Author's response to reviews

Title: Influence of pre- and post-usage flushing frequencies on bacterial water quality of non-touch water fittings

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Author's response to reviews: see over
To
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Authors’ reply to referees’ comments

MS: 1244420389950552: „Influence of pre- and post-usage flushing frequencies on bacterial water quality of non-touch water fittings”.

Dear Mrs. Harris,

The authors thank the Journal and the referees for having reviewed our manuscript “Influence of pre- and post-usage flushing frequencies on bacterial water quality of non-touch water fittings”. All four reviewers found our manuscript of interest and made very helpful comments. We have addressed the reviewers’ recommendations in our response and have addressed each comment in the re-submitted revised manuscript (R1). As requested by the journal’s copy editor, the manuscript was transformed into the journal’s style by using the provided format template. The competing interest as well authors’ contribution section was added. The revised manuscript was read and approved by all co-authors. We hope that the content of our revised paper meets the stringent quality criteria of your prestigious journal.

With our best Regards,

Univ.-Prof. Ojan Assadian, MD, DTMH

(For the author)
Referee #1

C1  The references are old. Most were published up to 2005 and only one was published after this (in 2007). Please update the references.

The respected reviewer is correct. We have updated the reference list and have modified the referencing according to the recently published literature.

C2.  It would be interesting if the authors included statements on conflict of interest, especially as to their relationship with the producer of the water fittings.

We have added a conflict of interest statement at the end of the manuscript’s text. The authors declare that they have no competing interests related to the presented work or the manufacturer of the water faucets tested in this study. The specific faucet model was chosen solely as it allowed pre-programming the flushing frequencies and modalities. The authors have no financial or other interests in relation to the manufacturer or the manufacturer’s sanitary fixtures.

C3.  The authors did not adequately evaluate the effect of pre-use flushing as fitting number 8 was not used at all. However, I agree with the authors that pre-use flushing even if effective would not be practical.

The respected reviewer puts emphasis on an issue, which was thoroughly discussed among the authors during preparation of the manuscript. In fact, the role of fitting no. 8 is different to the other seven fittings, as this fitting was incorporated into the study design in order to investigate the faith of water quality of a fitting, which is not used in the clinical setting. Indeed, when planning together with architects new hospitals or when hospitals are upgraded, there is the architect’s and users’ wish to install as much fittings as possible, just not to miss the possibility later to have the option of hand washing or to shower. From an infection control point, however, unused water fittings are usually those with unfavourable water
quality results. The reason why fitting no. 8 was incorporated into the study was to obtain published measurements to support or withdraw the infection control demand of having fewer, but then more frequently used water fittings in the hospital setting. Surprisingly, this fitting did not yield the highest bacterial counts, as would have been expected by the authors. There are a number of reasons for this outcome. Water faucets may be contaminated antegrade or retrograde. One possibility would be that because the fitting was not used frequently, no retrograde contamination occurred. Another reason may be that fitting no. 8 was the only fitting with a 30s pre-flush. This aspect, however, was not adequately addressed in the method section of the manuscript. We have corrected this matter and than the reviewer for highlighting the missed opportunity to present an important aspect adequately.

C4. I feel that the manuscript could be reduced by about 30%, especially the discussion.
We have followed the recommendation and have reduced the length of the discussion section from 992 words to 863 words.

Referee #2

C1. What was the idea to change the length of the water pipes?
The reason to change the length of the water pipes was to investigate the influence of the water pipe’s length on the total cfu count. The hypothesis behind this is that the larger the length of stagnating water is, the higher is the total cfu count. Our result, however, demonstrate that the length of the water pipe did not have a measurable effect on the total cfu counts.

C2. The authors concluded that 30 s preflushing with 18°C is very efficient preventing formation of cfu/ml. With this setup the pipe system has not been flushed with the 37°C
water therefore the conclusion “…that a 30 s preflushing is really helpful...” is not supported by the data. How long does it take that the whole pipe gets a temperature of 18°C. Was this time really determined or was the cold water just turned on? Was the 18°C the water temperature or the temperature of the pipe?

The respected reviewer is correct as the water will not have a temperature of 18°C immediately, but rather will reach this temperature gradually. During the unused period, the temperature of the water in the fitting and the water pipe will have ambient room temperature, e.g. 22°C. We measured the temperature of water during sampling and denoted the highest water temperature. The important aspect was to verify that the pre-set water temperature (e.g. 18°C or 37°C) was correctly achieved by the respective faucets programs. However, we have no data with demonstrated the time-kinetic how fast the final temperature was reached. Therefore, the temperature’s mean may have been higher or lower, yet, we cannot add information on the mean and SD of the temperature during sampling. The variation, however, may be negligible with no influence of the final results.

C3. The sentence on page 11 Pre-flushing of 30 seconds with cold water was the most effective method to bacterial accumulation in the tested non-touch fittings. does not make sense to me.

We sincerely thank the respected reviewer for pointing out this mistake. The sentence was now corrected to: “Pre-flushing of 30 seconds with cold water was the most effective method to prevent bacterial accumulation in the tested non-touch fittings.”

Referee #3

C1. An article of importance in its field

We sincerely thank the respected reviewer for this favourable comment.
C1. Please clarify the study aim - it is not consistent throughout and the cited literature indicates that accumulation of Pseudomonas aeruginosa is the focus of the paper but discussion is based on results for total bacterial counts (cfu). Also, one of the keywords is Pseudomonas aeruginosa. The cited literature (both in the introduction and discussion) primarily concerns Pseudomonas aeruginosa bacteria and their accumulation in tap fittings – particularly in the hospital setting. While the authors of this study tested for Pseudomonas aeruginosa these organisms were not detected. Conclusions made about flushing times of the tap fittings are based on total plate counts but there is no mention of how these relate to accumulation of Pseudomonas aeruginosa (i.e. no reference to the differences in accumulation and persistence of Pseudomonas aeruginosa in tap fittings and ease of removal etc compared with other bacteria). In the Introduction the aim of the study is defined as ‘..to investigate the number of frequently provided post-flushing times after use of non-touch fittings. Additionally, the effect of pre-rinsing with cold water before use was explored’. Later on (methods section), it is stated that the primary aim of the study ‘is to investigate possible differences in bacterial counts obtained from regularly used fittings (fittings no 3-7) with particular respect to no post-flushing and 2 seconds or 10 seconds of automated post flushing with cold water’. In the discussion paragraph 6 it is stated that ‘..the aim of the present experimental study was to examine the influence of frequency of usage, the duration of water stagnation, the influence of plastic materials, and post or pre-rinsing with cold water before or after use of non-touch fittings in a controlled laboratory setting..’. This aim should be rewritten to clarify the purpose of the experiments. Was it, as indicated by the literature cited, ‘to investigate the optimum flush (post- and/or pre-) time setting for removal of Pseudomonas aeruginosa ‘. If this is the case, based on results (i.e.
no detectable Pseudomonas aeruginosa) an argument would have to be made that total plate count bacteria (Pseudomonas aeruginosa being included amongst their number) are a sensitive indicator of bacterial accumulation and removal with flushing and that their use as a marker of Pseudomonas aeruginosa accumulation and removal is justified.

The respected reviewer raises an important issue. First, we thank the reviewer for highlighting that the aim of the study was not adequately stated in the introduction section. We have re-written the study’s aim based on the comments and based on the later made statements. Second, we were interested in the total cfu counts per mL sampled water. However, by reviewing the existing literature, we recognized that major emphasis is put on P. aeruginosa, and total cfu counts are highlighted to a lesser degree. Obviously, the reason for this is that P. aeruginosa is a known a multiply demonstrated facultative pathogen, while total cfu counts may or may not include pathogenic bacteria, diminishing the “dramaturgic” effect in published papers. However, we fully agree with the comment made by the reviewer that total plate counts are a sensitive marker of Pseudomonas aeruginosa accumulation and removal. Based on the reviewer’s comment, we have modified the discussion and we have incorporated the aspect that any effect on the total plate count may also affect P. aeruginosa counts, therefore emphasis was laid on the total cfu counts/ mL water.

C2. The methods are generally well described (with the exception of some editorial changes that would make reading this section easier)

We sincerely thank the respected reviewer for this favourable comment.

C3. Given that there is much discussion about P. aeruginosa persistence and accumulation – including reference in the discussion to the fact that other authors have found that the ‘source of contamination to be the magnetic valve made of plastic materials and the fitting outlet itself...’ I am surprised that the Methods section does not include a
description of type of valves in the fittings used in experimentation. This may be the reason that P. aeruginosa were not detected in this study. Perhaps if the valves were made of a plastic that supported P. aeruginosa growth authors of this study might have observed, even with few incoming P. aeruginosa bacteria, the accumulation of these bacteria after non-use periods and/or failure to remove them with flushing. Please include some detail about the valve material in the fittings used in the experiment.

The reviewer is correct! We have reviewed the magnetic valves of the investigated faucet. The fixture uses brass valve blocks! We have incorporated this important aspect into the discussion section and have added the information on the material of the valves into the method section. Furthermore, we have to acknowledge that one of our initial study questions was completely wrong, as we are not able to investigate the influence of the valve’s material on total cfu counts. We are only able to comment on the observations made with brass valves. Clearly, a comparative study investigating faucets with plastic or brass valves would be of high interest. Regretfully, we have no data to further elaborate on this aspect. However, we have included this aspect into the discussion section and hope to foster future research on this aspect.

C4. Why was it decided not to perform sample dilutions? How was the approximation of >1000 cfu/mL made? Please explain.

We filtered 500 mL of sample water and have processed 1 mL and 0.1 mL directly on agar. The > 1,000 cfu values derive from approximation of the 0.1 mL samples or directly without approximation of the 1 mL samples. Hence, all 1 mL values are direct measures.

C5. Also, the resolution of the plate count method is usually 250 - 300 colonies per plate – counts are reported above this – but no indication is given that these (counts above 250/300) are approximations. Please explain.
Please see our comments at C4.

C6. **Suggest that in results section that ‘None of the water samples yielded *P. aeruginosa*’ be replaced with *P. aeruginosa* results were <1 per 100mL for all samples**

We have followed the reviewer’s recommendation accordingly. The sentence was changed now as suggested by the reviewer.

C7. **Why are there no readings for fitting number 8 in weeks 1, 2, 3, 5, 6, 7, 8, and 20 in Table 2?**

The reason for this is that we were interested to measure from water fittings which were not frequently in use. Since sampling of fitting no. 8 would have introduced at least a minimum usage, the fitting was not samples throughout, and hence not used, in weeks 2-3 and 5-8. We measured only once before the test started, once after 4 weeks to obtain an orientation, and from week 9 onwards.

C8. **See comments about the aim of the study. The discussion and conclusions should directly address the aims of the study. As it stands the aim is not well defined leading to a discussion primarily about *P. aeruginosa* accumulation and removal - yet in this study there were no detections of *P. aeruginosa*. The relationship between total bacterial counts and *Pseudomonas aeruginosa* should be clarified if total bacterial counts are being used as surrogates for *P. aeruginosa* removal with flushing. Conclusions should related back to the aims once they are refined and be adequately supported by the data. If the aim is to be the one given in the discussion, results obtained need to be related back to the influence of plastic materials amongst other things requiring a description of plastic fittings and pipes and the material that they are made of.**

Please note our answers and comments at C1 and C3.
C9. The limitations of the work are not clearly stated because the reader is not informed about the relationship between numbers of total bacteria colony forming units and P. aeruginosa. If total bacteria colony counts are being used as a measure of the accumulation and subsequent removal with flushing of P. aeruginosa – the assumptions made in doing this must be stated.

Please see our comments at C1.

C10. Introduction, 2nd last paragraph: ‘Aside (of) *from* the materials used...’

This mistake was corrected.

C11. Discussion, paragraph 2, sentence 3: ‘similar findings were reported (from) *by* other authors’

Because of comments made by other reviewers this passage was omitted now completely.

C12. Choice of words sometimes is not ideal or wrong. Some examples (there are more):

Introduction, paragraph 4: ‘Regretfully, the (later) *latter* aspect together with the technical requirement...’

This mistake was corrected.

C13. Discussion, last paragraph: 'Such a concept would (bear) *have* further advantages...’

This mistake was corrected.

C14. Discussion, paragraph 6: sentence 1 ‘Therefore, it remained (open if) *debatable whether* bacterial colonization and accumulation are supported by a ..’
This mistake was corrected.

C15. Introduction, paragraph 2: CURRENTLY READS: ‘Among other microorganisms, *Pseudomonas aeruginosa* presumably originating from drinking water, water used for cleaning machines, wound cleaning, and other procedures bringing water in contact to patients, has been reported having a significant health impact in hospitals and nursing homes, resulting in longer hospital stays and deaths. Because of this, the World Health Organisation had placed particular emphasis on water safety in health care facilities, since to the high number of patients at risk the requirement on the microbiological quality of water is higher than in a domestic setting’. SUGGESTED CHANGE: ‘The World Health Organization had placed particular emphasis on water safety in health care facilities because of the concentration of susceptible persons in these, as compared with, domestic settings. Amongst other microorganisms, *Pseudomonas aeruginosa* has been reported as having a significant health impact in hospitals and nursing homes, resulting in longer hospital stays and deaths. Drinking water that is used for cleaning machines, wound cleaning, and other procedures that bring water in contact with patients provides a potential transmission route for these microorganisms...’

We thank the respected reviewer for these excellent suggestion. The proposed modifications have been incorporated now into the revised version of the manuscript as recommended.

C16. Introduction, paragraph 3: ‘Non-pathogenic micro-organisms (habitat) *may be* embedded and protected in biofilms, which may frequently be found in water pipes and water fixtures.’

Because of comments made by other reviewers this passage was omitted now completely.
C17. Discussion, paragraph 6: ‘Pre-flushing of 30 seconds with cold water was the most effective method to *prevent* bacterial accumulation in the tested non-touch fittings’

We thank the respected reviewer for highlighting this mistake. The sentence was corrected accordingly.