour of <i>Legionella pneumophila</i> . To exclude that the temperature in the bottom of the tanks may be much lower than the set one, it could be important to demonstrate by specific experiments the resistance at 80 – 90°C of the isolates.	Only a single isolate was collected from heating water tank with temperature set at 80 – 90°C. When we analysed this isolate its genotypic behaviour was similar to other isolates collected from heating water tank with temperature set at 50 – 60°C, and to be sure whether this isolate can survive this elevated temperature (80 – 90°C) a simple experiment was designed, this experiment involved inoculating this isolate on duplicate slants of CYE broth (charcoal yeast extract base) with BCYE growth supplement (ACES buffer/potassium hydroxyde, L-cysteine, ferric pyrophosphate, alpha-ketoglutarate) and MWY selective supplement (glycine, vancomycin, polymyxin B, anisomycin) and incubated at different temperatures ranging from 40–80°C for 24hr , after which the isolate was transferred to duplicate plates of CYE agar with BCYE growth supplement and MWY selective supplement and incubated at 35°C for 3–7 days to observe colony formation and it was confirmed that (NO growth was detected on plates inoculated with isolates grown for 24 hr over 60°C). Therefore it was suggested that that the temperature in the bottom of the tanks may be much lower than the set one resulted in the isolation of this strain (results not shown in the manuscript please advise on whether it should be added to the results and discussion section or not)



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<u>Minor</u> REVISION comments	Line 262 diveristy to correct with diversity. Marking that the RAPD seems not able to differentiate among the different isolates of serogroup 2-14: it is not clear if all these isolates belong to the same specific serogroup. It could be useful to identify the serogroup and to discriminate these strains to use another typing method such as PFGE	Diversity is corrected and highlighted in yellow in the manuscript. Our focus in this manuscript was more shifted towards <i>Legionella pneumophila</i> serogroup 1 since most of the isolates belonged to it and it's also the most common cause of Legionnaires' disease worldwide, but of course it would be useful in the future work to try and identify the serogroup and to discriminate these strains to use another typing method besides RAPD in case all these isolates belong to the same specific serogroup.
<u>Optional/General</u> comments	The main remark concern: the diffusion of <i>L. pneumophila</i> in domestic water system; the use of RAPD as method to evaluate the molecular diversity of isolates of <i>Legionella</i>; the value of temperature to control the dissemination of <i>Legionella</i>; the correlation with high tolerance of <i>L. pneumophila</i> serogroup 1 isolates to elevated temperature and their genetic diversity. However it is not clear if there is genetic diversity among all the <i>L. pneumophila</i> isolates belonging to serogroup 2-14.	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, but despite the limited number of tested isolates, the results observed in this study should be viewed with concern and necessitates continuous monitoring through surveillance studies besides trying to put more effort in properly identifying the <i>L. pneumophila</i> 2-14 serogroups and observe their genetic diversity using various DNA typing methods in future work.