## RESEARCH



# Management and microbial monitoring of final rinse water for flexible endoscopes in 290 hospitals in Jiangsu Province, China: a multicenter cross-sectional study



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## Abstract

**Background** There is a lack of a universally accepted standard or guideline for the frequency of disinfection in purified water pipelines. Furthermore, there is no standardized method for detecting microorganisms in the final rinse water utilized for endoscope cleaning. This study aims to examine the current management and microbial monitoring practices concerning the final rinse water used for flexible endoscope cleaning in medical institutions.

**Methods** A questionnaire was designed using a convenience sampling method to gather data on the maintenance and microbial monitoring of final rinse water for flexible endoscopes in 290 medical institutions across Jiangsu Province, China.

**Results** Purified water is used for endoscope rinsing by 93.45% of institutions, with 78.62% employing centralized water supply. Membrane filtration devices at the terminal are installed by 82.07%, mainly with a 0.2µm pore size (76.47%), and are replaced quarterly (32.77%). Disinfection devices are present at 52.76% of terminals, with varied disinfection frequencies; chlorine-containing disinfectants (48.15%) and peracetic acid (34.92%) are predominant. Inadequate disinfection, filter membrane neglect, sampling contamination, and biofilm formation are identified as reasons for non-compliant final rinse water. Actions include filter replacement, pipeline disinfection, and flushing. Microbial mornitoring occurs quarterly (70.96%), with faucet outlets as primary sampling sites. Standards are based on 10cfu/100ml (87.58%), using membrane filtration (40.81%) and nutrient agar plates (82.72%). A cultivation period of 2 days predominated (72.43%), with a temperature range of 35-37°C (76.47%).

**Conclusion** While purified water and terminal filters are common for final rinsing of endoscopes, there is variation in maintenance and supply line disinfection. Current microbiological methods' reliability is considered low, necessitating further research to establish unified standards for effective endoscope final rinse water management and monitoring.

Keywords Flexible endoscopy, Final rinse water, Management, Microbial monitoring

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## Introduction

With continuous updating and upgrading, flexible endoscopes have integrated examination and operation functions [1]. More and more complex features bring complexity to the structure, which in turn increases the difficulty of reprocessing [2] The risk of endoscopic-related infection has been listed as one of the top ten health technology hazards released by ECRI (Emergency Care Research Institute) Institute in 2018 and 2019 [3]. Endoscope-related infections are associated with factors such as failures in the reprocessing of flexible endoscopes, equipment-related issues (including endoscope automated cleaning and disinfection machines, pure water systems, and storage equipment), product malfunctions, and human errors [4]. At the same time, microbial contamination of final rinse water has also been associated with outbreaks [5] and pseudo-outbreaks [6-10], involving some flexible endoscopes.

The main purpose of final rinsing is to remove the residual chemical disinfectant to avoid adverse reactions to patients or workers. However, when the final rinse water is contaminated with bacteria or other pathogens, this step risks re-contaminating the endoscope and possibly causing infection to the patient. A multicenter study led by Zhongshan Hospital affiliated to Fudan University [11] and Tianjin Center for Disease Control and Prevention [3] (encompassing medical institutions at various levels) revealed that the rates for qualified final rinse waterr (water that meets the expected quality for the final rinse of endoscope reprocessing) were 63.09% (53/84) and 61.11% (111/180) respectively. The highest colony-forming units (cfu) per 100 mL of a single specimen reached as high as 91,000 cfu/100 mL. These results indicate it is essential to implement microbiological controls to ensure the final rinse water remains high quality.

There is currently no uniform standard for the maintenance requirements of the final rinse water processing system in China, and the microbial monitoring methods of the final rinse water are not consistent across medical institutions. The aim of this study was to investigate medical institutions in the Jiangsu Province and examine the water management and microbial monitoring for final rinsing of flexible endoscopes, to identify the existing issues and provide a basis for unifying requirements and establishing standards.

### Methods

## Objective

This study included a questionnaire developed to collect information on the management and microbial monitoring of final rinse water used for reprocessing flexible endoscopes in medical institutions. A total of 290 medical institutions in Jiangsu Province, China were included in this study. Out of these, 129 (44.48%) were tertiary hospitals and 161 (55.52%) were secondary hospitals. These i nstitutions were spread across 13 prefecture-level cities in Jiangsu Province. Specifically, there were 53 institutions in Nantong, 45 in Suzhou, 35 in Yancheng City, 28 in Huaian City, 21 in Taizhou City, and 18 in Nanjing and Changzhou each. Additionally, there were 15 institutions in Zhenjiang, 14 in Suqian, Yangzhou, and Wuxi, respectively, 8 in Xuzhou, and 7 in Lianyungang (Fig. 1).

#### Questionnaire design

To resolve practical difficulties arising in field operations, the questionnaire was designed based on relevant regulations and literature reports. It was refined through review feedback from two experts from endoscopy centers and four senior infection control specialists. The final version of the questionnaire comprised sections on: (1) the information about the final rinse water for flexible endoscopes; (2) configuration and maintenance of reprocessing processing equipment; (3) analysis of reasons for non-compliance and corresponding responses; (4) and the method of microbial monitoring for flexible endoscopes and final rinse water. See the supplementary table for details.

### Sampling method

The questionnaire was created using the 'Questionnaire Star' platform and a Quick Response(QR) code was generated. It was published to the WeChat Group of the Fifth Hospital Infection Management Professional Committee of Jiangsu Hospital Association on June 26, 2023. Participation was requested from secondary and higher-level medical institutions in the province, with each medical institution required to complete only one questionnaire and assign personnel for its completion. A convenience sampling method was employed, where all institutions in the WeChat group were invited to participate. This method was chosen for its practicality and efficiency in reaching a broad range of institutions within a limited timeframe. A total of 302 questionnaires were collected and underwent a rigorous data cleaning process. Two individuals independently reviewed each questionnaire, and telephone confirmation was conducted when necessary. During this process, ten duplicate questionnaires and two questionnaires with missing data were excluded, resulting in a final set of 290 valid questionnaires for analysis.

## Statistical analysis

Statistical analysis was conducted using the WPS2019 (Kingsun. Hong Kong, CHINA) for data processing and

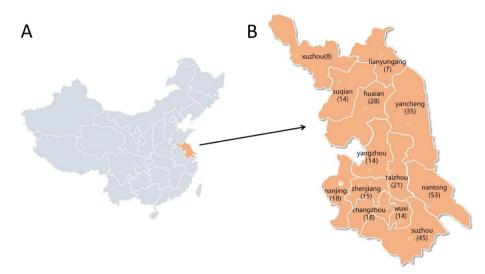


Fig. 1 Mapping representation of study area. A The location of Jiangsu Province in China. B Distribution of Hospitals Participating in This Study Across Jiangsu Province

the SPSS20.0 (IBM Corp. Armonk, NY,USA) for data analysis. Counting data were presented as frequency and percentage, and inter-group comparison was performed using the Chi-square test. A statistically significant difference was considered when P < 0.05.

## Results

### Current status of flexible endoscope and final rinse water

In 290 medical institutions, the types of flexible endoscopes used included gastroscopes (99.31%) and colonoscopes (96.21%), which were equipped in more than 95% of institutions, while fiberoptic bronchoscopes (56.21%) and laryngoscopes (51.03%) were equipped in more than 50% of institutions." Among them, the proportion of duodenoscopes, fiberoptic bronchoscopes, laryngoscopes and esophageal ultrasound endoscopes in tertiary hospitals was significantly higher than that in secondary hospitals. As to manual cleaning and disinfection workstations for flexible endoscopes, tertiary hospitals mostly had 3 to 5 stations (59.69%), while secondary hospitals mostly had fewer than 3 stations (75.78%). In tertiary hospitals, the majority (55.81%) had 1-5 units of automated cleaning and disinfection machines for endoscope reprocessing. The majority of secondary hospitals had no endoscope machines. Manual cleaning and disinfection workstations were mainly located in endoscope centers (97.59%), Ear, Nose, and Throat(ENT) outpatient clinics (30.00%), department of pulmonary and critical care medicine (13.10%) and operating rooms (13.10%). The proportion of purified water (including fully purified water and partially purified water) for final rinsing of flexible endoscope was 93.45%, and the proportion of centralized water supply (including fully centralized water supply and partially centralized water supply) was 78.62%. 78.97% of medical institutions were equipped with water storage tanks for final rinse water (including full configuration and part configuration). See Table 1 for details.

## Configuration and maintenance on terminal equipment of final rinse water for flexible endoscope

The installation of membrane filters at the terminal of water for final rinse is a critical step in ensuring the microbial quality of the rinse water. These filters are placed at the point where the water enters the faucet (before water discharge), reducing the risk of contamination during the endoscope reprocessing process. 82.07% of medical institutions installed membrane filters at the terminal of water for final rinse (before water discharge), with the main pore size of  $0.2\mu m$  (76.47%). The main replacement cycle of the membrane filters was quarterly (32.77%), no fixed cycle (26.05%), yearly (21.43%) and semi-annually (14.29%), mainly by third-party companies (51.06%) and hospital equipment/general affairs/logistics. 52.76% of medical institutions installed disinfection devices at the terminal of water for final rinse (before water discharge), with the main disinfection methods including chemical disinfection, ultraviolet disinfection, and ozone disinfection. As to the water supply pipeline disinfection frequency, 34.83% did not disinfect, 18.62% disinfected quarterly, 17.59% had no fixed disinfection frequency, and the main disinfectants were chlorinecontaining disinfectants (48.15%) and peracetic acid (34.92%). See Table 2 for details.

## Table 1 Current status of flexible endoscope and final rinse water in 290 medical institutions

Content	All hospita	als ( <i>n</i> =290)	Tertiary ho	ospital ( <i>n</i> =129)	Secondary Hospital (n=161)	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
Hospital level						
Tertiary hospital	129	44.48	/	/	/	/
Secondary hospital	161	55.52	/	/	/	/
Hospital grade						
Grade A	142	48.97	63	48.84	79	49.07
Grade B	67	23.10	33	25.58	34	21.12
Undetermined grade	81	27.93	33	25.58	48	29.81
Flexible endoscope type						
Gastroscope	288	99.31	128	99.22	160	99.38
Colonoscope	279	96.21	128	99.22	151	93.79
Duodenoscope	114	39.31	89	68.99	25	15.53
Fiberoptic bronchoscope	163	56.21	112	86.82	51	31.68
Laryngoscope	148	51.03	96	74.42	52	32.30
Esophageal ultrasound endoscope	47	16.21	42	32.56	5	3.11
Others (choledochoscope, nasopharyngoscope, etc.)	12	4.14	7	5.43	5	3.11
Number of manual cleaning and disinfection workstation			/	5.45	J	5.11
< 3	155		33	25.58	177	75.78
< 5 3 ~ 5		53.45			122	
> ~ 5	116 19	40.00 6.55	77	59.69	39	24.22 0
			19	14.73	0	0
Number of automated cleaning and disinfection machin			2.4	26.26	100	(2.11
0	134	46.21	34	26.36	100	62.11
1–5	132	45.52	72	55.81	60	37.27
6–10	21	7.24	20	15.50	1	0.62
> 10	3	1.03	3	2.33	0	0
Department distribution of manual cleaning and disinfed						
Endoscopy center	283	97.59	127	98.45	156	96.89
ENT outpatient clinic	87	30.00	64	49.61	23	14.29
Department of pulmonary and critical care medicine	38	13.10	26	20.16	12	7.45
Operating room	38	13.10	20	15.50	18	11.18
ICU	16	5.52	12	9.30	4	2.48
Department of ultrasound	6	2.07	5	3.88	1	0.62
Rehabilitation department	3	1.03	1	0.78	2	1.24
Other departments (oncology department, disinfec- tion and supply center, infection department, etc.)	39	13.45	24	18.6	15	9.32
Water source types of final rinse water for endoscope						
Fully purified water	228	78.62	101	78.29	127	78.88
Partially purified water	43	14.83	26	20.16	17	10.56
Unpurified water	19	6.55	2	1.55	17	10.56
Water supply mode of final rinse water for endoscope						
Fully centralized water supply	175	60.34	69	53.49	106	65.84
Partially centralized water supply	53	18.28	34	26.36	19	11.80
All non-centralized water supply	62	21.38	26	20.16	36	22.36
Equipped with water storage tank for final rinse water						
Full configuration	176	60.69	72	55.81	104	64.60
Partial configuration	53	18.28	35	27.13	18	11.18
All not configured	61	21.03	22	17.05	39	24.22

## Analysis of the reasons for unqualified water used for the final rinsing of flexible endoscopes and corresponding measures

According to the relevant guidelines [12], the gualified standard for microbial culture results of final rinse water for flexible endoscopes is≤10cfu/100 ml. Results exceeding this threshold are considered unqualified. Among the 290 medical institutions, 18 did not detect the final rinse water for flexible endoscope, and 3 did not have the unqualified water for final rinsing. Excluding these, a total of 269 medical institutions were included in the partial analysis, including 125 tertiary hospitals and 144 secondary hospitals (Table 3). In the analysis of the reasons for the unqualified results of final rinse water for endoscopes, the possible factors included ineffective disinfection of the water supply pipeline, no replacement of terminal filter membrane, sampling contamination and biofilm formation at the faucet end of water supply pipeline. It is important to note that these factors are based on institutional analysis and perception, and definitive evidence, such as laboratory testing for biofilm formation or other confirmatory measures, was not consistently available across all cases. Replacement of terminal filter membrane, disinfection of water supply pipeline, and flushing of water supply pipeline were the main measures taken when the water used for final rinsing was deemed unqualified. See Table 3 for details.

## Current situation of microbial detection methods for flexible endoscope and final rinse water

Only 3.45% of medical institutions had independent laboratories for testing final rinse water, and 27.24% of medical institutions outsource the testing of endoscope and terminal rinse water samples to third-party companies; Infection control specialists were responsible for testing and cultivating throughout the entire process in only 14.14% of medical institutions. The detection frequency of final rinse water was mainly once every quarter (70.96%), the outlet of faucet and irrigation port accounted for 90.81% and 28.68% of the sampling sites, and the largest sampling quantities were 10 ml (38.6%), 50 ml (33.46%) and 100 ml (14.14%), respectively. The standard of eligibility was primarily based on 10cfu/100 ml (87.58%). The membrane filtration method accounted for 40.81% of the inoculation methods. A cultivation period of 2 days predominated (72.43%), with a temperature range of 35-37°C (76.47%). The selection of culture dishes primarily favored nutrient agar plates (82.72%) See Table 4 for details.

## Discussion

The WS 507–2016 Technical regulations for cleaning and disinfection of flexible endoscopes [12] (referred to as technical specifications) defines the final rinsing as

the process of rinsing the endoscope with purified water. Research findings indicate that 93.45% of flexible endoscopes are rinsed with purified water (fully or partially), with tertiary hospitals using purified water in 98.45% of cases and secondary hospitals in 89.44% of cases. This showed that the majority of medical institutions surveyed in the Jiangsu Province are using purified water as the final rinse water, which was higher than the results of a study conducted by Zhu Xuanrui et al. [13] in 2020 in 72 medical institutions in Jilin Province (87.9% for tertiary hospitals and 80.8% for secondary hospitals), as well as than the results of a study conducted by Liu Feng et al. [14] in 2020 in 318 medical institutions in Shandong Province (53.88% for tertiary hospitals and 30.84% for secondary hospitals). Furthmore, this study found that the frequency of testing for final rinse water was primarily quarterly (70.96%) and monthly (14.34%), which is higher than the results of a survey [15] conducted in 165 medical institutions across 39 countries, where the primary frequencies were monthly (22%) and semiannually (15%). At present, there is no clear requirement for the sampling frequency of final rinsing water, and medical institutions may set it according to their specific circumstances.

While purified water equipment is highly effective in removing microorganisms and impurities during the initial purification process, the quality of purified water can be compromised as it travels through extensive pipelines to reach the end-use point. Potential contamination risks include biofilm formation, pipeline degradation, and prolonged water stagnation within the pipes. These factors may contribute to microbial overgrowth, ultimately undermining the integrity of the purified water. To address this, additional filtration at the end of the final rinse water and the maintenance of the purified water pipeline are common and necessary practices. These ensure that the water used for the final rinse meets the required microbial and chemical standards, even after potential contamination during distribution. This step is critical for maintaining the safety and efficacy of endoscope reprocessing [12].

In this study, it was found that 82.07% of the final rinse water outlets for flexible endoscope rinsing were equipped with a filter membrane..Among these, 84.50% were in tertiary hospitals and 80.12% were in secondary hospitals, which were higher than the results reported by Zhu Xuanrui et al. [13] (81.80% in tertiary hospitals and 53.80% in secondary hospitals).According to the technical specifications [12], the pore size of the filter membrane used in the production of purified water should be  $\leq 0.2\mu$ m and should be replaced regularly.The research discovered that 93.28% of hospitals complied with the technical specifications, with 96.33% of tertiary

Content	Total ( <i>n</i> =290)		Tertiary hospital ( <i>n</i> =129)		Secondary Hospital (n=161)	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%
Filter membrane device installed at the terminal of final rinse	water (befor	e water discharge)				
Yes	238	82.07	109	84.5	129	80.12
No	52	17.93	20	15.5	32	19.88
Pore size of terminal filter membrane for final rinse water ( $n=$	238)					
0.1µm	40	16.81	19	17.43	21	16.28
0.2µm	182	76.47	86	78.9	96	74.42
0.4µm	10	4.20	0	0	10	7.75
Other pore size	6	2.52	4	3.67	2	1.55
Replacement cycle of terminal filter membrane for final rinse	water (n=23	3)				
Weekly	1	0.42	0	0	1	0.78
Monthly	9	3.78	4	3.67	5	3.88
Quarterly	78	32.77	40	36.7	38	29.46
Half-yearly	34	14.29	21	19.27	13	10.08
Yearly	51	21.43	22	20.18	29	22.48
Replaced but not in a fixed cycle	62	26.05	21	19.27	41	31.78
No replacement	3	1.26	1	0.92	2	1.55
Filter membrane replacement department ( $n=235$ )	5	1.20		0.92	2	1.55
Third-party company	120	51.06	57	52.78	63	49.61
Hospital equipment/general affairs/logistics department	100	42.55	46	42.59	54	42.52
Replaced by the department	15	6.38	5	4.63	10	7.87
Is there a disinfection device at the terminal of the final rinse		0.50	5	4.05	10	7.07
Yes	153	52.76	73	56.59	80	49.69
No	135	47.24	73 56	43.41	80 81	49.09 50.31
No Number of terminal disinfection method types used for final			20	43.41	01	50.51
			50	71.00		60.75
1	107	69.93	52	71.23	55	68.75
2	34	22.22	15	20.55	19	23.75
3	12	7.84	6	8.22	6	7.50
Type of terminal disinfection device for final rinse water (n =153)						
UV	65	42.48	27	36.99	38	47.5
Ozone	55	35.95	23	31.51	32	40.00
Chemical disinfection	89	58.17	50	68.49	39	48.75
Other disinfection methods	2	1.31	0	0	2	2.50
Disinfection frequency of water supply pipeline						
Daily	5	1.72	3	2.33	2	1.24
Monthly	25	8.62	12	9.30	13	8.07
Weekly	27	9.31	16	12.40	11	6.83
Quarterly	54	18.62	28	21.71	26	16.15
Half-yearly	10	3.45	7	5.43	3	1.86
Yearly	17	5.86	10	7.75	7	4.35
Disinfect but not at a fixed frequency	51	17.59	23	17.83	28	17.39
Not disinfected	101	34.83	30	23.26	71	44.10
Type of chemical disinfectant used for disinfection of water s	upply pipelin	e ( <i>n</i> =189)				
Chlorine-containing disinfectant	91	48.15	37	37.37	54	60.00
Peracetic acid	66	34.92	42	42.42	24	26.67
Hydrogen peroxide	13	6.88	7	7.07	6	6.67
Other chemical disinfectants (ozone, chlorine dioxide, acidified water, etc.)	19	10.05	13	13.13	6	6.67

 Table 2
 Configuration and maintenance of terminal equipment on final rinse water for flexible endoscope in 290 medical institutions

## Table 2 (continued)

Content	Total ( <i>n</i> =290)		Tertiary hospital ( <i>n</i> =129)		Secondary Hospital (n=161)	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
Department for water supply pipeline disinfection ( $n=189$ )						
Third-party company	63	33.33	35	35.35	28	31.11
Hospital equipment/general affairs/logistics department	79	41.80	46	46.46	33	36.67
Replaced by the department	44	23.28	17	17.17	27	30.00
Infection control department	3	1.59	1	1.01	2	2.22

 Table 3
 Analysis of the reasons for unqualified water used for the final rinsing of flexible endoscopes and corresponding measures in 290 medical institutions

Content	Total ( <i>n</i> =269)		Tertiary hospital (n=125)		Secondary Hospital ( <i>n</i> =144)	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
Reasons for unqualified results of final rinse water for endosco	pe					
Ineffective disinfection of water supply pipeline	226	84.01	108	86.40	118	81.94
Terminal filter membrane not installed	94	34.94	43	34.40	51	35.42
Terminal filter membrane not replaced	204	75.84	90	72.00	114	79.17
Biofilm formed at the faucet end of the water supply pipeline	200	74.35	96	76.80	104	72.22
Sampling contamination	203	75.46	96	76.80	107	74.31
Inoculation contamination	129	47.96	70	56.00	59	40.97
Measures to be taken if the water used for final rinsing of endo	oscope unqu	ualified				
Flush the water supply pipeline	189	70.26	92	73.60	97	67.36
Disinfect water supply pipeline	222	82.53	105	84.00	117	81.25
Replace the terminal filter membrane	229	85.13	103	82.40	126	87.50
Other measures <sup>a</sup>	13	4.83	6	4.80	7	4.86
No measures taken if endoscopic test qualified	47	17.47	19	15.20	28	19.44
No measures taken (regardless of endoscopic examination results)	48	17.84	20	16.00	28	19.44

<sup>a</sup> Other measures include re-sampling to remove contamination, disinfection of faucets, multi-sectoral joint meetings, etc.

hospitals and 90.70% of secondary hospitals meeting the requirements. Most of medical institutions could meet technical requirements for membrane pore size. It is important to note that the filter membrane traps bacteria instead of killing them, and therefore, it needs to be regularly replaced to maintain its filtering effect. If it is changed too infrequently or never changed, filter membrane will become a source of contamination [16, 17]. In this research, only 1.26% of medical institutions never replace their filters. The majority of medical institutions had replacement frequencies concentrated on a quarterly basis (32.77%), no fixed cycle (26.05%), and yearly (21.43%). This replacement cycle is lower than the findings [13] of Zhu Xuanrui et al. The replacement frequency of the filter membrane in various medical institutions needs to be integrated with the requirements of the manual, water consumption, water quality and the monitoring results of the final rinsing water.

The maintenance of the purified water pipeline is crucial for controlling microbial indicators. However, there is currently a lack of multicenter studies on the disinfection interval of the purified water pipeline, and there is no unified standard or guideline in China [18]. Some studies [19, 20] recommend monthly disinfection of the water supply pipeline system to ensure the quality of purified water. However, further research and discussion are needed to determine its applicability, considering various factors such as raw water quality, temperature, and environmental conditions. This study found that the disinfection frequency of water supply pipelines varied, with some medical institutions indicating no disinfection was being performed..Among those medical institutions that do perform disinfection, the frequency was inconsistent Table 4 Current situation of detection methods on final rinse water of flexible endoscope in 290 medical institutions

Content	Total ( <i>n</i> =290)		Tertiary hospitals (n = 129)		Secondary Hospital (n=161)	
	Quantity	Percentage(%)	Quantity	Percentage (%)	Quantity	Percentage(%)
Test area on detection and culture of final rinse water						
With independent laboratory in infection control	10	3.45	9	6.98	1	0.62
No independent laboratory for infection control, but areas dedicated to monitoring of environmental hygiene in the microbiology lab	100	34.48	58	44.96	42	26.09
No independent laboratory for infection control, and no special area dedicated to monitoring of environmental hygiene in the microbiology lab	96	33.1	58	44.96	38	23.6
Send to the third party company for inspection	79	27.24	4	3.1	75	46.58
Other circumstances (sent to higher-level hospitals for inspection)	5	1.72	0	0	5	3.11
What position of personnel completes the detection and cultur	re of final rir	ise water?				
Infection control specialists responsible for the entire process	41	14.14	22	17.05	19	11.8
Microbiology lab personnel responsible for the task, but infection control specialists involved in some testing and cultivation work	86	29.66	55	42.64	31	19.25
Microbiology lab personnel responsible for the task and no involvement from infection control specialists	81	27.93	48	37.21	33	20.5
Completed by a third party company	82	28.28	4	3.1	78	48.45
The awareness of the testing methods of third-party companie	s (Include in	oculation method	l, use of plat	e, culture time, cu	lture tempe	rature)? ( <i>n</i> =79)
Fully aware	11	13.92	3	75	8	10.67
Partially aware	55	69.62	1	25	54	72
Completely unaware	13	16.46	0	0	13	17.33
The awareness of the detection method of microbiology lab (Ir	nclude inocu	lation method, us	e of plate, c	ulture time, culture	e temperatu	ıre)? ( <i>n</i> =196)
Fully aware	89	45.41	60	51.72	29	36.25
Partially aware	102	52.04	56	48.28	46	57.5
Completely unaware	5	2.55	0	0	5	6.25
Detection frequency of final rinse water( $n=272$ )						
Monthly	39	14.34	27	21.26	12	8.28
Quarterly	193	70.96	86	67.72	107	73.79
Half-yearly	11	4.04	2	1.57	9	6.21
Yearly	7	2.57	1	0.79	6	4.14
Unregularly	22	8.09	11	8.66	11	7.59
Sampling department of final rinse water ( $n=272$ )						
Infection control	215	79.04	105	82.68	110	75.86
Clinical department	137	50.37	69	54.33	68	46.9
Others (CDC, third party companies, etc.)	16	5.88	7	5.51	9	6.21
Sampling site of final rinse water( $n=272$ )						
Inlet of water supply pipeline	78	28.68	38	29.92	40	27.59
Outlet-faucet(for rinsing)	247	90.81	118	92.91	129	88.97
Outlet-irrigation port(For irrigation)	78	28.68	31	24.41	47	32.41
Other sites	8	2.94	4	3.15	4	2.76
What is the amount of water sampled for final rinsing $(n=272)$						
1ml	2	0.74	2	1.57	0	0
5ml	35	12.87	16	12.6	19	13.1
10ml	105	38.6	55	43.31	50	34.48
50ml	91	33.46	41	32.28	50	34.48
100ml	39	14.34	13	10.24	26	17.93

## Table 4 (continued)

Content	Total ( <i>n</i> =290)		Tertiary hospitals (n = 129)		Secondary Hospital ( <i>n</i> =161)	
	Quantity	Percentage(%)	Quantity	Percentage (%)	Quantity	Percentage(%)
Criteria for the qualification of final rinse water( $n=272$ )						
≤ 10cfu/100 ml	239	87.58	112	88.19	127	87.59
≤ 100 cfu/ml	31	11.4	14	11.02	17	11.72
≤ 20 cfu/ml	2	0.74	1	0.79	1	0.69
Several inoculation methods used for final rinse water ( $n=272$ )						
1	181	66.54	91	71.65	90	62.07
2	58	21.32	28	22.05	30	20.69
3	29	10.66	7	5.51	22	15.17
Uncertain	4	1.47	1	0.79	3	2.07
Inoculation method of final rinse water ( $n=272$ )						
Smear method	116	42.65	52	40.94	64	44.14
Pouring method	157	57.72	72	56.69	85	58.62
Filter membrane method	111	40.81	44	34.65	67	46.21
Time of culture for final rinse water ( $n=272$ )						
2d	197	72.43	99	77.95	98	67.59
3d	1	0.37	1	0.79	0	0
5d	23	8.46	7	5.51	16	11.03
7d	48	17.65	20	15.75	28	19.31
Uncertain	3	1.1	0	0	3	2.07
Temperature of culture for final rinse water ( $n=272$ )						
17–23°C	43	15.81	24	18.9	19	13.1
28–30° <b>℃</b>	19	6.99	4	3.15	15	10.34
35–37 <b>°C</b>	208	76.47	99	77.95	109	75.17
Uncertain	2	0.74	0	0	2	1.38
Types of plates for culturing final rinse water ( $n=272$ )						
Nutritional agar plate	225	82.72	105	82.68	120	82.76
R2A plate	40	14.71	18	14.17	22	15.17
Others	3	1.1	2	1.57	1	0.69
Uncertain	4	1.47	2	1.57	2	1.38
Is pathogenic microorganism species regularly detected in fina	al rinse water	r? (n=272)				
Regularly	87	31.99	27	21.26	60	41.38
Unregularly, detected only when needed	173	63.6	96	75.59	77	53.1
Never	12	4.41	4	3.15	8	5.52

indicating a lack of clear guidance for each medical institution. Therefore, it is crucial to provide relevant evidence-based research and standards to guide them in this matter.

Studies have shown that the primary causes of contamination in final rinse water is the failure to regularly replace the filter [7, 8, 21, 22], improper filter pore size [23], filter missing [24] and ineffective disinfection of water supply pipelines [25]. This is consistent with the findings of this survey. In this study, the main measures taken to rectify poor testing results were disinfection of the water supply pipeline and replacement of the terminal filter membrane. Furthermore, it has been reported that continuous hypochlorous acid disinfection [26] and the installation of an ozone and ultraviolet dual system [27] can significantly enhance the qualified rate of microbial detection in the final rinse water by not only producing qualified final rinsing water but also disinfecting the water pipeline. Therefore, these factors and measures can serve as valuable references when dealing with contamination in the final rinse water.

The filtration membrane method is recommended for the detection of pure water according to relevant regulations both domestically [28] and internationally [29]. This method is highly effective due to its sensitivity, capability to process large sample volumes (which increases the likelihood of detecting low bacterial concentrations), and its ability to reduce interfering substances (e.g., disinfectant residues). By concentrating bacteria on a membrane, it allows for more reliable detection of low bacterial concentrations compared to traditional methods. Its direct culturing approach minimizes errors, and its standardized procedure ensures consistent results, making it an ideal choice for monitoring water quality in medical settings. In this study, only 40.81% of medical institutions utilized the filtration membrane method for the microbial detection of endoscopic final rinsing water. This finding underscores the necessity for further standardization and promotion of the filter membrane method. Additionally, regarding the sample volume for the final rinse water, domestic guidelines do not provide clear recommendations. However, the relevant British guidelines [29] recommend a volume of 100 ml, as a larger sample size increases the likelihood of detecting microorganisms in the water. This offers a valuable reference for further standardizing the sample volume selection when using the filtration membrane method for sampling.

Currently, there is no standardized culture method for detecting microbes in the final rinse water for endoscopes (including culture temperature, culture time, and plate type). There are three main methods commonly used in the laboratory for culturing. The first method is based on the culture method of drinking water [30], which involves culturing the water at 35–37°C for 48 hours on a nutrient agar plate [31, 32]. The second method is based on the culture method of dialysis water (YY0572) [33], where the water is cultured at 18-23°C for 7 days on low-nutrient agar plates such as R2 A [34, 35]; The third method refers to the culture method described in the Chinese Pharmacopoeia for purified water [28], which involves culturing the water at 28-32°C for 5 days on low-nutrient agar plates like R2 A [11, 18]. The British guideline [29] also recommends culturing the water at 28-32°C for 5 days on low-nutrient agar plates.

Culturing purified water using a low-nutrient medium, lower temperature, and longer duration is more suitable as excessive nutrients can inhibit the growth of heterotrophic bacteria and reduce the detection rate [36]. Previous studies [34] have shown that culturing on lownutrient agar plates at 20°C for 7 days yields a significantly higher microbial detection rate compared to culturing on normal nutrient agar at 36°C for 48 hours. However, there is currently no comparison between the second and third methods, which is an area that requires further investigation. This research has identified significant differences in the microbial detection methods used for the final rinse water of endoscopes. The membrane filtration method -

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accounted for less than half of the inoculation methods, with a culture time of mainly 2 days and a culture temperature of mainly 35–37°C. Nutrient agar plates were predominantly selected for culturing These findings indicate that approximately 80% of medical institutions are potentially using unreliable detection methods for the final rinse water, highlighting the need for verification and improvement of these methods.

#### Conclusions

This research discovered that the ratio of purified water and terminal filter membrane configuration is relatively high for the final rinse water of flexible endoscopes. However, there are notable variations in the frequency of filter membrane replacement and disinfection of the water supply pipeline. The reliability of the current microbial detection method is limited, underscoring the necessity for further research to establish standardized requirements and protocols. This will enhance the effectiveness of monitoring the final rinse water used in endoscope reprocessing.

## **Supplementary Information**

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Supplementary Material 1.

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#### Authors' contributions

Z.L. and B.L. (as co-first authors) wrote the main manuscript text. Q.Z., Z.G., F.Z., W.C., and Y.Z. contributed to data analysis and interpretation. X.D. and J.D. prepared figures and tables. W.Z. supervised the project and revised the manuscript. All authors reviewed the manuscript.

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#### Data availability

No datasets were generated or analysed during the current study.

#### Declarations

#### Ethics approval and consent to participate

This study does not require ethical approval as it does not involve patient information or harm patients'rights. The research is limited to the analysis of endoscope rinse water and does not include human subjects or personal data.

#### **Consent for publication**

This study does not require informed consent as it does not involve human subjects, patient data, or any procedures that would necessitate consent. The research is limited to the analysis of endoscope rinse water and does not include personal information.

#### **Competing interests**

The authors declare no competing interests.

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