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# Analysis of microbial contamination of household water purifiers

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## Abstract

Household water purifiers are increasingly used to treat drinking water at the household level, but their influence on the microbiological safety of drinking water has rarely been assessed. In this study, representative purifiers, based on different filtering processes, were analyzed for their impact on effluent water quality. The results showed that purifiers reduced chemical qualities such as turbidity and free chlorine. However, a high level of bacteria ($10^2$–$10^6$ CFU/g) was detected at each stage of filtration using a traditional culture-dependent method, whereas quantitative PCR with propidium monoazide (PMA) treatment showed $10^6$–$10^8$ copies/L of total viable bacteria in effluent water, indicating elevated microbial contaminants after purifiers. In addition, high-throughput sequencing revealed a diverse microbial community in effluents and membranes. *Proteobacteria* (22.06–97.42%) was the dominant phylum found in all samples, except for purifier B, in which *Melainabacteria* was most abundant (65.79%). For waterborne pathogens, *Escherichia coli* ($10^0$–$10^6$ copies/g) and *Pseudomonas aeruginosa* ($10^0$–$10^5$ copies/g) were frequently detected by qPCR. Sequencing also demonstrated the presence of *E. coli* (0–6.26%), *Mycobacterium mucogenicum* (0.01–3.46%), and *P. aeruginosa* (0–0.16%) in purifiers. These finding suggest that water from commonly used household purifiers still impose microbial risks to human health.

**Keywords** Household treatment purifiers · Microbial contamination · Membrane filtration · Waterborne pathogens · High-throughput sequencing · PMA-qPCR

## Introduction

In the past decades, source water has been increasingly contaminated by various chemicals and emerging pollutants, which represents a great concern for public health (Hu et al. 2018; Shi et al. 2018). People's demand for safe and healthy drinking water is increasing, creating a challenge for public water suppliers. The microbiological safety of drinking water is crucial for human health. It is estimated that five million people lose their lives because of waterborne illness each year (WHO 2006). To control microbiological risks, water is generally disinfected to kill pathogens before entering the drinking water distribution system. However, some persistent bacteria remain in water, and even proliferate and grow in pipelines (Douterelo et al. 2018; Vaz-Moreira et al. 2017). Traditional treatment techniques do not efficiently remove all chemical and microbiological contaminants. Therefore, point of use (POU) or household water treatment methods can be useful to improving the quality and safety of drinking water.

Household water purifier can include multi-step activated carbon or special absorbent material filters and key step membrane filters. Activated carbon treatment can effectively adsorb organic substances and residual disinfectants, improving water quality and taste (Korotta-Gamage and Sathasivan 2017). In addition, membrane filtration is another process that physically removes various contaminants, including bacteria, viruses, suspended solids, and heavy metals (Shirasaki et al. 2017; Wang et al. 2013). Commonly used membrane filtration technologies include microfiltration (MF), ultrafiltration (UF), and reverse osmosis (RO), which can filter out 0.5–5-, 0.005–0.5-, and 0.0007–0.005-μm particles, respectively (Hoslett

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et al. 2018). However, biofilms generated on the surfaces of moist activated carbon can detach and enter effluents, causing public health problems (Gibert et al. 2013). Similarly, membrane filtration systems are also easily fouled by attached microorganisms during routine operation (Shao et al. 2018; Wei et al. 2011). Membrane fouling of household water purifiers can decrease membrane flux, and biofilms released from membrane surface can lead to excessive bacteria in effluents (Su et al. 2009). A previous study showed that the clinical relevance of *Pseudomonas aeruginosa* isolated from filters of household water treatment systems (Mombini et al. 2019). However, there is limited information on microbial contaminants and membrane fouling in household water purifiers.

The quality of drinking water is traditionally assessed by measuring cultivable bacteria and attempting to detect fecal indicator bacteria (Gillespie 2016). However, only a slight fraction (below 1%) of bacteria in drinking water system can be measurable by culture-dependent methods; a large proportion of bacteria are in a viable but non-culturable (VBNC) state (Hammes et al. 2008). Therefore, PCR-based techniques have been developed to rapidly, accurately, and more comprehensively survey microorganisms. For instance, high-throughput sequencing (HTS) is widely used to show the microbial diversity in drinking water systems (Bae et al. 2019; Gerrity et al. 2018; Lin et al. 2014). In addition, quantitative PCR (qPCR) can target at specific microbes, including pathogens (Cui et al. 2017). Furthermore, propidium monoazide (PMA)–modified PCR method can be applied to distinguish live from dead cells by inhibiting amplification of DNA from dead cells, which represent real health risks associated with drinking water (Gensberger et al. 2014).

This study aimed to investigate microbial contaminations in household water purifiers, as well as microbial health risks to humans. Specific objectives were the following: (1) enumerate total bacteria both in the water phase and in filter units of each treatment process; (2) compare total, viable, and culturable bacteria in water and membrane samples; (3) characterize microbial communities using HTS technology; and (4) quantify common waterborne pathogens.

## Materials and methods

### Water sample collection and water quality measurement

Four representative household water purifier devices designed with different treatment processes and service times were obtained from residents. Detailed descriptions are provided in Table 1. The influent and effluent water quality (i.e., temperature, pH, free chlorine, dissolved oxygen (DO), turbidity, and organic matter indices) was measured three times following the National Standards for Drinking Water Quality (GB 5749-2006) before the devices were disassembled. Several indices such as temperature, DO, and free chlorine were measured on site at the time of sampling. To obtain representative samples, the water was left running for 5 min before collection. After this, 1.5 L of influent and effluent water was collected in sterile bottles. The number of total bacteria was determined by spread plating on nutrient agar (NA) medium at 37 °C for 48 h or on $R_2A$ agar at 28 °C for 5 days (Liu et al. 2019). Moreover, 10 L of influent and effluent water was filtered through 0.22-μm membrane filters (Millipore, Billerica, MA, USA) in duplicate at each sample site for later DNA extraction.

**Table 1** Description of household water purifiers examined in this study

| Purifiers | Service time | Membrane technology | Producing country |
|---|---|---|---|
| A | 2 years | UF | China |
| B | 1 year | UF | America |
| C | 5 years | RO | China |
| D | 1 year | UF and RO | China |

UF is ultrafiltration; RO is reverse osmosis

### Microorganism collection from padding materials and membrane filters

To detect microorganisms attached on different purifiers, each treatment unit was split using a saw. Diagrams of each purifier are shown in Fig. S1. In purifier A, drinking water was successively treated with a polypropylene (PP) cotton filter → pre-activated carbon (AC) filter → AC filter → composite material (CM) filter → ultrafiltration (UF) membrane filter, and finally post-activated carbon (AC) filter. The treatment process of Purifier B was simpler, with only three steps: PP cotton filter, UF membrane filter, and compressed AC filter. In purifier C, drinking water was continuously treated with five major steps: PP cotton filter → pre-AC filter → pre-AC filter → reverse osmosis (RO) membrane filter → post-AC filter. Purifier D employed a series of treatment processes: PP cotton filter, compressed AC filter, UF membrane filter or RO membrane filter, and post-AC filter.

To obtain the bacteria adhering to membranes, the membranes were cut into pieces using sterile scissors. Both the packing material and membrane samples were placed in a sterile saline solution for sonic treatment (KQ-500DE, China) at 30 min and 40 kHz to separate biomass from the matrix (Shi et al. 2013). After that, the suspension was left standing for 5 min to remove large particles, and the supernatant was used to determine the amount of biomass in biofilms. The number of total bacteria was determined on NA or $R_2A$ agar plates in the above incubation conditions.

## PMA treatment and DNA extraction

To extract total genomic DNA, the supernatants from membrane samples were concentrated by filtering through membrane filters with a 0.22-μm pore size in duplicate. The second aliquots of membrane filters were intended for pretreatment by evenly dropping 500 μL PMA dye (Biotium Inc., USA) on the surfaces of filters at a final PMA concentration of 50 μg/mL, which was demonstrated to be an efficient concentration based on previous studies (Chen et al. 2017; Hu et al. 2019). In brief, filters were incubated in the dark for 5 min and then subsequently held on ice and horizontally exposed to a 650-W halogen light at a distance of approximately 20 cm for 4 min (Gensberger et al. 2014; Liu et al. 2018). Finally, both the pre-treated and non-treated samples were cut into small pieces and used for total DNA extraction with a FastDNA SPIN Kit (MP Biomedicals, USA) following the manufacturer's instructions. DNA concentration and purity were measured using a ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA).

## Illumina high-throughput sequencing

To determine the diversity and composition of bacterial communities in water and membrane samples, DNA was amplified using forward primer 515F (5′GTGCCAGC MGCCGCGGTAA-3′) and reverse primer 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) targeting the V4–V5 regions of 16S rRNA (Zhang et al. 2018). In detail, DNA was first amplified by PCR at 95 °C for 5 min, followed by 30 cycles at 95 °C for 40 s, 58 °C for 40 s, 72 °C for 40 s, and a finial extension at 72 °C for 7 min. PCR products were purified using an AxyPrepDNA Gel Kit (Axygen, CA, USA), and sequencing was performed on an Illumina MiSeq platform using standard procedures (Novogene Bioinformation Technology Co., Ltd., China).

Raw sequences were filtered and analyzed using a pipeline in Quantitative Insights into Microbial Ecology (QIIME) software to exclude low-quality and chimeric sequences as described in a previous report (Wang et al. 2018). After this, the sequences were clustered into operational taxonomic units (OTUs) with a 97% threshold. Species diversity was evaluated in mothur (http://www.mothur.org). A representative sequence for each OTU was aligned using the Silva database and Ribosome Database Project classifier (Song et al. 2018). Hierarchical clustering was conducted to visualize similarities in bacterial communities based on unweighted UniFrac metrics. The raw sequencing data has been submitted to the NCBI Sequence Read Archive (SRA) with the project accession code of PRJNA576308.

## Quantification of total bacteria and potential pathogens

Total bacteria were quantified based on 16S rRNA using SYBR Green qPCR. For the SYBR Green qPCR assay, each 20-μL reaction mixture included 10 μL of SYBR Premix Ex Tap (Takara, Japan), 0.4 μL of each primer (10 μmol/L), and 1 μL of template DNA. The PCR cycling procedures were the following: 95 °C for 30 s, followed by 35 cycles of 95 °C for 5 s, 60 °C for 30 s, 72 °C for 30 s, and then 1 cycle of 72 °C for 7 min. In addition, a TaqMan probe was designed to target five potential pathogens, namely *P. aeruginosa*, *Salmonella enterica*, *Shigella*, *Escherichia coli*, and *Legionella pneumophila* with 6-carboxyfluoresein (FAM) as the fluorescent reporter on the 5′ end and 6-carboxytetramethylrhodamine (TAMRA) as the quencher dye on the 3′ end. For the real-time qPCR assay, each 20-μL reaction mixture contained 10 μL of 2× PCR mixture, 1.0 μL of each primer, 0.5 μL of each 10 μM probe, and 1 μL DNA template. The reaction procedures included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and, 60 °C for 1 min. All qPCRs were performed on a 7300 real-time PCR system (ABI 7300, Applied Biosystems, USA). The primer sequences used are listed in Table S1. Both DNA templates and negative controls (DNA replaced with nuclease-free water) were run in triplicate. In addition, a melting curve was prepared to verify primer specificity. All standard qPCR curves were constructed from 10-fold serial dilutions of the plasmid carrying target genes ranging from $10^2$ to $10^7$ gene copies per microliter. The number of target gene copies was calculated based on Ct values compared with the standard curves described above.

## Observation of microbes on membranes by scanning electron microscopy

The microbial morphology of microbes on membranes was directly observed by scanning electron microscopy (SEM). Membrane samples were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for 1 h at 4 °C and washed three times with PBS at room temperature. Each sample underwent graded dehydration with 30%, 50%, 70%, and 90% alcohol for 10 min and then treatment with 100% pure ethyl alcohol for 20 min. Finally, the samples were dried overnight in a critical point dryer before analysis by SEM (SEM, Hitachi S-4800, Japan).

## Statistical analysis

The physicochemical and biological data from the influent and effluent water samples were compared statistically with an analysis of variance (ANOVA) and a significance level of $p < 0.05$ using IBM SPSS Statistics 22.0 software.

## Results

### Water quality characteristics

As shown in Table 2, levels of free chlorine in effluent water (0.00–0.06 mg/L) were significantly lower than those in all influent water (0.41–0.74 mg/L). pH values were around neutral (6.71–7.38) for all water samples, except for effluent water from RO membranes in purifier D (6.26 ± 0.37). DO ranged from 8.28 to 8.70 mg/L, but DO in the effluents of purifier C (RO 5.06 ± 0.11) and purifier D (UF 4.23 ± 0.10, RO 3.79 ± 0.88) were significantly less. Turbidity of 0.08–0.53 NTU was observed in influent water samples, but this was less in effluent water samples (below 0.02 NTU), except for effluent from the UF membrane in purifier D (0.09 NTU). The values of $NO_3$-N and total organic carbon (TOC) were slightly decreased after UF membrane filtration, but were significantly reduced after RO membrane filtration. As for temperature, it ranged from 22.33 to 23.43 °C in three independent samplings. Finally, it should be noted that no bacteria were detected from influent water cultivated in NA medium, but high levels of bacteria (0.67–2.59 × $10^5$ CFU/100 mL) were detected in effluent water, especially after RO treatment.

### Microbial contaminants in each processing unit

To analyze microbial contaminants at each filtration step in purifiers, filtration units were disassembled. The bacteria attached padding materials and membranes were enumerated (Fig. 1). No bacteria grew on NA medium, but 3.33 × $10^2$–3.67 × $10^3$ CFU/L bacteria were detected in influent water by $R_2A$ plating. Similarly, more bacteria were found on $R_2A$ plates than on NA medium at other treatment steps. Subsequently, high levels of bacteria (6.12 × $10^2$–3.78 × $10^5$ CFU/g) were retained at the first step of PP cotton filtration, and bacterial concentrations were higher on external surfaces (2.58 × $10^3$–3.78 × $10^5$ CFU/g) than on internal surfaces (6.12 × $10^2$–3.11 × $10^5$ CFU/g) because the water in these systems flowed from outside to inside. In addition, we found that the color of PP cotton filters became yellow by the naked eye (data not shown). Moreover, 1.51 × $10^4$–2.88 × $10^5$ CFU/g of bacteria was maintained in one-step or multi-step pre-activated carbon or complex material filters in all purifiers except purifier D. In purifier D, although the number of bacteria in pre-activated carbon was only 1.59 × $10^1$ CFU/g on NA medium, there were still 1.03 × $10^6$ CFU/g bacteria grown on $R_2A$ medium. It should also be noted that there were no pre-activated carbon filters in purifier B.

The concentration of bacteria increased to 1.12 × $10^3$–1.24 × $10^6$ CFU/g at the membrane filtration step. Microbial attachment on membrane filters was directly verified by SEM (Fig. 2). Microbes were scattered or assembled in small groups on the surfaces of membranes. However, a lower level (1.12 × $10^3$ CFU/g) of bacteria was detected on a UF membrane from purifier B on NA medium, which might be due to remaining free chlorine as there was no pre-activated carbon filter in this purifier. It should be noted that still 3.33 × $10^0$–5.98 × $10^3$ CFU/g of bacteria persisted in post-activated carbon filters. Finally, the number of bacteria in effluent water (2.0 × $10^4$–2.30 × $10^7$ CFU/L) was significantly higher than that in influent water (3.33 × $10^2$–3.67 × $10^4$ CFU/L) when counted on $R_2A$ medium.

### Viability of bacteria in water and membrane samples

In this study, three methods, including traditional qPCR, PMA-modified qPCR, and culture, were employed to quantify microbial contaminants. As shown in Fig. 3, a high level of 1.26 × $10^5$–2.28 × $10^6$ copies/L total bacteria was detected in influent water by traditional qPCR, and the concentration of

**Table 2** Characteristics of water quality

| Samples | pH | Free chlorine (mg/L) | DO (mg/L) | Turbidity (NTU) | $NO^{3-}$N (mg/L) | TOC (mg/L) | Temperature (°C) | HPC (CFU/100 mL) |
|---|---|---|---|---|---|---|---|---|
| A-I | 7.36 ± 0.04 | 0.46 ± 0.01 | 8.61 ± 0.05 | 0.53 ± 0.01 | 1.00 ± 0.01 | 0.82 ± 0.08 | 22.33 ± 2.32 | ND |
| A-E | 7.35 ± 0.06 | 0.05 ± 0.01 | 8.55 ± 0.03 | ND | 0.90 ± 0.00 | 0.79 ± 0.09 | 22.47 ± 2.71 | 9.67 ± 4.93 |
| B-I | 7.36 ± 0.03 | 0.74 ± 0.04 | 8.61 ± 0.01 | 0.12 ± 0.01 | 1.81 ± 0.02 | 0.92 ± 0.04 | 22.63 ± 2.34 | ND |
| B-E | 7.38 ± 0.07 | 0.06 ± 0.01 | 8.70 ± 0.08 | 0.02 ± 0.00 | 1.38 ± 0.00 | 0.91 ± 0.01 | 22.60 ± 2.78 | 0.67 ± 1.15 |
| C-I | 7.16 ± 0.14 | 0.41 ± 0.01 | 8.63 ± 0.03 | 0.09 ± 0.01 | 1.19 ± 0.00 | 1.11 ± 0.18 | 23.07 ± 2.12 | ND |
| C-E | 7.12 ± 0.04 | ND | 5.06 ± 0.11 | 0.01 ± 0.00 | 0.08 ± 0.07 | 0.12 ± 0.02 | 22.97 ± 1.61 | (1.97 ± 0.12) × $10^5$ |
| D-I | 7.13 ± 0.11 | 0.58 ± 0.09 | 8.28 ± 0.27 | 0.08 ± 0.01 | 1.16 ± 0.02 | 0.72 ± 0.09 | 23.03 ± 2.29 | ND |
| D-UW | 6.71 ± 0.02 | 0.01 ± 0.01 | 4.23 ± 0.10 | 0.09 ± 0.04 | 1.39 ± 0.11 | 0.47 ± 0.01 | 23.43 ± 2.25 | (9.33 ± 1.77) × $10^4$ |
| D-RW | 6.26 ± 0.37 | 0.02 ± 0.01 | 3.79 ± 0.88 | 0.01 ± 0.00 | 0.16 ± 0.01 | 0.08 ± 0.01 | 22.96 ± 1.79 | (2.59 ± 0.22) × $10^5$ |

Results were the average value of three independent sampling event (mean ± SD); *ND* is not detected; HPC (heterotrophic plate count) indicates the number of total bacteria on NA agar plate. Samples A-I, B-I, C-I, and D-I, and A-E, B-E, C-E, D-UW, and D-RW are influent and effluent water from purifiers A, B, C, and D, respectively. UW is UF effluent; RW is RO effluent

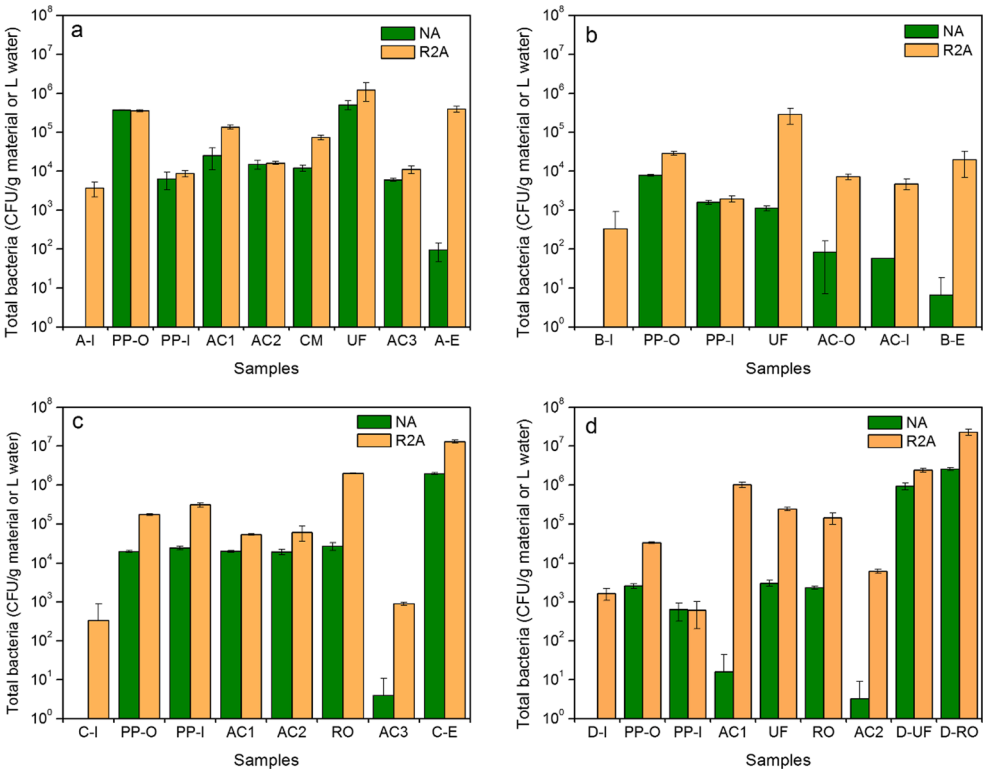


**Fig. 1** The number of total bacteria from different purifiers determined by incubation in NA and $R_2A$ media. **a** Filter A, **b** filter B, **c** filter C, and **d** filter D

total viable bacteria, including cultivable and VBNC cells, was almost at the same level ($1.16 \times 10^5$–$2.34 \times 10^6$ copies/L) revealed by PMA-modified qPCR. However, the concentration of cultivable cells on $R_2A$ medium ranged $3.33 \times 10^2$–$3.67 \times 10^3$ CFU/L in influent water. Besides, PMA-qPCR can reveal real active bacterial cells including cells in VBNC state and cultivable cells in household water purifiers. For example, only $9.67 \times 10^1$, $6.67 \times 10^0$, and $1.97 \times 10^6$ CFU/L were detected by microbiological culture on NA plates in effluent water from purifiers A, B, and C,

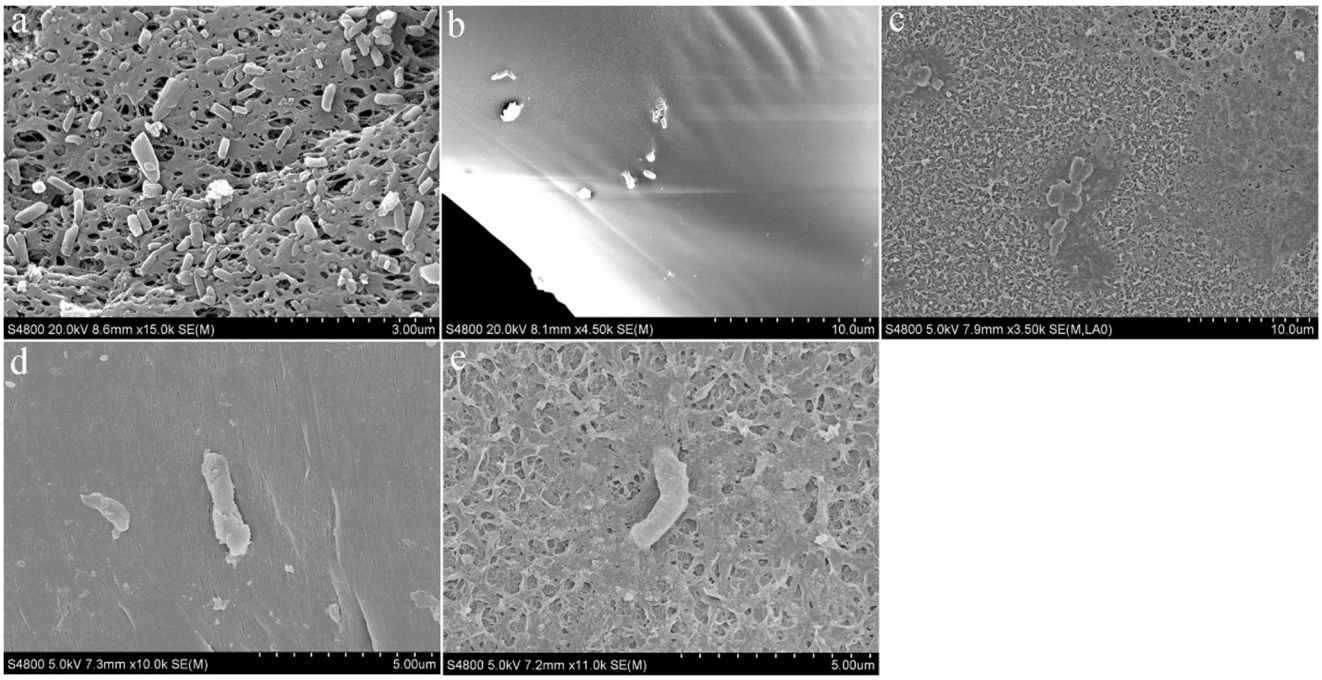


**Fig. 2** Scanning electron micrographs of microorganisms attached to surfaces of different filters membranes. **a** Ultrafiltration (UF) membrane from filter A, **b** UF membrane from filter B, **c** reverse osmosis (RO) membrane from filter C, **d** UF membrane from filter D, and **e** RO membrane from filter D

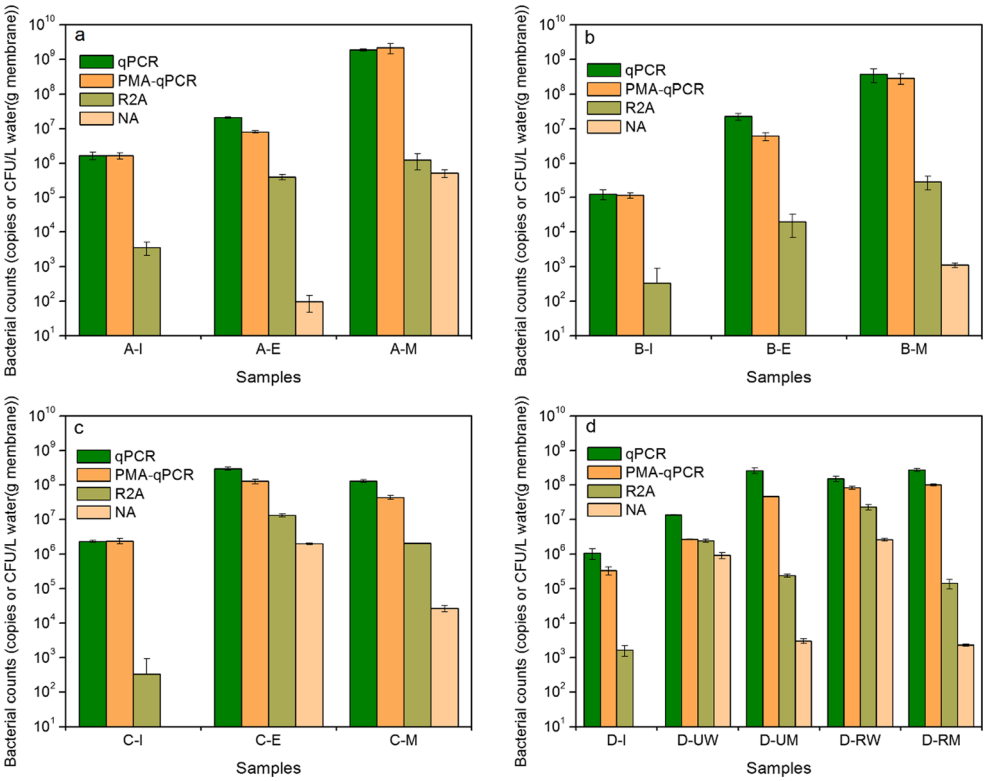


**Fig. 3** Concentrations of total bacteria from different purifiers determined by molecular and culture-dependent technologies. **a** Filter A, **b** filter B, **c** filter C, and **d** filter D

respectively, but total viable bacteria from these purifiers based on PMA-qPCR remained at $8.08 \times 10^6$, $6.06 \times 10^6$, and $1.28 \times 10^8$ copies/L. According to previous studies (Klappenbach et al. 2000; Stoddard et al. 2014), the copy number of rRNA operons per bacterial genome varies from 1 to as many as 15. Thus, the concentration of total viable bacterial cells ranged from $5.39 \times 10^5$ to $8.08 \times 10^6$, from $4.04 \times 10^5$ to $6.06 \times 10^6$, and from $8.51 \times 10^6$ to $1.28 \times 10^8$ cells/L, respectively. So, the difference between the total viable bacterial cells and cultivable cells was 2–5 order of magnitude; i.e., 76.82–100% of the viable cell entered the VBNC state. However, considering the concentration of cultivable cells on R2A plates, which was $3.97 \times 10^5$, $2.00 \times 10^4$, and $1.32 \times 10^7$ CFU/L, thus, the proportion of microorganisms in VBNC state ranged from 26.35 to 95.09%, from 95.05 to 99.67%, and from 0 to 89.66%. In addition, the percentage of microorganisms in VBNC state from UF effluent of purifier D ranged from 0 to 9.62% by R2A plate and from 0 to 64.90% by NA plate. These values might be lower than actual ones because of experimental error. Therefore, a large part of bacteria entered the VBNC state in this study.

Moreover, the concentrations of bacterial cells in effluent water and membrane samples were significantly higher than those in influent water for all purifiers. From purifier C, PMA-qPCR showed $1.28 \times 10^8$ copies/L total viable bacteria in effluent water and $4.36 \times 10^7$ copies/g on membrane compared with $2.34 \times 10^6$ copies/L in influent water. In purifier D, $2.26 \times 10^6$ copies/L and $8.29 \times 10^6$ copies/L total viable bacteria were detected in effluent water filter through UF and RO membranes, respectively. Finally, many bacterial cells ($4.36 \times 10^7$–$2.18 \times 10^9$ copies copies/g) on membrane samples were also detected by PMA-qPCR.

## Bacterial diversity and community composition in water and membrane samples

As shown in Table 3, 14 16S rRNA gene (V4–V5) libraries were constructed to reveal bacterial communities in influent water, effluent water, and membrane samples from four household water purifiers. After removing low-quality sequences and chimeras, 964,561 effective sequences were obtained. The sequence number of each sample was normalized and 202–400 OTUs were identified. Notably, microbial community diversity and species richness in effluent water and membranes were significantly higher than those in influent water. For example, the Shannon index for effluent water and membranes from purifier C were 5.05 and 5.07, respectively, while this value was 2.17 in influent water. Similar trends were observed in other samples. Furthermore, the coverage index of all samples was over 99.6%, suggesting that sequencing depth was enough to reveal the bacterial community in these samples.

Bacterial communities from all samples were primarily dominated by *Proteobacteria* (22.06–77.04%), except for those in effluent water from purifier B (B-E 6.61%) (Fig. 4). In particular, a high proportion (97.42%) of bacterial

**Table 3** Bacterial diversity of each sample from different purifiers based on high-throughput sequencing of 16S rRNA gene

| Samples | Sequences | OTUs | Shannon | Simpson | Ace | Chao1 | Coverage (%) |
|---|---|---|---|---|---|---|---|
| A-I | 74,098 | 268 | 2.17 | 0.45 | 246.74 | 245.37 | 99.8 |
| A-E | 42,643 | 269 | 5.05 | 0.94 | 258.61 | 252.95 | 99.9 |
| A-M | 80,038 | 387 | 5.07 | 0.92 | 365.02 | 377.36 | 99.8 |
| B-I | 78,846 | 287 | 3.68 | 0.73 | 271.25 | 286.31 | 99.8 |
| B-E | 74,809 | 255 | 3.52 | 0.85 | 236.69 | 234.37 | 99.8 |
| B-M | 80,144 | 400 | 5.76 | 0.96 | 437.16 | 444.14 | 99.7 |
| C-I | 65,565 | 202 | 0.69 | 0.14 | 189.89 | 177.08 | 99.8 |
| C-E | 80,159 | 289 | 4.33 | 0.88 | 253.19 | 252.91 | 99.8 |
| C-M | 80,107 | 397 | 5.58 | 0.96 | 477.26 | 484.16 | 99.6 |
| D-I | 69,592 | 176 | 1.36 | 0.33 | 192.89 | 188.19 | 99.9 |
| D-UW | 60,882 | 219 | 4.07 | 0.88 | 240.85 | 242.38 | 99.9 |
| D-UM | 60,087 | 294 | 5.37 | 0.96 | 318.60 | 313.59 | 99.9 |
| D-RW | 64,608 | 231 | 3.30 | 0.80 | 250.95 | 247.00 | 99.9 |
| D-RM | 52,983 | 354 | 5.06 | 0.90 | 367.06 | 373.33 | 99.9 |

*OTUs* is operational taxonomic units. Sample names A-I, B-I, C-I, and D-I mean the influent water from purifiers A, B, C, and D, respectively; A-E, B-E, C-E, D-UW, and D-RW mean the effluent water from purifiers A, B, C, and D, respectively; A-M, B-M, C-M, D-UM, and D-RM mean the membrane samples from purifier A, B, C, and D, respectively; UM is ultrafiltration membrane, RM is reverse osmosis membrane, UW is UF effluent, and RW is RO effluent

sequences from influent water for purifier C (C-I) were classified as *Proteobacteria*. The phylum *Cyanobacteria* was also relatively abundant in influent water samples from purifier A (15.74%) and purifier B (9.28%). For sample B-E, *Melainabacteria* was the most abundant phylum, occupying 65.79% of total sequences. In addition, *Acidobacteria* from influent water showed low abundant (0.07–0.09%), whereas these bacteria were highly abundant on membrane samples (6.41–23.75%) and relatively less abundant in effluent water samples (0.22–5.79%). Similarly, *Planctomycetes* and *Bacteroidetes* displayed a higher abundance in effluent water and membrane samples than in influent water. The cluster analysis based on unweighted UniFrac metrics determined that the microbial community from effluent water was more likely to cluster with that on membranes (Fig. S2). Furthermore, the bacterial community in purifier D was relatively distinct from that in the other purifiers.

The top 50 genera in bacteria communities analyzed in this study are listed in Fig. 5. Genera whose abundance was above 5% in these samples were *Bacterium clone* SRAO 22 (0–30.21%), *Reyranella* sp. (0.01–14.71%), *Paenibacillus borealis* (0–12.08%), *Trachydiscus minutus* (0.01–9.32%), *Desulfosporosinus meridiei* (0–7.46%), and *Gemmata* sp. 28IL (0–5.54%). In addition, with an alignment against the

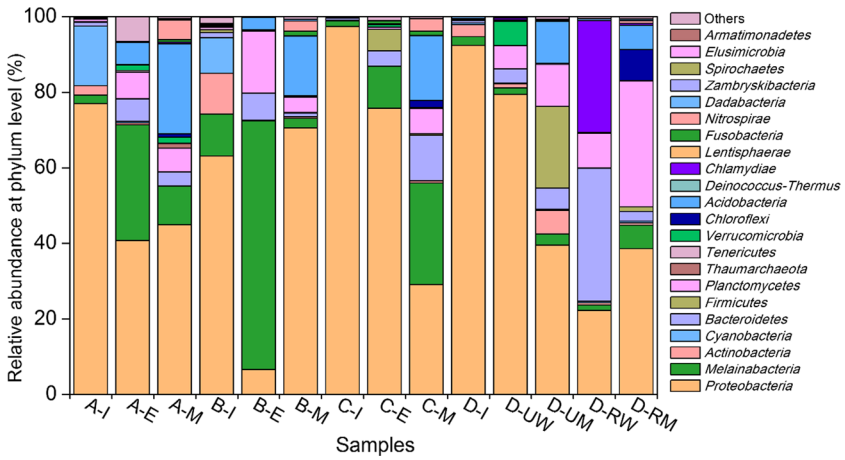


**Fig. 4** Microbial communities in water and membrane samples from different purifiers at the phylum level. A-I, B-I, C-I, and D-I mean the influent water from purifiers A, B, C, and D, respectively; A-E, B-E, C-E, D-UW, and D-RW mean the effluent water from purifiers A, B, C, and D, respectively; A-M, B-M, C-M, D-UM, and D-RM mean the membrane samples from purifiers A, B, C, and D, respectively; UM is an ultrafiltration membrane, RM is a reverse osmosis membrane, UW is UF effluent, and RW is RO effluent

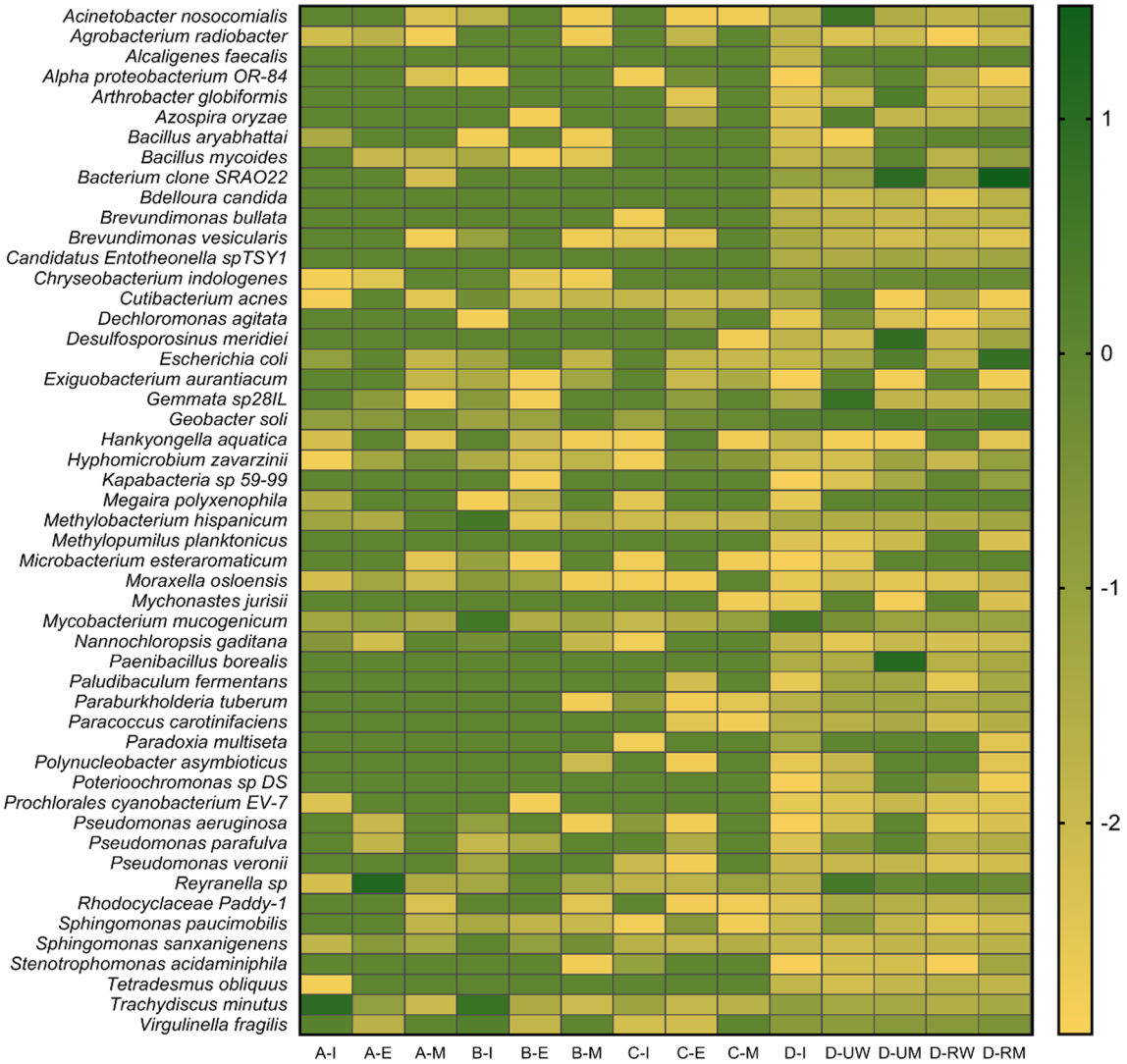


**Fig. 5** Heatmap showing the top 50 genera detected in water and membrane samples from different purifiers. Values indicate the $\log_{10}$-transformed relative abundance of bacteria in each genus. The sample names are the same as those mentioned above

pathogen database, potential pathogens were detected in these samples. It is worth noting that *E. coli* was abundant in UF (1.60%) and RO (6.26%) membranes from purifier D. *Mycobacterium mucogenicum* was more frequently detected in influent water (2.84%) and effluent water of UF membranes (0.34%) from purifier D, and influent water (3.46%) in purifier B. Moreover, the abundance of *P. aeruginosa*, a major pathogen in nosocomial infections, accounted for 0–0.16% of total bacteria in the communities tested.

## Quantification of potential waterborne pathogens

Of the five pathogens tested, only *E. coli* and *P. aeruginosa* were detected in these samples by qPCR (Fig. 6). The Ct values of *S. enterica*, *Shigella*, and *L. pneumophila* were all below or near the detection limit for all samples. Six samples, including A-I, A-M, C-E, D-UW, D-UM, and D-RM, were positive for *E. coli* detection using qPCR, and the gene copy numbers of this strain varied in the range of $5.11 \times 10^1$–$2.23 \times 10^6$ copies per liter water or gram membrane. Especially, the concentrations of *E. coli* on UF and RO membranes from purifier D were $2.23 \times 10^6$ and $1.16 \times 10^6$ copies/g, respectively, although the concentrations of viable *E. coli* were a little lower ($1.02 \times 10^5$ and $3.21 \times 10^5$ copies/g) for these two samples tested by PMA-qPCR. In purifier D, *E. coli* was negative in influent water (D-I), whereas a level of $1.52 \times 10^2$ copies/L was detected in the effluent water of UF filters (D-UW).

In *P. aeruginosa*, the expression of Exotoxin A is under the control of the regulatory gene *regA* (Storey et al. 1991; Wolz et al. 1994). Previous study used *regA* gene to detect this important waterborne pathogen *P. aeruginosa* in municipal wastewater system and showed high sensitivity and specificity (Lee et al. 2006). So, *regA* gene was also selected to quantify

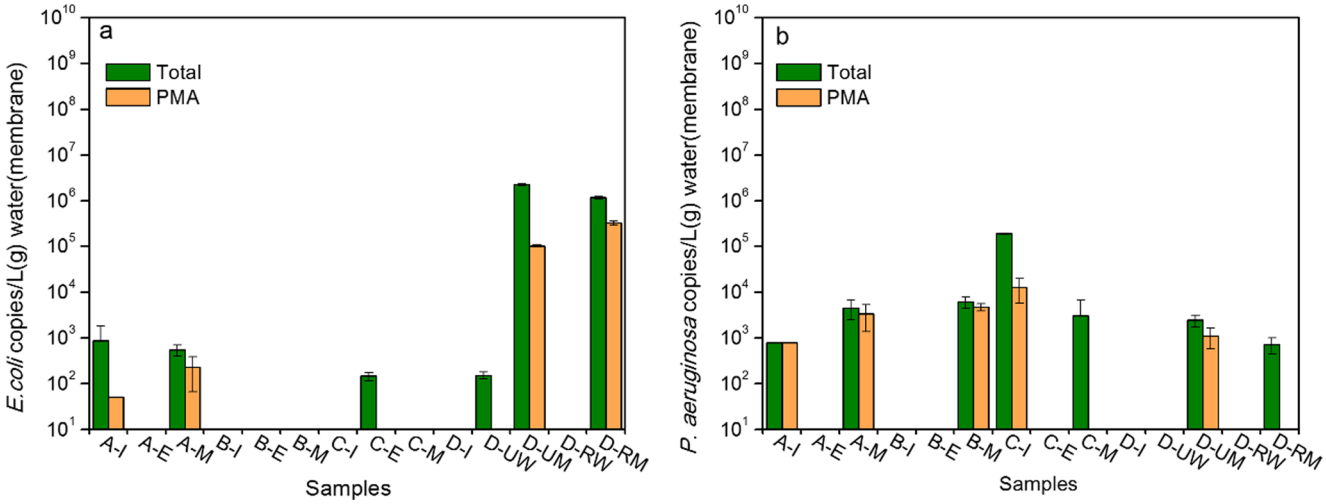


**Fig. 6** Concentrations of potential pathogens in the water phase and membrane samples. **a** *Escherichia coli* and **b** *Pseudomonas aeruginosa*. The sample names are the same as those mentioned above

the concentration of *P. aeruginosa* in household water purifiers in this study. It was found that the concentrations of the *reg* gene, including those for *P. aeruginosa*, were near or below the detection limit in all water samples, except for influent water from purifier C with $1.88 \times 10^5$ copies/L. It is also important to note that *P. aeruginosa* was most likely to be found on membranes ranging from $2.44 \times 10^3$ to $6.14 \times 10^3$ copies/g, but it was not detected in effluent water. Similarly, *M. mucogenicum* was identified at a low abundance by sequencing, but the presence of this organism was not further verified, because this bacterium should be contained at or beyond Biosafety Level-2 for DNA extraction and standard plasmid construction.

## Discussion

Providing safe drinking water for consumers presents a great challenge as source water quality continues to deterioration. Household water purifiers are widely used to ensure safe and high-quality drinking water. Multi-step activated carbon filters and membrane filters are widely coupled in purifiers to improve drinking water quality. However, little information on best procedures to ensure the microbial safety of drinking water is available. In this study, free chlorine and turbidity were removed significantly after activated carbon filtration. AC filters are commonly used for pre-filtration or post-filtration in household water purifier processes because of their large surface area, microporous structure, and high surface reactivity (El Gamal et al. 2018; McQuillan et al. 2018). They can efficiently adsorb various organic and odor compounds, significantly decreasing free chlorine in effluent water (Hoslett et al. 2018). In addition, both UF and RO membrane filters can reduce turbidity, but RO membranes exhibit better removal of organic compounds such as $NO_3$-N and TOC than UF because of their smaller pore sizes (Warsinger et al. 2018). Previous studies have indicated that a physical separation process, i.e., membrane filtration (Albergamo et al. 2019; Schurer et al. 2019), together with a biological process, i.e., activated carbon filtration (Korotta-Gamage and Sathasivan 2017), can effectively remove organic compounds. Our results indicate that household water purifiers indeed ameliorate water quality, either in taste or in chemical characteristics.

It should be noted that no bacteria were detected from influent water cultivated in NA medium, but they were detected in effluent water, especially after RO treatment. Some factors such as residual disinfectants, non-cultivability of microorganisms, or inadequate growth conditions may result in the failure to detect bacteria in influent water samples (Gillespie et al. 2014; Li et al. 2018). As mentioned above, 0.41–0.74 mg/L of free chlorine was still present in influent water, and this could inhibit microbial regrowth. However, free chlorine was depleted with step-by-step filtering. In these cases, surviving or injured microbes can attach, regrow, and proliferate on the surfaces of padding materials and membranes, illustrating the microbial health risks of effluent water. Besides, although no bacteria grew on NA medium, yet more bacteria were found on $R_2A$ plates than on NA medium in influent water as well as other samples. In general, low-nutrient $R_2A$ medium can be used to recover many species of bacteria, and is more suitable to determining total counts of heterotrophic bacteria in drinking water systems when compared with NA medium (Deininger and Lee 2005).

A high level of microbial contaminants was detected at each stage of filtration. PP cotton filters, as the first step of purifiers, play a key role in intercepting with microorganisms or particulate matter. To ensure filtration efficiency, PP cotton filter should be changed every 2–6 months according to the manufacturer's instructions. Moreover, activated carbon filters provided a good place for bacteria to attach and proliferate in

the absence of free chlorine (Gibert et al. 2013), but the adsorption performance of activated carbon was affected by surface area and microporous structure (McQuillan et al. 2018). In household purifiers, granular activated carbon provides limited adsorbing sites, easily leaking carbon for the next step. In fact, carbon powder was observed on the surfaces of membrane filters (Fig. S3). Similarly, the microorganisms leaked from activated carbon filters can be intercepted by follow-up membrane filtration (Hong et al. 2018).

Fouling is an inevitable and long-standing problem in membrane technology for drinking water treatment. Previous studies (Gaveau et al. 2017; Helling et al. 2017; Wang et al. 2008) showed that bacteria commonly leaked through membrane filters. In particular, the concentration of microorganisms in effluent water from RO filters was relatively high. RO membranes are capable to removing organic compounds and microbes because of their small pore size. However, RO filters produce large volumes of concentrated water with low water productivity. Some consumers only use effluents for drinking aims. In this case, microorganisms easily regrow and proliferate in moist environments such as filters and water-storing container because of low usage rate and long-time stagnation (Su et al. 2009). This factor is often ignored, despite the microbial health risks to human that these conditions pose. In contrast, a simple process with one-stage filtration followed by one-stage post-activated carbon filtration in purifier B is likely more appropriate from the perspective view of microbial health risk. Activated carbon and membranes are compact and can be used to obtain safe effluents. Therefore, it can be inferred that both pre- and post-activated carbon filters, as well as membrane filters, provide appropriate surfaces for microbial growth and then allow microbes to be released or leak to effluent water. This was in line with previous studies (Wang 2017; Wu et al. 2012; Wu and Li 1997; Zhou et al. 2012), which investigated hundreds of purifiers from different areas of China including Shanghai, Hangzhou, and Tianjin, and found an excessive rate, i.e., the total number of bacteria in effluent above 100 CFU/mL and ranging from 13.2 to 87.5% (Table S2). That was to say, household water purifiers did not lower but elevated microbial risks, presenting a considerable problem for human health.

Our results show that the concentration of total bacteria was a little higher than that of total viable bacteria and significantly higher than cultivable cells in effluent water and on membrane samples. Microbes were continuously exposed to the two most common environmental stressors in household water purifiers, i.e., free chlorine and oligotrophic conditions, allowing bacteria to easily enter the VBNC state with low metabolic activity and no division (Gensberger et al. 2014). VBNC cells are often undetected using commonly used culture-based methods and standards, leading to an underestimate of the real microbial population size (Gillespie 2016). Although traditional quantitative PCR is sensitive and specific, it detects DNA from living, non-cultivable, and dead bacteria, leading to false positive results (Liu et al. 2018; Zacharias et al. 2015). Accordingly, PMA-qPCR can be used to differentiate between intact and compromised cells (Slimani et al. 2012; Telli and Doğruer 2019) and is more suitable for assessing real health risks of household water purifier condition. In this study, PMA-qPCR revealed $10^6$–$10^8$ copies/L of total viable bacteria in effluent water, and the difference between the total bacterial cells and cultivable cells was 2–5 order of magnitude, indicating that a large part of bacteria entered the VBNC state in this condition. It was reported that bacteria in the VBNC state still maintain metabolic activity and have the potential to resuscitate and regrow, regain virulence when the environmental conditions are favorable (Kibbee and Örmeci 2017; Pinto et al. 2011). In particular, many kinds of pathogen including *Vibrio parahaemolyticus* (Liu et al. 2018), *E. coli* (Kibbee and Örmeci 2017), and *L. pneumophila* (Slimani et al. 2012) were found to be able to enter VBNC state. It would be a significant concern for public health once the VBNC cells undergo a rapid resuscitation to the fully culturable state (Oliver et al. 1995).

In general, the Shannon and Simpson indices are often used to indicate microbial community diversity, and the Ace and Chao1 indices are used to represent species richness (Zhang et al. 2018). Greater community diversity and richness were observed in effluent water and on membranes. Therefore, it is reasonable to suggest that multi-stage pre-activated carbon treatment increases the microbial diversity of effluent water. In addition, *Proteobacteria* was predominant in all sample except for those in effluent water from purifier B (B-E), which was the most common group found in drinking water treatment and distribution systems (Bautista-de los Santos et al. 2016; Huang et al. 2014; Perrin et al. 2019). Moreover, *Cyanobacteria* was abundant in influent water samples because of the use of surface water as a source of drinking water (Fuente et al. 2019). *Melainabacteria*, as the most abundant phylum in sample B-E (65.79%), were classified as members of the non-photosynthetic, anaerobic, and nitrogen fixers and were believed to represent an ancient lineage of the *Cyanobacteria* (Celikkol-Aydin et al. 2016). Effluent water and membrane samples presented a higher proportion of *Melainabacteria* than influent water, indicating this group could be enriched during purifier treatment. Knowledge about the presence of *Melainabacteria* in drinking water systems is limited, but these bacteria are known to be present in the human gut (Gerrity et al. 2018; Zamyadi et al. 2019). Finally, microbial communities in effluent water are more similar to those on membranes than those in influent water based on a cluster tree, suggesting purifier treatment procedures may shift communities to those in effluent water.

In terms of potential pathogens, the presence of *E. coli*, *P. aeruginosa*, and *Mycobacterium* were detected by using high-

throughput sequencing and TaqMan qPCR. *E. coli* is the most commonly used fecal bacteria, indicating fecal contamination of drinking water (Coleman et al. 2013; Ikonen et al. 2017). In addition, *P. aeruginosa*, as a major pathogen in nosocomial infections, was frequently detected in drinking water environments (Bressler et al. 2009; De and Galván 2001; Moritz et al. 2010). Moreover, *M. mucogenicum* may cause severe disease and even death in immunocompromised individuals, and its presence has been demonstrated in water environments such as potable water used in hospital (Fernandez-Rendon et al. 2012; Loret and Dumoutier 2019). It was interesting to find that more *E. coli* and *P. aeruginosa* was attached to the surfaces of membranes than that found in influent and effluent water, suggesting these bacteria may have been captured and enriched on membrane surfaces.

The results of the present study suggest that using household water purifiers does not lower but elevates microbial risk. Some efforts can be made by both manufacturers and consumers to improve the performance of household water purifiers, such as replacing filters regularly before over-saturation or installing a back-washing program to prolong the life span of a filter (Shao et al. 2018). Moreover, advanced technologies such as UV-LED treatment can be introduced as a final step to minimize microbial contamination in purifiers (Lui et al. 2016).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Ethical statement** This article does not contain any studies with human participants or animals performed by any of the authors.

## References

Albergamo V, Blankert B, Cornelissen ER, Hofs B, Knibbe WJ, van der Meer W, de Voogt P (2019) Removal of polar organic micropollutants by pilot-scale reverse osmosis drinking water treatment. Water Res 148:535–545. https://doi.org/10.1016/j.watres.2018.09.029

Bae S, Lyons C, Onstad N (2019) A culture-dependent and metagenomic approach of household drinking water from the source to point of use in a developing country. Water Res X:100026. https://doi.org/10.1016/j.wroa.2019.100026

Bautista-de los Santos QM, Schroeder JL, Blakemore O, Moses J, Haffey M, Sloan W, Pinto AJ (2016) The impact of sampling, PCR, and sequencing replication on discerning changes in drinking water bacterial community over diurnal time-scales. Water Res 90:216–224. https://doi.org/10.1016/j.watres.2015.12.010

Bressler D, Balzer M, Dannehl A, Flemming HC, Wingender J (2009) Persistence of *Pseudomonas aeruginosa* in drinking-water biofilms on elastomeric material. Water Sci Technol Water Supply 9:81–87. https://doi.org/10.2166/ws.2009.026

Celikkol-Aydin S, Gaylarde CC, Lee T, Melchers RE, Witt DL, Beech IB (2016) 16S rRNA gene profiling of planktonic and biofilm microbial populations in the Gulf of Guinea using Illumina NGS. Mar Environ Res 122:105–112. https://doi.org/10.1016/j.marenvres.2016.10.001

Chen L, Zhu X, Zhang M, Wang Y, Lv T, Zhang S, Yu X (2017) Profiling total viable bacteria in a hemodialysis water treatment system. J Microbiol Biotechnol 27(5):995–1004. https://doi.org/10.4014/jmb.1612.12002

Coleman BL, Louie M, Salvadori MI, McEwen SA, Neumann N, Sibley K, Irwin RJ, Jamieson FB, Daignault D, Majury A, Braithwaite S, Crago B, McGeer AJ (2013) Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. Water Res 47:3026–3036. https://doi.org/10.1016/j.watres.2013.03.008

Cui Q, Fang T, Huang Y, Dong P, Wang H (2017) Evaluation of bacterial pathogen diversity,abundance and health risks in urban recreational water by amplicon next-generation sequencing and quantitative PCR. J Environ Sci 57:137–149. https://doi.org/10.1016/j.jes.2016.11.008

De VJ, Galván M (2001) *Pseudomonas aeruginosa* as an indicator of health risk in water for human consumption. Water Sci Technol 43:49–52. https://doi.org/10.2166/wst.2001.0710

Deininger RA, Lee J (2005) Rapid detection of bacteria in drinking water, Ukraine, Modern tools and methods of water treatment for improving living standards, pp 71–78

Douterelo I, Fish KE, Boxall JB (2018) Succession of bacterial and fungal communities within biofilms of a chlorinated drinking water distribution system. Water Res 141:74–85. https://doi.org/10.1016/j.watres.2018.04.058

El Gamal M, Mousa HA, El-Naas MH, Zacharia R, Judd S (2018) Bio-regeneration of activated carbon: a comprehensive review. Sep Purif Technol 197:345–359. https://doi.org/10.1016/j.seppur.2018.01.015

Fernandez-Rendon E, Cerna-Cortes JF, Ramirez-Medina MA, Helguera-Repetto AC, Rivera-Gutierrez S, Estrada-Garcia T, Gonzalez-y-Merchand JA (2012) *Mycobacterium mucogenicum* and other non-tuberculous *mycobacteria* in potable water of a trauma hospital: a potential source for human infection. J Hosp Infect 80:74–76. https://doi.org/10.1016/j.jhin.2011.10.003

Fuente A, Muro-Pastor AM, Merchán F, Madrid F, Pérez-Martínez JI, Undabeytia T (2019) Electrocoagulation/flocculation of *cyanobacteria* from surface waters. J Clean Prod 238:117964. https://doi.org/10.1016/j.jclepro.2019.117964

Gaveau A, Coetsier C, Roques C, Bacchin P, Dague E, Causserand C (2017) Bacteria transfer by deformation through microfiltration membrane. J Membr Sci 523:446–455. https://doi.org/10.1016/j.memsci.2016.10.023

Gensberger ET, Polt M, Konrad-Köszler M, Kinner P, Sessitsch A, Kostić T (2014) Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality. Water Res 67:367–376. https://doi.org/10.1016/j.watres.2014.09.022

Gerrity D, Arnold M, Dickenson E, Moser D, Sackett JD, Wert EC (2018) Microbial community characterization of ozone-biofiltration systems in drinking water and potable reuse applications. Water Res 135:207–219. https://doi.org/10.1016/j.watres.2018.02.023

Gibert O, Lefèvre B, Fernández M, Bernat X, Paraira M, Calderer M, Martínez-Lladó X (2013) Characterising biofilm development on granular activated carbon used for drinking water production. Water Res 47:1101–1110. https://doi.org/10.1016/j.watres.2012.11.026

Gillespie S (2016) Chapter 3 - current status of molecular microbiological techniques for the analysis of drinking water. In: Nigel C, Martin D, Thompson K (eds) . Academic Press, San Diego, pp 39–58

Gillespie S, Lipphaus P, Green J, Parsons S, Weir P, Juskowiak K, Jefferson B, Jarvis P, Nocker A (2014) Assessing microbiological water quality in drinking water distribution systems with disinfectant residual using flow cytometry. Water Res 65:224–234. https://doi.org/10.1016/j.watres.2014.07.029

Hammes F, Berney M, Wang Y, Vital M, Köster O, Egli T (2008) Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res 42: 269–277. https://doi.org/10.1016/j.watres.2007.07.009

Helling A, Kubicka A, Schaap I, Polakovic M, Hansmann B, Thiess H, Strube J, Thom V (2017) Passage of soft pathogens through microfiltration membranes scales with transmembrane pressure. J Membr Sci 522:292–302. https://doi.org/10.1016/j.memsci.2016.08.016

Hong S, Tang X, Wu N, Chen H (2018) Leakage of soluble microbial products from biological activated carbon filtration in drinking water treatment plants and its influence on health risks. Chemosphere 202: 626–636. https://doi.org/10.1016/j.chemosphere.2018.03.123

Hoslett J, Massara TM, Malamis S, Ahmad D, van den Boogaert I, Katsou E, Ahmad B, Ghazal H, Simons S, Wrobel L, Jouhara H (2018) Surface water filtration using granular media and membranes: a review. Sci Total Environ 639:1268–1282. https://doi.org/10.1016/j.scitotenv.2018.05.247

Hu Y, Jiang L, Zhang T, Jin L, Han Q, Zhang D, Lin K, Cui C (2018) Occurrence and removal of sulfonamide antibiotics and antibiotic resistance genes in conventional and advanced drinking water treatment processes. J Hazard Mater 360:364–372. https://doi.org/10.1016/j.jhazmat.2018.08.012

Hu D, Lin W, Zeng J, Wu P, Zhang M, Guo L, Ye C, Wan K, Yu X (2019) Profiling the microbial contamination in aviation fuel from an airport. Biofouling 35(8):856–869. https://doi.org/10.1080/08927014.2019.1671977

Huang K, Zhang XX, Shi P, Wu B, Ren H (2014) A comprehensive insight into bacterial virulence in drinking water using 454 pyrosequencing and Illumina high-throughput sequencing. Ecotoxicol Environ Saf 109:15–21. https://doi.org/10.1016/j.ecoenv.2014.07.029

Ikonen J, Pitkänen T, Kosse P, Ciszek R, Kolehmainen M, Miettinen IT (2017) On-line detection of *Escherichia coli* intrusion in a pilot-scale drinking water distribution system. J Environ Manag 198: 384–392. https://doi.org/10.1016/j.jenvman.2017.04.090

Kibbee RJ, Örmeci B (2017) Development of a sensitive and false-positive free PMA-qPCR viability assay to quantify VBNC *Escherichia coli* and evaluate disinfection performance in wastewater effluent. J Microbiol Methods 132:139–147. https://doi.org/10.1016/j.mimet.2016.12.004

Klappenbach JA, Dunbar JM, Schmidt TM (2000) rRNA operon copy number reflects ecological strategies of bacteria. Appl Environ Microbiol 66(4):1328–1333. https://doi.org/10.1128/AEM.66.4.1328-1333.2000

Korotta-Gamage SM, Sathasivan A (2017) A review: potential and challenges of biologically activated carbon to remove natural organic matter in drinking water purification process. Chemosphere 167: 120–138. https://doi.org/10.1016/j.chemosphere.2016.09.097

Lee DY, Shannon K, Beaudette LA (2006) Detection of bacterial pathogens in municipal wastewater using an oligo nucleotide microarray and TaqMan real-time PCR. J Microbiol Methods 65(3):453–467. https://doi.org/10.1016/j.mimet.2005.09.008

Li W, Zhang J, Wang F, Qian L, Zhou Y, Qi W, Chen J (2018) Effect of disinfectant residual on the interaction between bacterial growth and assimilable organic carbon in a drinking water distribution system. Chemosphere 202:586–597. https://doi.org/10.1016/j.chemosphere.2018.03.056

Lin W, Yu Z, Zhang H, Thompson IP (2014) Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation. Water Res 52:218–230. https://doi.org/10.1016/j.watres.2013.10.071

Liu Y, Zhong Q, Wang J, Lei S (2018) Enumeration of *Vibrio parahaemolyticus* in VBNC state by PMA-combined real-time quantitative PCR coupled with confirmation of respiratory activity. Food Control 91:85–91. https://doi.org/10.1016/j.foodcont.2018.03.037

Liu L, Xing X, Hu C, Wang H, Lyu L (2019) Effect of sequential UV/free chlorine disinfection on opportunistic pathogens and microbial community structure in simulated drinking water distribution systems. Chemosphere 219:971–980. https://doi.org/10.1016/j.chemosphere.2018.12.067

Loret JF, Dumoutier N (2019) Non-tuberculous *mycobacteria* in drinking water systems: a review of prevalence data and control means. Int J Hyg Environ Health 222:628–634. https://doi.org/10.1016/j.ijheh.2019.01.002

Lui GY, Roser D, Corkish R, Ashbolt NJ, Stuetz R (2016) Point-of-use water disinfection using ultraviolet and visible light-emitting diodes. Sci Total Environ 553:626–635. https://doi.org/10.1016/j.scitotenv.2016.02.039

McQuillan RV, Stevens GW, Mumford KA (2018) The electrochemical regeneration of granular activated carbons: a review. J Hazard Mater 355:34–49. https://doi.org/10.1016/j.jhazmat.2018.04.079

Mombini S, Rezatofighi SE, Kiyani L, Motamedi H (2019) Diversity and metallo-β-lactamase-producing genes in *Pseudomonas aeruginosa* strains isolated from filters of household water treatment systems. J Environ Manag 231:413–418. https://doi.org/10.1016/j.jenvman.2018.10.068

Moritz MM, Flemming HC, Wingender J (2010) Integration of *Pseudomonas aeruginosa* and *Legionella pneumophila* in drinking water biofilms grown on domestic plumbing materials. Int J Hyg Environ Health 213:190–197. https://doi.org/10.1016/j.ijheh.2010.05.003

Oliver JD, Hite F, McDougald D, Andon NL, Simpson LM (1995) Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment. Appl Environ Microbiol 61(7): 2624–2630. https://doi.org/10.1177/002215540205000815

Perrin Y, Bouchon D, Delafont V, Moulin L, Héchard Y (2019) Microbiome of drinking water: a full-scale spatio-temporal study to monitor water quality in the Paris distribution system. Water Res 149:375–385. https://doi.org/10.1016/j.watres.2018.11.013

Pinto D, Almeida V, Santos MA, Chambel L (2011) Resuscitation of *Escherichia coli* VBNC cells depends on a variety of environmental or chemical stimuli. J Appl Microbiol 110(6):1601–1611. https://doi.org/10.1111/j.1365-2672.2011.05016.x

Schurer R, Schippers JC, Kennedy MD, Cornelissen ER, Salinas-Rodriguez SG, Hijnen W, van der Wal (2019) Enhancing biological stability of disinfectant-free drinking water by reducing high molecular weight organic compounds with ultrafiltration posttreatment. Water Res 164: 114927. https://doi.org/10.1016/j.watres.2019.114927

Shao S, Wang Y, Shi D, Zhang X, Tang CY, Liu Z, Li J (2018) Biofouling in ultrafiltration process for drinking water treatment and its control by chlorinated-water and pure water backwashing. Sci Total Environ 644:306–314. https://doi.org/10.1016/j.scitotenv.2018.06.220

Shi P, Jia S, Zhang XX, Zhang T, Cheng S, Li A (2013) Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. Water Res 47:111–120. https://doi.org/10.1016/j.watres.2012.09.046

Shi P, Zhou S, Xiao H, Qiu J, Li A, Zhou Q, Pan Y, Hollert H (2018) Toxicological and chemical insights into representative source and drinking water in eastern China. Environ Pollut 233:35–44. https://doi.org/10.1016/j.envpol.2017.10.033

Shirasaki N, Matsushita T, Matsui Y, Murai K (2017) Assessment of the efficacy of membrane filtration processes to remove human enteric viruses and the suitability of bacteriophages and a plant virus as

surrogates for those viruses. Water Res 115:29–39. https://doi.org/10.1016/j.watres.2017.02.054

Slimani S, Robyns A, Jarraud S, Molmeret M, Dusserre E, Mazure C, Facon JP, Lina G, Etienne J, Ginevra C (2012) Evaluation of propidium monoazide (PMA) treatment directly on membrane filter for the enumeration of viable but non cultivable Legionella by qPCR. J Microbiol Methods 88:319–321. https://doi.org/10.1016/j.mimet.2011.12.010

Song J, Chen L, Chen H, Sheng F, Xing D, Li L, Zhang Y, Rittmann B (2018) Characterization and high-throughput sequencing of a trichlorophenol-dechlorinating microbial community acclimated from sewage sludge. J Clean Prod 197:306–313. https://doi.org/10.1016/j.jclepro.2018.06.061

Stoddard SF, Smith BJ, Hein R, Roller BRK, Schmidt TM (2014) rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Res 43:D593–D598. https://doi.org/10.1093/nar/gku1201

Storey DG, Raivio TL, Frank DW (1991) Effect of *regB* on expression from the P1 and P2 promoters of the *Pseudomonas aeruginosa* regAB operon. J Bacteriol 173(19): 6088–94. https://doi.10.1128/jb.173.19.6088–6094.1991

Su F, Luo M, Zhang F, Li P, Lou K, Xing X (2009) Performance of microbiological control by a point-of-use filter system for drinking water purification. J Environ Sci 21:1237–1246. https://doi.org/10.1016/S1001-0742(08)62410-9

Telli AE, Doğruer Y (2019) Discrimination of viable and dead *Vibrio parahaemolyticus* subjected to low temperatures using Propidium Monoazide – quantitative loop mediated isothermal amplification (PMA-qLAMP) and PMA-qPCR. Microb Pathog 132:109–116. https://doi.org/10.1016/j.micpath.2019.04.029

Vaz-Moreira I, Nunes OC, Manaia CM (2017) Ubiquitous and persistent *Proteobacteria* and other gram-negative bacteria in drinking water. Sci Total Environ 586:1141–1149. https://doi.org/10.1016/j.scitotenv.2017.02.104

Wang D (2017) Study on the effects of bacterial contamination of household water purification equipment and water contact material on microbial growth. Master, Chinese Center for Disease Control and Prevention

Wang Y, Hammes F, Düggelin M, Egli T (2008) Influence of size, shape, and flexibility on bacterial passage through micropore membrane filters. Environ Sci Technol 42:6749–6754. https://doi.org/10.1021/es800720n

Wang R, Guan S, Sato A, Wang X, Wang Z, Yang R, Hsiao BS, Chu B (2013) Nanofibrous microfiltration membranes capable of removing bacteria, viruses and heavy metal ions. J Membr Sci 446:376–382. https://doi.org/10.1016/j.memsci.2013.06.020

Wang JH, Lu J, Zhang YX, Wu J, Zhang C, Yu X, Zhang Z, Liu H, Wang WH (2018) High-throughput sequencing analysis of the microbial community in coastal intensive mariculture systems. Aquac Eng 83:93–102. https://doi.org/10.1016/j.aquaeng.2018.10.001

Warsinger DM, Chakraborty S, Tow EW, Plumlee MH, Bellona C, Loutatidou S, Karimi L, Mikelonis AM, Achilli A, Ghassemi A, Padhye LP, Snyder SA, Curcio S, Vecitis CD, Arafat HA, Lienhard JH (2018) A review of polymeric membranes and processes for potable water reuse. Prog Polym Sci 81:209–237. https://doi.org/10.1016/j.progpolymsci.2018.01.004

Wei G, Liang H, Ma J, Mei H, Chen ZL, Han ZS, Li GB (2011) Membrane fouling control in ultrafiltration technology for drinking water production: a review. Desalination 272:1–8. https://doi.org/10.1016/j.desal.2011.01.051

WHO (2006) Guidelines for drinking water quality, First Addendum to Third Edition. World Health Organization Recommendations 1

Wolz C, Lehmann R, Vasil ML (1994) A new extracellular protein of *Pseudomonas aeruginosa* PA103 regulated by *regA*. Microbiology 140(Pt 7):1755–1761. https://doi.org/10.1099/13500872-140-7-1755

Wu X, Li C (1997) Investigation on microbial contamination of drinking water caused by inferior water purifier. J Environ Health 21

Wu L, Cui W, Zhang Y, Ge G, Chen Z, Wang G, Li Z (2012) Survey on hygienic situation of outlet water from household water filters in Shanghai. J Environ Occup Med 129:475–480

Zacharias N, Kistemann T, Schreiber C (2015) Application of flow cytometry and PMA-qPCR to distinguish between membrane intact and membrane compromised bacteria cells in an aquatic milieu. Int J Hyg Environ Health 218:714–722. https://doi.org/10.1016/j.ijheh.2015.04.001

Zamyadi A, Romanis C, Mills T, Neilan B, Choo F, Coral LA, Gale D, Newcombe G, Crosbie N, Stuetz R, Henderson RK (2019) Diagnosing water treatment critical control points for cyanobacterial removal: exploring benefits of combined microscopy, next-generation sequencing, and cell integrity methods. Water Res 152:96–105. https://doi.org/10.1016/j.watres.2019.01.002

Zhang Y, Ping Y, Zhou R, Wang J, Zhang G (2018) High throughput sequencing-based analysis of microbial diversity in dental unit waterlines supports the importance of providing safe water for clinical use. J Infect Public Health 11:357–363. https://doi.org/10.1016/j.jiph.2017.09.017

Zhou Z, Hu B, Bao R, Gao X, Shen Y, Cui Y, Wei S, Zhou Q (2012) Impact factor for microbiological contamination of water purifier. Chin J Nosocomiol 22:2580–2582