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Exploring the efficacy of combination of point-of-use filters and peracetic acid disinfection in reducing total viable counts in final rinse water of endoscopes

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The final rinse water pollution of endoscopes in medical institutions is a severe growing problem, posing a latent risk to nosocomial infection. This study aimed to explore the efficacy of combination of point-of-use (POU) filters and peracetic acid (PAA) disinfection in reducing total viable counts (TVCs) in final rinse water of endoscopes. Eight final rinse water faucets of endoscope centers were randomly selected and none of the faucets had POU filters prior to the study. Four faucets were assigned to experimental group and had POU filters installed, the other four faucets were set as control group and did not have POU filters installed. During monitoring, both the experimental and control groups were used 0.3% PAA to disinfect the purified water system twice. TVCs in final rinse water was monitored weekly for 13 weeks. 231 final rinse water samples were collected, of which 111 and 120 samples were in the experimental and control groups, respectively. The mean TVCs and positive rate of samples for experimental group were both significantly lower than that of control group [10 *vs.* 50,450 colony-forming units (CFU)/100 mL, and 27.7% *vs.* 98.3%, $P < 0.001$]. The TVCs in control group was from 2.0×10^3 to 2.5×10^5 CFU/100 mL, and the positive rates of samples were almost 100%. However, the TVCs and positive rates of samples in experiment group were always at a low-level, with a maximum TVCs of 47 CFU/100 mL, corresponding to a maximum positive rate of 50%, by the 13th week of monitoring. Our findings demonstrated that PAA disinfection alone exhibited limited efficacy in controlling TVCs in final rinse water of endoscopes, whereas its combined application with POU filters significantly enhances the control effect of TVCs.

Keywords Point-of-use filtration, Peracetic acid disinfection, Endoscope center, Total viable counts, Combination

Abbreviations

CFU	Colony-forming units
IQR	Interquartile range
PAA	Peracetic acid
POU	Point-of-use
SD	Standard deviation
TVCs	Total viable counts
PVC-U	Unplasticized polyvinyl chloride

Hospital centralized water supply systems have served as reservoirs for waterborne pathogens, and colonization of waterborne pathogens in water supplies have been correlated with the incidence of healthcare-associated infections (HAIs)[1–4]. As the final rinsing process after endoscope disinfection, the contamination of endoscope

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final rinse water can easily lead to endoscopic contamination and iatrogenic infections[5]. Previous study revealed that contamination of the endoscopic final rinse water increased probability of endoscopic reprocessing failure[6]. Endoscope reprocessing failures have been reported by the ECRI institute as one of the top 10 health technology hazards[7]. Ji et al. investigated 59 endoscope units in China and found that 56.52% flushing channel samples of gastrointestinal endoscopes were positive for culture, but use of purified water could reduce the risk of endoscope reprocessing failure[8]. Multinational guidelines recommend rinsing endoscopes and accessories with purified or sterile water after high-level disinfection[9,10]. Monitoring and reducing the total viable counts (TVCs) for final rinse water of endoscopy centers were essential to prevent infection caused by gastrointestinal endoscopes[11].

The most common method for controlling the TVCs in water is to regularly disinfect the water systems with chemical disinfectants[12]. At present, the commonly used disinfectant include chlorine, chlorine dioxide, chlorine ammonia, and ozone disinfection, etc., but these methods have the problem of residual toxic disinfection by-products[13,14]. Peracetic acid (PAA) as a widely used alternative oxidant and disinfectant in water treatment, can rapidly and effectively eliminate trace contaminants and produce fewer harmful by-products[15]. However, PAA disinfection must be repeated at certain intervals, and TVCs of the final rinse water cannot be effectively controlled. Point-of-use (POU) water filter has obvious benefits to lower the number of waterborne pathogens[16], and was a simple, successful, and highly cost-effective strategy to control the outbreak of nosocomial infection[17,18]. After an outbreak of invasive fusariosis in a children's cancer hospital in Brazil, many control measures were instituted, but the outbreak was only controlled one year after the first case, when all faucets and showers were equipped with POU filters filtering 0.2 μm[19]. A large outbreak of legionella infection occurred in an American hospital despite using copper-silver ionization system, while the legionella epidemic was effectively controlled by equipping with POU water filters[20].

In the present study, we aimed to explore the influence of combining the PAA chemical disinfection for the centralized water supply system with installing POU filters for the final rinse water of endoscopes on controlling the TVCs in the final rinse water.

Materials and methods

Study design

The study was performed at a endoscopic center of a tertiary general hospital in Wuhan, China, from March to June 2022. Eight final rinse water faucets of endoscope centers were randomly selected and none of the faucets had POU filters prior to the study. Four faucets were assigned to experimental group and had POU filters installed, the other four faucets were assigned to control group and did not have POU filters installed. During monitoring, the pure water storage tanks, water transmission pipelines [constructed from unplasticized polyvinyl chloride (PVC-U)], and each terminal point of both the experimental and control groups were disinfected twice by using 0.3% PAA, with each immersion cycle lasting 40 min (Fig. 1). TVCs in final rinse water was monitored weekly, starting from the day of PAA disinfection and POU filter installation, lasted for 13 weeks (Fig. 1).

The POU filter (Pall-Aqua-Safe Water Filter, AQ14F1S) is prepared with polyether sulfone membrane of 0.2 μm, whose filter element is able to intercept microorganisms and particles in the water and purify water, based on the principle of physical interception. Moreover, silver ion bacteriostatic is added to the filter shell

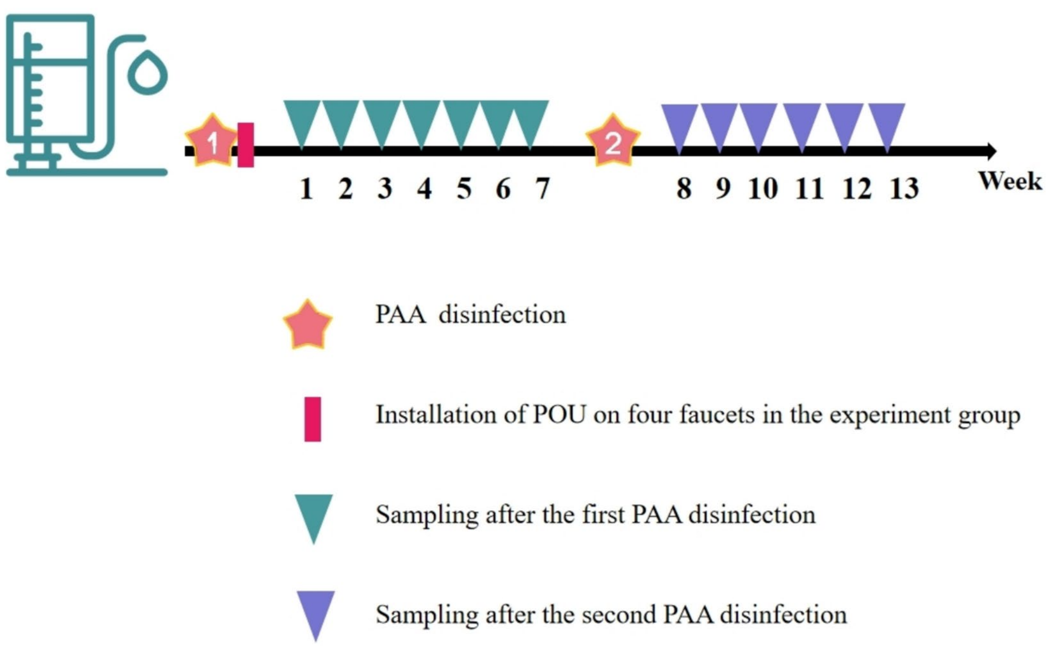

Fig. 1. The flowchart of the experiment design.

to prevent bacteria from growing on the filter. Nurses and cleaning personnel at the endoscopic center were instructed not to touch or clean the filters, which were not changed during the monitoring period.

Sample collection

After disinfecting with 75% ethanol and 60 s water flush, all samples (100 mL per sample) were collected by using pre-sterilized glass bottles, and were stored at a low-temperature refrigerator immediately until testing within two hours of collection. We totally collected 231 samples, of which 111 collected from faucets with filters installed were assigned to the experimental group, and the other 120 collected from faucets without filters installed were set as the control group. Meanwhile, we simultaneously collected 24 source water samples from water tank of centralized water supply system and 20 endoscopic final rinse water samples from faucets without POU filters on the second day after PAA disinfection.

Microbiological analysis

Water samples were treated by the membrane filtration method. 1.0 mL of original water, diluted 10-fold and 100-fold water samples were poured culture, respectively, and the remaining 99 mL of samples were filtered (pore size of 0.45 μm, Millipore, USA). After filtration, the membranes were attached to R2A agar mediums and incubated at 17–23 °C for seven days for colony counting[21]. After culturing, if the filter membrane could not be counted, the result of the poured plate was adopted; otherwise, the sum of the number of bacterial colonies on the plate and the filter membrane was counted. The TVCs of 0 colony-forming units (CFU)/100 mL was considered qualified.

Statistical analysis

The distribution of characteristic variables was expressed as the mean (standard deviation, SD), number (percentage), or median (interquartile range, IQR). The differences in continuous variables between experiment and control groups were tested by Mann–Whitney *U* test or paired-chi square (Fisher exact). The threshold for significance was $P<0.05$. In this study, all statistical analyses were performed with Statistical Analysis System 9.4 (SAS Institute, Carry, North Carolina, https://support.sas.com/software/94/) and GraphPad Prism 8 software (San Diego CA, https://www.graphpad.com/scientific-software/prism).

Results

The flowchart of the study was shown in Fig. 2. The TVCs and positive rate of samples for experimental group were both significantly lower than that of the control group (10 *vs.* 50,450 CFU/100 mL and 27.7% *vs.* 98.3%, $P<0.001$, Table 1).

The TVCs for 24 water source samples was significantly lower than 20 endoscopic final rinsing water samples (6.2 *vs.* 128.2 CFU/100 mL, $P=0.001$). During the surveillance, TVCs among experimental group and control

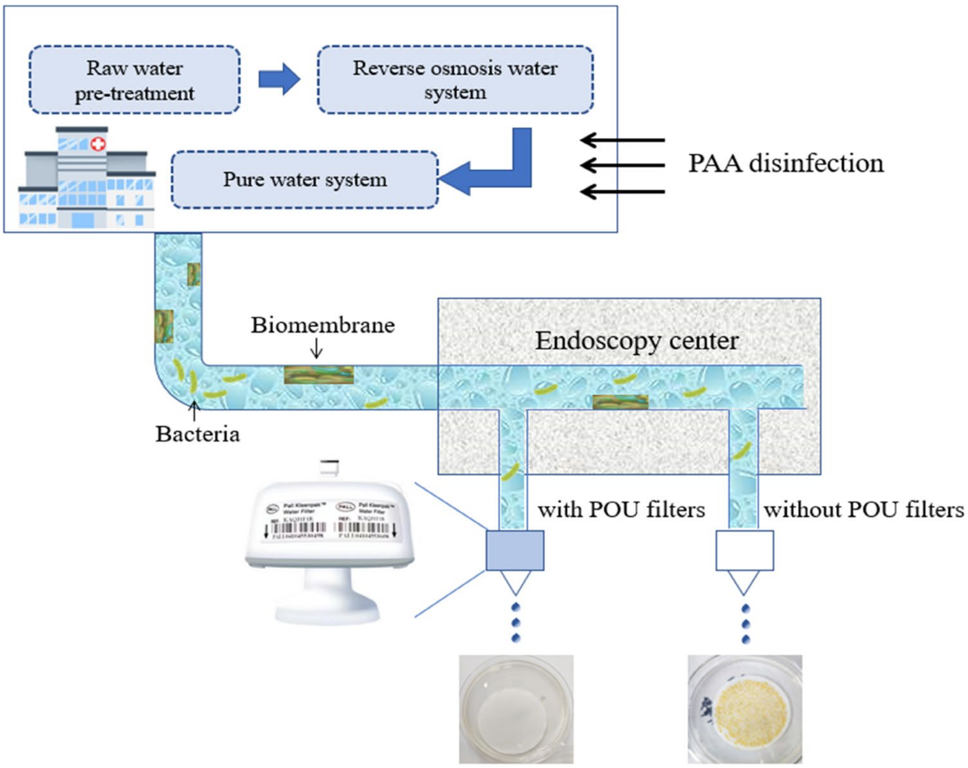

Fig. 2. The flowchart of the study.

Variables	Experiment group (n = 112)	Control group (n = 120)	P^a
Positive samples, no. (%)	31 (27.7)	118 (98.3%)	< 0.001
TVCs (CFU/100 mL)			
Mean ± standard deviation	10 ± 27	50,450 ± 128,473	< 0.001
Median (interquartile range)	0 (0, 5)	17,500 (4000, 43,000)	< 0.001

Table 1. Characteristics of pathogen contamination in the experiment and control group. *CFU* colony-forming units, *TVCs* total viable counts. [a]Mann–Whitney *U* test and paired-chi square (Fisher exact) test were used to compare the differences in the experiment and control group.

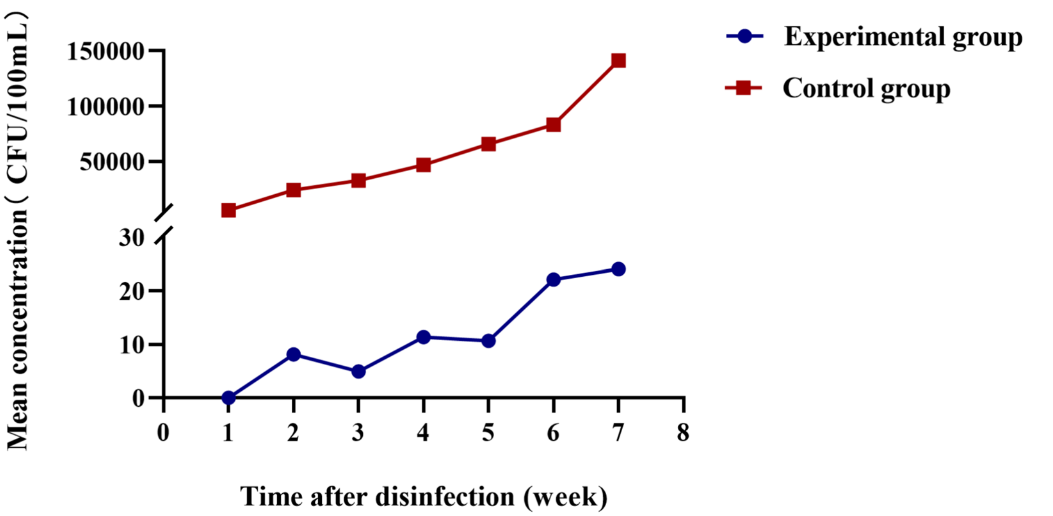

Fig. 3. Trend of the total viable counts in the experimental group and control group by the time after disinfection.

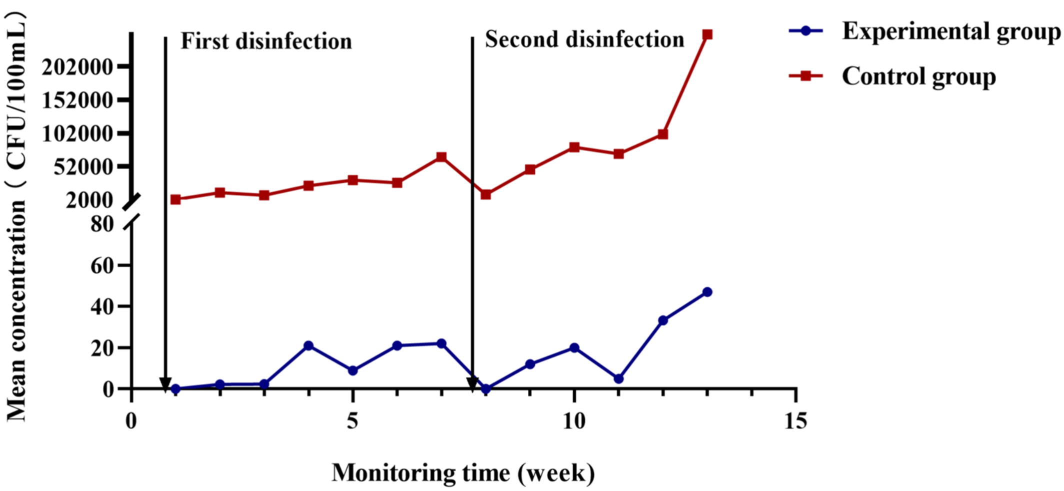

Fig. 4. Trends of the total viable counts in the experimental group and control group by the monitoring time.

group both gradually increased with the time after disinfection, but the former always lower than the later (Fig. 3).

As shown in Fig. 4, the TVCs for the control group ranged from 2.0×10^3 to 2.5×10^5 CFU/100 mL, and was nearly unaltered or slightly increased within 2 weeks after PAA disinfection. However, the TVCs gradually increased as the disinfection interval extended, but decreased after the second PAA disinfection, while when the 13th week of the monitoring period had the highest TVCs, which was 2.5×10^5 CFU/100 mL, followed by the 12th week, which was 1.0×10^5 CFU/100 mL. The TVCs for the experiment group ranged from 0 to

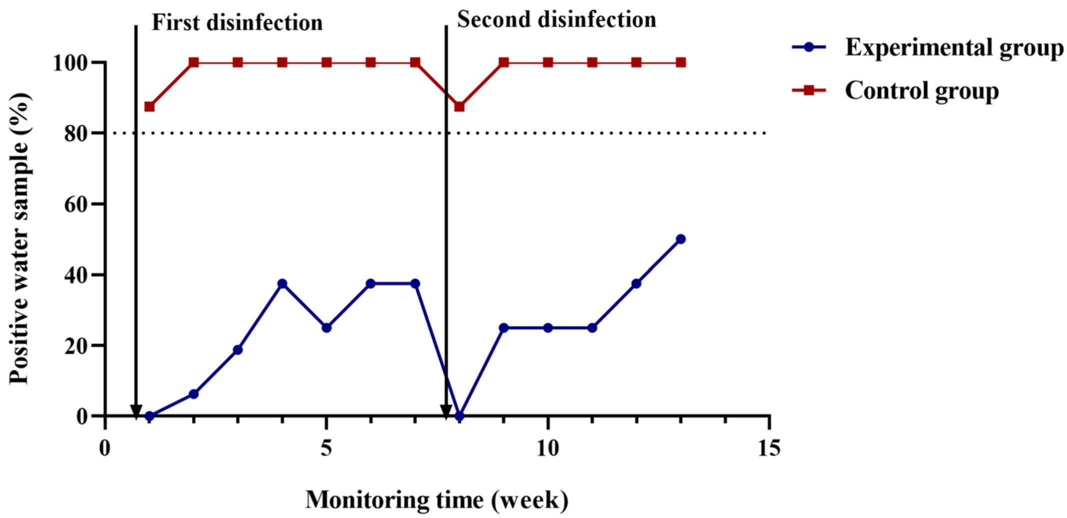

Fig. 5. Trends of the positive rates of samples in the experiment group and control group by the monitoring time.

47 CFU/100 mL, and was unchanged or slightly increased during the monitoring time; it is worth noting that a reduction to 0 CFU/100 mL in TVCs occurred after the second disinfection.

As shown in Fig. 5, the positive rates for the control group were almost 100%, but the positive rates for the experiment group were always at a low to medium level, with the 13th week of the monitoring period having the highest positive rate, which was 50%.

The predominant bacteria detected in water samples for control group were *Pseudomonas fluorescens, Sphingomonas paucimobilis, Ralstonia picketti, Stenotrophomonas maltophilia, Brevundimonas vesicularis, Burkholderia cepacia and Methylobacteriu*. While for the experiment group, only *Ralstonia pickettii* and *Burkholderia cepacia* were detected.

Discussion

To our knowledge, this is the first study to investigate the effect of decreasing the TVCs in the final rinse water of endoscopes by combination of POU filter and PAA disinfection. Severe microbial pollution of medical water has become a difficult problem for medical institutions[22,23]. Contamination of water system by potentially infectious microorganisms, such as bacteria, viruses and protozoa, is a source of nosocomial infections[24,25]. Our research found that TVCs in the final rinse water of endoscopes easily exceed the standard, but it is practicable to combine POU filters installation at the final of branches with PAA disinfection for pipeline systems to control the bacterial count in the final rinse water.

Removing microbial contaminants from water through chemical disinfection is a way to guarantee the safe management of medical water[26,27]. PAA has powerful antibacterial activity and PAA chemical disinfection can effectively destroy multiple pathogenic microorganisms in water, which is regarded as the best alternative to chlorine disinfection and is widely used in disinfection of water systems in medical institutions[28]. However, in this study, we found that after PAA disinfection, bacterial contamination still occurred during transportation in waterway system, resulting in significantly higher TVCs in the rinse water from branch terminal than that in the source water. In our study, the TVCs in the final rinse water of endoscope centers began to increase after one week of disinfection of centralized water supply system and transmission pipelines. Therefore, due to the instability and limited duration of the effect, PAA disinfection must be repeated at a certain time interval[29]. Previous study indicated that high-frequency disinfection would corrode pipeline system components, cause biofilm attachment, and produce a large number of disinfection by-products; long-term use will also lead to selective resistance of bacteria to disinfectants[26,30–32]. In this study, PVC-U was selected as the pipeline material due to its documented resistance to PAA corrosion. Throughout the experimental period, no corrosion of the PVC-U material caused by PAA exposure was observed. The biofilm shedding in water system releases attached bacteria into water, resulting in altered number of bacterial colonies[33], which may explain the large change in the number of colonies after the second PAA chemical disinfection among control group in this study. Consequently, the effectiveness of only using PAA chemical disinfection to control the TVCs in final rinse water is questionable.

As an efficient and rapid intervention method, POU filters can effectively reduce the bacterial content of final water[34], and reduce the risk of waterborne infection[35,36]. A study at a cancer center in Northwestern Pennsylvania revealed that no legionella was detected in POU filtered samples within 12 weeks, and the total bacteria was decreased by an average of 1.86 logarithm compared with controls[37]. Parkinson et al. found that POU filters installed on both faucets and showers eliminated legionella and reduced heterotrophic plate count concentrations for 12 weeks[38], which was consistent with our results that the bacterial count in the final rinse water of the experiment group was significantly lower than that of the control group. Bielefeldt et al. found that the initial disinfection efficiencies of POU filters ranged from 3 to 4.5 logarithm experimentally, but the effectiveness decreased to 0.2–2.5 logarithm after loading multiple batches of highly contaminated water, indicating that

POU filters were effective, but showed loss of effectiveness with time[39]. In addition, water chemistry (pH, OM, inorganic ions), adsorbent characteristics (pore distribution, particle size, functional group) can affect the adsorption efficiency of POU filters, and lead to variable result in real world application[40].

The combination of POU filters and PAA disinfection obtain better disinfection effects and economic applicability. Previous studies indicated that the number of bacteria in the downstream of POU filters and risk of reverse pollution both gradually increased after a period of use; downstream of filter membranes and filter outlets were susceptible to the pollution of liquid splashing, drain aerosol backwash, and other ways, resulting in the increase of the bacterial[41]. It is debatable whether it is economical to invest medical resources in preventing healthcare-associated infections related to opportunistic aquatic pathogens, especially since their short maximum lifetime and relatively high prices have limited their use[36,37]. In this study, we found that installation of POU filter combined with PAA chemical disinfection could significantly reduce the number and variety of bacteria for lasting about 12 weeks, this duration exceeded the 30-day timeframe typically advised in the product manual. Therefore, combination of regular chemical disinfection of central water supply systems with POU filters in essential departments such as endoscopy center is an economical and feasible method control the bacteria in downstream of POU within the required range and prevent nosocomial infection[36,38,42].

Most of the bacteria detected in the study belonged to the *Proteobacteria*, which are widely distributed in hospital environments and water, and some of them are opportunistic pathogens. *Burkholderia cepacia* can cause bloodstream infections and has high infectivity[43,44]. *Stenotrophomonas maltophilia* can lead to pneumonia, bloodstream infections, or catheter-related infections in immunocompromised individuals[45]. *Pseudomonas fluorescens* is occasionally reported to cause pneumonia[46] and bloodstream infections[47]. *Ralstonia pickettii* and *Brevundimonas vesicularis* have low virulence and rarely cause nosocomial infections. The literature on nosocomial infections reported by *Methylobacterium* and *Sphingomonas paucimobilis* is limited. *Stenotrophomonas maltophilia* and *Burkholderia cepacia* have prominent resistance, among which *Stenotrophomonas maltophilia* has significant resistance to carbapenems, quinolones, tetracyclines, β-lactams and other antibiotics; *Burkholderia cepacia* is a multidrug-resistant bacterium that exhibits extensive resistance to β- lactam and aminoglycoside antibiotics. In our study, bacterial identification revealed no significant changes in antibiotic resistance profiles before and after the combination of POU filters and PAA disinfection. We acknowledge that prolonged PAA exposure may theoretically induce biofilm adaptation. But our integrated POU filtration system's regular replacement protocol effectively disrupts potential resistance development cycles. Nevertheless, further long-term studies are necessary to conduct more detailed analyses on strain typing and antibiotic resistance monitoring.

This study is the first to introduce the concept of controlling the TVCs in the final rinse water of endoscopes by combining POU filters with PAA chemical disinfection of pipeline systems, and provides an important reference for controlling bacterial reproduction and biofilm formation in water system. In addition, this study used the R2A culture method for the detection of bacteria in water, which has higher sensitivity and stronger reliability of results[21,48,49]. In some countries the microbial level is expected to be < 10 CFU/100 mL[50] or 0 CFU/100 mL[51], and in our study we have adopted strict standards that the TVCs = 0 CFU/100 mL was considered as qualified. However, several limitations of our study should be acknowledged. First, the quality of purified water is affected by many factors, such as source water, storage temperature and environment, and season[26,52], hence the interval of disinfection in pipe network should be further studied and discussed according to regional and seasonal differences. Second, due to the hospital's routine use of PAA chemical disinfection, we cannot test without PAA disinfection to evaluate the impact of filtration. However, the TVCs in the control group measured one week before PAA disinfection was significantly higher than the TVCs measured one week after PAA disinfection (62,625 vs. 2354 CFU/100 mL, $P < 0.001$), which could be used as a before-and-after comparison. Third, as a widely used chemical disinfectant, the side effects of PAA cannot be ignored, which although were not analyzed in the current research but need to be accessed in the further studies. Forth, for the branches of central pipeline water supply system, only endoscope center was selected. It is necessary to further study the long-term effects of installing POU filters on the number of pathogenic microorganisms and related nosocomial infections in other departments or hospitals.

Conclusion

The central water supply system is prone to excessive microorganisms at the branches. Combination of POU filters with PAA chemical disinfection of pure water pipelines is able to effectively reduce the TVCs detected in final rinse water of endoscopes. Our study provides an effective reference for medical institutions with central water supply systems to control the bacterial content in final rinse water.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
XC, HM and LC contributed equally to this work. XC, YF and LX made substantial contributions to the conception or design of the work; XC, HM and YW took responsibility for the integrity of the data and the accuracy of the data analysis. XC and HM drafted the manuscript. LC, FG and HX conducted the statistical analyses. LX managed the project and provided guidance. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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