

Comparison of channel sampling methods and brush heads in surveillance culture of endoscope reprocessing: A propensity score matching and paired study

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Abstract

Background: Endoscopy-related infections have caused multiple outbreaks. The importance of surveillance culture is gradually recognized, but sampling techniques are not consistent in many guidelines. It is unclear whether the Flush-Brush-Flush sampling method (FBFSM) is more sensitive than the conventional flush sampling method (CFSM) and whether different sampling brushes have different effects.

Methods: The propensity score matching method was done with two matching ways, 1:1 nearest neighbor propensity score matching and full matching was used to analyze the surveillance culture data collected by FBFSM and CFSM. We fit a confounder-adjusted multiple generalized linear logistic regression model to estimate the marginal odds ratio (OR). A paired study was applied to compare the sampling effect of polyurethane foam (PU) head brush and polyamide (PA) head brush.

Result: From 2016 to 2020, 316 reprocessed endoscope samples were collected from all 59 endoscopy centers in Tianjin. About 279 (88.3%) reprocessed endoscopes met the threshold of Chinese national standards (<20 CFU/Channel). The qualified rate of reprocessed endoscopes sampling by CFSM (91.8%) and FBFSM (81.6%) was statistically different ($p < 0.05$). The adjusted OR by full matching for FBFSM was 7.98 (95% confidence interval: 3.35-21.78). Forty one pairs of colonoscopes, after reprocessing from 27 centers, were tested by PA and PU brushes, and no difference was found in microbial recovery.

Conclusion: FBFSM was confirmed to be a more sensitive sampling technique. PU and PA brushes had no significant difference in sampling effect.

Keywords: Brush head, contamination, endoscope reprocessing, flush-brush-flush sampling method, surveillance culture

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Submitted: 12-Aug-2021 **Revised:** 09-Oct-2021 **Accepted:** 15-Oct-2021 **Published:** 02-Dec-2021

Access this article online	
Quick Response Code:	Website: www.saudijgastro.com
	DOI: 10.4103/sjg.sjg_437_21

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How to cite this article: Ji XY, Ning PY, Fei CN, Song J, Dou XM, Zhang NN, *et al.* Comparison of channel sampling methods and brush heads in surveillance culture of endoscope reprocessing: A propensity score matching and paired study. Saudi J Gastroenterol 2022;28:46-53.

INTRODUCTION

In the past few decades, the increase in endoscopes' functions and pathogenic microorganisms' evolution has made reprocessing endoscopes more difficult.^[1] In particular, some modern and complicated endoscopic interventions such as endoscopic retrograde cholangiopancreatography (ERCP), break through the mucosal barrier and increase the risk of infection caused by endoscopy.^[2] Gastrointestinal endoscopy has been confirmed to be an essential risk factor for the spread of carbapenem-resistant *Enterobacteriaceae* (CRE) and its related superbugs.^[3] It was thought that microbiologic monitoring of flexible endoscope channels was the most important direct method for assuring that the reprocessing procedures being used were reliable.^[4] Given cases of infection caused by failure of duodenoscope reprocessing,^[2,5] and the expert consensus that surveillance culture can help identify specific endoscopic defects that hamper effective reprocessing, the U.S. Food and Drug Administration (FDA), the U.S. Centers for Disease Control and Prevention (CDC), and American Society for Microbiology (ASM), released protocols on voluntary, standardized duodenoscope surveillance sampling and culturing in 2018.^[6,7] Endoscope reprocessing guidelines issued by some countries or regions recommend routine surveillance [Table 1].

The sampling methods in these guidelines are different. Some use sterile water or saline with or without a neutralizing

agent to flush the endoscope biopsy channel, which we call the conventional flush sampling method (CFSM).^[12,13,15,16] Some use a sampling brush while flushing, which we call the Flush-Brush-Flush sampling method (FBFSM),^[4,11,14,17] and some use a sampling pump to assist flushing, which we call pump assistant sampling method (PASM).^[12] Theoretically, it was believed that FBFSM might have a better microbial recovery rate,^[17] but a few studies had not confirmed this conclusion.^[18,19] In the study of a simulated accumulation biofilm model, Alfa *et al.*^[20] found in 2017 that the use of the FBFSM could increase the recovery mean levels of *P. aeruginosa* from polytetrafluoroethylene buildup biofilm compared with CFSM. However, owing to the limited sample size, it does not provide a statistically significant difference between the two sampling methods, explained by the authors. In contrast, Brandabur *et al.*^[18] conducted a large-scale study on 4032 samples, and the results showed that no significant difference was detected because the standard deviation in a unique sampling method was higher than that between different sampling methods. Some experts even claimed that anterograde sampling was not sensitive enough. They sampled the biopsy suction channel retrogradely using the suction button to suck back the sample fluid, used for flushing, to the proximal channel opening.^[21-23] To our knowledge, few direct comparative sampling methodological studies have been reported.^[18,24] Most were observational, non-randomized controlled trials with small sample sizes.^[18,25,26]

Table 1: Summary of sampling methods, threshold, and frequencies in various guidelines (sorted by year of publication)

Guideline	Year	Frequency	Method	Threshold
BSG, United Kingdom ^[8]	2020	Sampling when an outbreak is known or suspected, routine surveillance not recommended	-	-
ASGE, ^[9] SGNA, ^[6] United States	2018	Uniform or intermittent surveillance for duodenoscopes	-	-
KSGE, Korean ^[10]	2017	-	-	-
SFERD, Netherlands ^[11]	2016	Quarterly	20-mL sterile saline flush + brush +20-mL sterile saline	<20 CFU/20 mL
China ^[12]	2012	Quarterly	Flushing with or without pump using 50-mL liquid with surface activity and neutralizer ingredients	<20 CFU/channel
JGETS ^[13] , Japen	2012	At least once a year	100-mL sterile saline flush	<20 CFU/channel
GENCA-GESA-AGEA, Australia ^[14]	2010	Duodenoscopes every 4 weeks. Bronchoscopes every 4 weeks. All other gastrointestinal scopes every 4 months.	10-ml sterile water or normal flush + brush + 10-mL sterile water or normal flush	<10 CFU
ESGE-ESGENA, Europe ^[15]	2008	Intervals no longer than 3 months.	0.9% 20-mL sterile saline with or without neutralizer flush	<20 CFU/channel
CTINILS, France ^[16]	2007	-	Flushing with 100-200 mL liquid with surface activity and neutralizer ingredients	<25 CFU/channel
APIC, United States ^[17]	2000	Sampling when an outbreak is known or suspected, routine surveillance not recommended	Sterile saline flush or brush	No vegetative bacteria
MACID, Manitoba of Canada ^[4]	2000	Every 4-6 months	5-mL sterile water flush + brush +5- mL sterile water flush	<20 CFU/0.1 mL

Note:- not mentioned; CFU, colony-forming units



Figure 1: Photos of sampling brushes (a): PU brush (b): PA brush

Since 2016, we have used the observational research method for many years in Tianjin, the largest coastal city in northern China, comparing the microbial recovery effects of different sampling techniques (CFSM, FBFSM, and PASM). However, the small sample size collected each year and the significant differences in the endoscopes' background characteristics may lead to a particular selection bias and insufficient evidence for the sampling techniques' estimated effects. Although our previous research reported that FBFSM or PASM had been associated with the impact of endoscope microbial recovery,^[1,27] it remains unclear if associations were attributable to selection bias.

Accordingly, this study has two goals. One is to estimate whether FBFSM is more sensitive than CFSM using propensity score matching based on the data obtained from observational studies, and the other is to compare the effect of two types of sampling brushes on the recovery of microbe, through a paired test.

MATERIAL AND METHODS

Ethical approvals

This study has been reviewed by the Tianjin Centers for Disease Control and Prevention at three levels, including the leadership of scientific research authorities, academic and ethics committees, and project leaders.

Sampling brushes

Two types of sampling brushes [Figure 1], which we customized from Jiangsu GuardKing Medical Equipment Co., Ltd., were applied in the Flush-Brush-Flush sampling method (FBFSM). The head of the yellow brush is made of polyurethane foam (PU), and the white one a polyamide (PA). The heads of these two sampling brushes

are 5 mm in diameter and 20 mm in length. The handles are all made of polyoxymethylene, with a certain degree of flexibility, and the length is 2.3 meters. All sampling brushes are made of disposable paper-plastic packaging and are used after sterilization by ethylene oxide.

Research process

Since 2016, our study has adopted CFSM and FBFSM to monitor reprocessed endoscopes as routine work, covering all 59 endoscopy centers in Tianjin. Before 2019, the municipal and district CDCs' staff randomly selected 1–2 endoscopes from one center for testing by CFSM or FBFSM. From 2019 to 2020, we, the municipal CDC, conducted a paired study. We randomly selected two colonoscopies in the same center and compared the sampling efficiency of the PA and PU brushes.

Methods for sample collection of channels

The CFSM was implemented following the Chinese national standard "Hospital Disinfection and Sanitation Standards (GB15982-2012)", that was, 50 mL of the eluent containing neutralizer (ECN) was used for flushing the endoscope biopsy channel.^[27] The FBFSM was not mentioned in Chinese national standards, but it was a sampling method proposed by our comprehensive literature and guidelines. The FBFSM was based on the CFSM. First, 25 mL ECN was flushed through the biopsy channel and collected at the distal tip into a sterile bottle. Second, one PU or PA brush was inserted into the biopsy channel port, passed through, and then head cutoff into the sample bottle. Last, 25 mL ECN was flushed through the biopsy channel and collected at the distal tip into the above sterile bottle. Samples were collected using the aseptic technique to prevent contamination. When the endoscope was disinfected with glutaraldehyde/ortho-phthalaldehyde, the formula of ECN was phosphate buffer saline (PBS) with 5 g/L glycine, 10 g/L peptone, 8.5 g/L sodium chloride, and 1 g/L Tween 80. When using acidic electrolytic water/peracetic acid/others, the formula is PBS with 5 g/L sodium thiosulfate, 10 g/L peptone, 8.5 g/L sodium chloride, and 1 g/L Tween 80.

Testing technique

All samples had counts performed in two steps. First, 15–20 mL of the melted common nutrient agar medium cooled to 40°C to 45°C with 1 mL of the original sample was poured. This step was counted as CFU/plate. Second, concentration was filtrated over a 0.45- μ m filter (Microsart® @filter, Sartorius, Germany) with the remaining water sample. The membrane filter was aseptically removed, transferred to a nutrient agar plate, and was incubated at 36°C for 48 hours. This second

step was presented as CFU/membrane. The final report result (CFU/channel) depended on the value of CFU/membrane. If CFU/membrane was not countable, then CFU/channel was reported as CFU/plate × 50. Otherwise, it was notified as CFU/plate + CFU/membrane.^[12] The CFU/channel less than 20 shows a total CFU result that is within acceptable limits (i.e., <20 CFU/channel), considered to meet the Chinese National Standard “Hygienic Standard for Disinfection in Hospital (GB15982-2012)” issued by the National Health Commission of China.^[12]

Statistical analysis

The qualified status of the endoscope was presented in percentages, and the comparison of the rates was carried out by the Chi-square test. Median, interquartile range (IQR), and range were given for microbial culture results, which was almost skewed data, and the Wilcoxon rank-sum test was used for comparison.

Several endoscopes’ reprocessing related information was recorded by using a questionnaire while sampling, including the type and age of the endoscope, the type of disinfectant used for reprocessing, whether manual or automatic endoscope reprocessing, whether centralized management of endoscope, the frequency of enzymatic cleaners replacement, self-test and culture method in surveillance, and how alcohol was used during drying.

We used all the data collected from 2016 to 2020, including the results from routine work and the paired study, to analyze whether the FBFSM could improve the recovery of contaminating organisms. We regarded FBFSM as treatment, CFSM as control, the reprocessing of the endoscope-whether a failure as an outcome, and the information collected by the questionnaire as series of covariates. As all the data were not derived from randomized controlled trials, it was necessary to match the covariates to estimate the treatment’s marginal effect. We used propensity score matching to estimate the average marginal effect of the treatment on outcome, accounting for confounding by the included covariates. We attempted two matching methods, 1:1 nearest neighbor propensity score matching without replacement, and full matching with a propensity score estimated using logistic regression.

We adopted the R version 4.0.3,^[28] making use of the “MatchIt”^[29] package for estimating and conditioning the propensity scores to build a confounder-adjusted multiple generalized linear logistic regression model. We used the cobalt^[30] package in R to assess balance on the resulting propensity score-matched and weighted samples. The

difference (Diff), standardized mean differences, or raw differences in proportions were used to assess the balance calculated. The standardized mean differences for binary and multi-category treatments are defined as

$$\text{Diff} = \frac{(\bar{x}_{FBFSM} - \bar{x}_{CFSM})}{\sqrt{(s_{FBFSM}^2 + s_{CFSM}^2) / 2}} \quad (1)$$

where \bar{x} and s denote the sample mean and variance of the covariate in FBFSM and CFSM group subjects, respectively. The raw differences in proportions for continuous covariates are defined as

$$\text{Diff} = \frac{(\hat{p}_{FBFSM} - \hat{p}_{CFSM})}{\sqrt{\frac{\hat{p}_{FBFSM}(1 - \hat{p}_{FBFSM}) + \hat{p}_{CFSM}(1 - \hat{p}_{CFSM})}{2}}} \quad (2)$$

where \hat{p} denotes the prevalence of the dichotomous variable in FBFSM and CFSM group subjects, respectively.^[31] Diff, a threshold of 0.1, has been proposed by Stuart *et al.*^[32] who found that the threshold of 0.1 was more effective at assessing imbalance, leading to biased effect estimation.

As the outcome variable (0 = ≤20 CFU/channel, 1 = >20 CFU/channel) was binary, a conditional logistic regression of the outcome on the treatment was used to calculate the matched odds ratio (OR) and 95% confidence intervals (CIs). Wilcoxon matched-pairs signed-rank test was applied to compare the sampling effect between PA and PU brushes. All statistical calculations used were performed using R software. A *P* value of 0.05 was considered significant.

RESULTS

Endoscope microbial culture results after reprocessing

A total of 316 endoscopes from 59 endoscopy centers were sampled from 2016 to 2020, of which 109 (34.5%) used FBFSM and 207 (65.5%) used CFSM. The microbial culture result of 279 (88.3%) reprocessed endoscopes was below 20 CFU/channel, which was in line with Chinese national standards and was considered qualified. The total number of bacterial colonies (CFU/channel) cultured with endoscope flushing samples collected by FBFSM ranges from 0 to 14900, with an IQR of 0[0-2], whereas by CFSM from 0 to 85000, with an IQR of 0[0-3]. According to Wilcoxon rank-sum test, the difference was statistically significant (*p* < 0.05). The qualified rate of endoscopes using CFSM was 91.8%, whereas it was 81.6% for using FBFSM, the difference was statistically significant (*P* < 0.05).

Propensity-score matching for FBFSM and CFMS

Data on endoscope reprocessing collected by the questionnaire and laboratory tests were integrated into a dataset for later analysis. The propensity-score matching method was applied to estimate the average marginal effect of the treatment, FBFSM, on the reprocessed endoscope’s microbiological results. Table 2 shows the means of continuous baseline covariates and prevalences of dichotomous baseline covariates and standardized differences comparing baseline covariates between CFMS and FBFSM in the original unmatched and matched sample.

We began by attempting 1:1 nearest neighbor matching without replacement. It can be seen from Figure 2 and Table 2 that the balance was acceptable. Only one covariate (the endoscope age) did not have a good balance with the standardized mean differences greater than 0.1. We instead tried full matching, which yielded adequate balance. The differences for all covariates were below 0.1 and no units were discarded [Figure 2].

Estimating the FBFSM treatment effects in propensity-score matched samples

For binary outcomes (0 = ≤20 CFU/channel, 1 = >20 CFU/channel), we used logistic regression model to estimate the marginal odds ratio (OR) for FBFSM. Table 3 showed that the adjusted OR by full matching for FBFSM was 7.98 (95%CI: 3.35-21.78). The odds for FBFSM to detect the endoscope reprocessing failure was about 6.98 times higher than that of the odds for CFMS.

Comparing the recovery effect of PA brush and PU brush

In 2020, we collected 41 pairs of colonoscopes after reprocessing from 27 centers. Each pair of endoscopes was

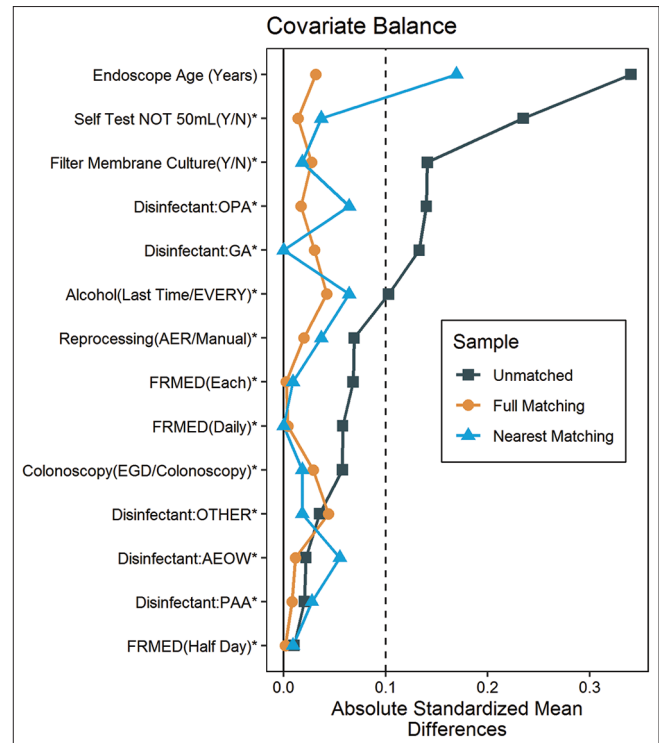


Figure 2: Covariate balance shown by Love plot. Note: The vertical dashed line corresponding to the value of 0.1, the absolute value of standardized mean differences, is the threshold for assessing imbalance

Table 2: Differences in covariates between CFMS and FBFSM before and after matching

covariate	Unmatched			Full Matching			Nearest Matching		
	CFMS n=207	FBFSM n=109	Diff.	CFMS n=207	FBFSM n=109	Diff.	CFMS n=109	FBFSM n=109	Diff.
Disinfectant (%)									
GA	27.05	13.76	-0.133	16.77	13.76	-0.030	13.76	13.76	0.000
OPA	22.71	36.70	0.140	38.41	36.70	-0.017	30.28	36.70	0.064
AEOW	43.48	41.28	-0.022	40.11	41.28	0.012	46.79	41.28	-0.055
PAA	3.86	1.83	-0.020	2.68	1.83	-0.008	4.59	1.83	-0.028
OTHER	2.90	6.42	0.035	2.03	6.42	0.044	4.59	6.42	0.018
Endoscope (%) (gastroscope/colonoscope)									
Colonoscopy	58.45	64.22	0.058	67.13	64.22	-0.029	62.39	64.22	0.018
Frequency of enzymatic cleaners replacement (%)									
Each scope	92.27	99.08	0.068	98.82	99.08	0.003	98.17	99.08	0.009
Half Day	1.93	0.92	-0.010	0.76	0.92	0.002	1.83	0.92	-0.009
Daily	5.80	0.00	-0.058	0.42	0.00	-0.004	0.00	0.00	0.000
Alcohol for Dry (%) (Last Time/EVERY)									
Last Time	22.22	11.93	-0.103	7.71	11.93	0.042	5.50	11.93	0.064
Filter Membrane Culture (YES/NO)									
YES	39.13	53.21	0.141	50.46	53.21	0.028	51.38	53.21	0.018
Self Test (%) (50 mL/NOT 50 mL)									
NOT 50 mL	38.16	14.68	-0.235	13.28	14.68	0.014	18.35	14.68	-0.037
Reprocessing (AER/Manual) (%)									
AER	18.84	11.93	-0.069	13.92	11.93	-0.020	15.60	11.93	-0.037
Endoscope Age (Years) (mean±SD)	4.34±3.07	5.80±4.31	0.340	5.67±3.52	5.80±4.31	0.032	5.07±3.19	5.80±4.31	0.169

Note: Diff.: the standardized mean differences, for binary and multi-category treatments, or raw differences in proportions, for continuous covariates.

Table 3: The result of the logistic regression model

	Estimate	Std. Error	Z	P	OR	95%CI
Full matching FBFSM	2.077	0.470	4.422	<0.001	7.98	3.35~21.78
Nearest matching FBFSM	1.660	0.519	3.201	0.002	5.26	2.05~16.32

collected from the same endoscopy center, and the brand, model, age, and reprocessing methods of the endoscope were the same. The range of endoscope microbial culture results collected by PA and PU were 0 to 400 CFU/channel and 0 to 300 CFU/channel, respectively. The medians were 1 CFU/channel (IQR: 0-6.5) and 2 CFU/channel (IQR: 0-6.5), respectively. The Wilcoxon pairs signed-rank test [Figure 3] on the microbial culture results showed no statistical difference between the two sampling brushes ($Z = -0.216, P = 0.6144$).

DISCUSSION

Some scholars believed that surveillance culture was complicated, time-consuming, and expensive, may not detect atypical organisms, and the results were not available until after the potential exposure has occurred.^[33] In addition, the adenosine triphosphate bioluminescence test is accepted as a method to monitor manual cleaning compliance, but not accepted as a substitute for culture to monitor contamination in fully reprocessed endoscopes. There is no other effective verification method. The microbial culture was considered the gold standard for quality control of endoscope reprocessing.^[34,35] It is worth noting that some guidelines recommend that action be taken when there is even 1 CFU of an “Organism of Concern”(e.g, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Enterococci*, and *Beta-hemolytic Streptococci*), whereas this study has focused on “total CFU” only, and does not include the type of microorganism detected on culture. Our research results showed that of the 316 reprocessed endoscopes examined, 279 (88.3%) met Chinese national standards (≤ 20 CFU/channel). Compared with our previous research reports, the qualified reprocessing rate has increased (82.07% reported in 2018,^[1] 84.64% reported in 2020^[27]). In the past few years, on-site routine surveillance has played a vital role in publicizing national standards and promoting hospitals to implement the standard reprocessing protocol. It can also be seen from the results of historical data analysis that the qualified rates of different sampling methods were different and statistically significant (CFSM: 91.8% vs. FBFSM: 81.6%). Different sampling technology may play a critical role in the recovery of microbes, as shown in Table 1, which was not unified in national guidelines or literature

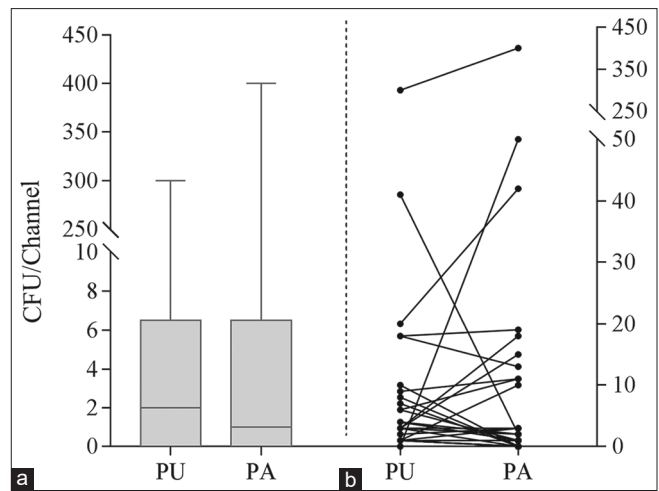


Figure 3: The microbial culture results of the two brushes. (a): box plots (b): paired scatter plots

reports. The improved sampling method is beneficial to objectively reflect the reprocessing of endoscopes and improve the surveillance quality.^[34] Aumeran *et al.*^[24] failed to detect any contamination by saline flushing routine for surveillance cultures of duodenoscopes, when they deal with an outbreak due to multi-resistant *Klebsiella pneumoniae* contaminating duodenoscopes. When they used a brush and rinsed with a Tween 80-lecithin-based solution instead of a salt solution, they isolated the outbreak strain from the contaminated endoscope.

Currently, few studies comparing different sampling techniques have been published, and there are some limitations such as a nonexperimental study, small sample size, or different background characteristics between groups.^[25,34] The experimental research makes the treatment and control groups the same in terms of background characteristics through random assignment, setting up controls, and so on, allowing for straightforward comparison of outcomes. The background characteristics of the nonexperimental data source usually contain a lot of confounding factors. Propensity-score matching is an increasingly popular technique for estimating causal effects in nonexperimental studies by controlling the observed confounding variables through matching methods.^[36]

We first attempted the most common 1:1 nearest neighbor matching to balance the background characteristics. Balance checks showed that the matching was adequate,^[37] except for one covariate (the endoscope age) with the standardized mean differences less than 0.2 and greater than 0.1. In general, standardized mean differences should be as close to zero as possible.^[32] We also tried full matching on the propensity score, which yielded a better adequate balance with the standardized mean difference

of all covariates being less than 0.1. Full matching used all CFSM and FBFSM group units; so no units were discarded by the matching. The adjusted OR by full matching for FBFSM was 7.98 (95% CI: 3.35-21.78). The odds for FBFSM to detect the endoscope reprocessing failure was about 6.98 times higher than that for CFSM. In addition to being consistent with some guidelines, this result was also compatible with many research reports, confirming that FBFSM was superior to CFSM in microbial recovery efficiency.^[25,34,38] A study reported by Babb *et al.*^[39] in 1982 was considered to be the earliest research on brush-assisted sampling that we can find. Babb *et al.*^[39] claimed that although brushing was difficult to standardize, the results obtained were more meaningful than bacterial counts from washings. Ma *et al.*^[34] compared the three sampling techniques of biopsy channel flushing, entire channel flushing, and FBFSM, and the results showed that the detection rate of bacteria by FBFSM (50%) or the entire channel flushing (41.7%) was significantly higher than that by the biopsy channel flushing (8.3%). Cattoir *et al.*^[25] reported that the qualified rate of endoscopes (<25 CFU/channel) was significantly lower when the saline + PULL THRU™ (a patented endoscope channel cleaning brush, Medivators, Minneapolis, MN) method (60%) was used instead of saline alone (82.5%).^[16]

Among PU and PA brushes the PA brush was an ordinary disposable cleaning brush for cleaning the biopsy channel, and the head of the PU brush was specialized for this research. In our paired study, no statistical difference was found between PA and PU brushes. We analyzed the reason to be the relatively small sample size, only 41 pairs, or the low microbial load of the reprocessed endoscope, with the IQR only 0–6.5 CFU/channel. Gazdik *et al.*^[40] reported that the use of flocked swabs for sampling duodenoscopes can significantly improve the recovery of Gram-negative bacteria, demonstrating a 2-fold increased recovery rate compared to the Olympus cleaning brush that CDC recommended.

There were several limitations in our study. First the investigation of covariate information related to endoscope reprocessing was not constant every year. For example, some key covariates (i.e., the number of daily reprocessing, final rinse water quality, etc.) were only available in 2019 or 2020, so this information cannot be matched by propensity scoring. Also, all our studies were field trials. Although propensity-score matching was carried out to balance the background characteristics, there may still be undiscovered confounding factors. Further work is required to build a simulated-use buildup biofilm model to compare different sampling methods or brush heads.

In this study, we analyzed the surveillance culture data of endoscope reprocessing collected from 2016 to 2020 using propensity-score matching. FBFSM was confirmed to be a more sensitive sampling technique, which could release more viable organisms attached to the channel's inner lumen. However, we found that PU and PA brushes had no significant difference in the sampling effect.

Financial support and sponsorship

X. Y. J. received grant support from the Tianjin Municipal Health Science and Technology Project (No. ZC20021) issued by the Tianjin Municipal Health Commission.

Conflicts of interest

There are no conflicts of interest.

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