Reviewer's report

Title: Identification of bacteria in drinking water and purified water during the monitoring of a typical water purification system.

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Reviewer: Prof Pierre Payment

Level of interest: not specified

Advice on publication: Other (see below)

The following texts from the resubmitted paper are irrelevant to this paper whose subject is pharmaceutical water. They should be deleted.
Delete:
Page 4 lines 11 to 24
Page 5 (all)
Page 6 (all)
Page 8 lines 20 to 22 (table)
Page 9 table at top of page
Page 20 lines 1 to 8
Page 27 line 21-23: Suggestion: "The analysis of treated water for heterotrophic bacteria including Pseudomonas species is valuable to prevent the formation of biofilms and to reduce the amount of pyrogen in the water."
Page 28 lines 1 to 10: Delete

The following paper could be integrated in the text and references:
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16S Ribosomal DNA-Based Analysis of Bacterial Diversity in Purified Water Used in Pharmaceutical Manufacturing Processes by PCR and Denaturing Gradient Gel Electrophoresis
Mako Kawai,1 Eiichi Matsutera,1 Hisashi Kanda,1 Nobuyasu Yamaguchi,2 Katsuji Tani,2 and Masao Nasu2*
The bacterial community in partially purified water, which is prepared by ion exchange from tap water and is used in pharmaceutical manufacturing processes, was analyzed by denaturing gradient gel electrophoresis (DGGE). 16S ribosomal DNA fragments, including V6, -7, and -8 regions, were amplified with universal primers and analyzed by DGGE. The bacterial diversity in purified water determined by PCR-DGGE banding patterns was significantly lower than that of other aquatic environments. The bacterial populations with esterase activity sorted by flow cytometry and isolated on soybean casein digest (SCD) and R2A media were also analyzed by DGGE. The dominant bacterium in
purified water possessed esterase activity but could not be detected on SCD or R2A media. DNA sequence analysis of the main bands on the DGGE gel revealed that culturable bacteria on these media were Bradyrhizobium sp., Xanthomonas sp., and Stenotrophomonas sp., while the dominant bacterium was not closely related to previously characterized bacteria. These data suggest the importance of culture-independent methods of quality control for pharmaceutical water.

**Competing interests:**

None declared.