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Original article

Best practice in microbiological surveillance of endoscopes: Evaluation the efficacy of different sampling strategies for reprocessed endoscopes

Nourhan Hussein [1], Moustafa Ragab [2], Gehan Fahmy [3], Iman El-Kholy [4]*

1- *Faculty of Graduate Studies and Environmental Research, Ain Shams University, Cairo, Egypt*
2- *Community and Environmental Medicine Department, Faculty of Graduate Studies and Environmental Research, Ain Shams University, Cairo, Egypt*
3- *Professor of clinical microbiology, Infection Control Consultant, Ain –Shams University Specialized Hospital, Cairo, Egypt*
4- *Ain –Shams University Specialized Hospital, Cairo, Egypt*

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ABSTRACT

Background: Every year, more than 17.7 million endoscopic procedures take place. Although endoscopic procedures are generally low-risk, multidrug-resistant bacteria have been identified as a contributing factor to endoscope-associated infection(EAI). From here, the value of microbiological surveillance is gradually becoming a challenge to determine the best practice for endoscope surveillance. **Methods:** We aim to compare various guidelines for microbiological surveillance of endoscopes. Different types of endoscopes are sampled after undergoing high-level disinfection (HLD) by the conventional flushing sampling method (CFSM), flush-brush-flush sampling method (FBFSM), and rapid protein test. Different brush heads were used while sampling by the FBFSM. **Results:** There was a high significant difference between the two used methods, being the highest among FBFSM and the lowest among CFSM, with detection ranges (2–72 cfu) and (0–30 cfu), respectively, and the FBFSM were able to isolate more high concern bacteria than the CFSM. Additionally, a pull-through brush when added to the sampling process of FBFSM, improved the culture outcomes significantly. **Conclusion:** FBFSM was confirmed to be more sensitive than CFSM in bacterial detection and the usage of the pull-through brush in the case of FBFSM increased its sensitivity in the detection of microorganisms. Microbiological surveillance can't be replaced by rapid protein test.

Introduction

Flexible endoscopes are vital medical instruments that allow visualisation of inside organs and tissues. They are essential in the diagnosis and treatment of many conditions. They have extremely intricate structures made up of numerous long, narrow tubular tubes and fibrotic bundles, a suction system, and an air and water system. As they are reusable devices, there have been significant worries concerning the spread of diseases by endoscopes [1,2].

Every year, more than 17.7 million endoscopic operations are carried out. Although endoscopic procedures are generally low-risk, there is a chance of adverse events, such as infection with multidrug-resistant bacteria [3].

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* *Corresponding author:*Nourhan Hussein
E-mail address: *Nourhan.hussein@iesr.asu.edu.eg*

Contaminated flexible endoscopes have been linked to many outbreaks of diseases related to healthcare as they may introduce harmful microorganisms into the human body. The flexible endoscope is a difficult tool to use because of its complex structure and potential for significant microbial contamination, especially with the biofilm formation that makes it harder to disinfect [4].

Endogenous flora (the patient's own microbes) or exogenous microbes, which may come from previous patients or contaminated reprocessing equipment, are both responsible for endoscopic procedure-related infections. Due to low frequency, a lack of clinical symptoms, or inadequate surveillance, the true rate of transmission during endoscopy may be underestimated [5,6]. In recent years, the composite infection rate was calculated to be 0.2% after Gastro-Intestinal (GI) endoscopic operations, and after Endoscopic Retrograde Cholangiopancreatography (ERCP), it was found to be 0.8% [3].

Moreover, additional factors, including improper drying and bad storage conditions, might also affect endoscope contamination. Remaining damp inside the canals encourages the growth of leftover microbes such as *Pseudomonas aeruginosa, Klebsiella pneumonia,* and others. The majority of endoscopy-related infections are preventable with precise and careful endoscope reprocessing [7].

High-level disinfection (HLD) is required for reprocessing endoscopes since they fall under the Spaulding categorization system's semi-critical device level [4,8] This is the reason why these medical devices require a series of processes, including pre-cleaning, cleaning, high-level disinfection, and water rinsing, followed by drying [9,10].

Microbiological surveillance plays a vital role in guaranteeing the safety of reprocessing endoscopes by confirming their quality and detecting any contaminated devices. This surveillance aims to identify any possible problems, validate the quality of the endoscope reprocessing, and evaluate its efficacy [6,11].

Microbiological surveillance is applied by different guidelines. These guidelines use different sampling techniques. Some employ what we refer to as the conventional flush sampling method (CFSM) to flush the endoscope biopsy channel using sterile water or saline, either with or without a neutralising agent. Some flush using a brush sampling (called the Flush-Brush-Flush sampling method, or FBFSM) to help with flushing [2].

The value of surveillance culture is gradually becoming understood; however, many standards and different guidelines have inconsistent sampling practices. It is unclear whether the conventional flush sampling method (CFSM) or the flush-brush-Flush sampling method (FBFSM) is more sensitive [11].

Any small change in the sampling technique, sampling dilutes, or brushing using a different type of brush could influence the recovery rate [12, 13].

Because improperly reprocessed endoscopes are more likely to be found, the methods with higher recovery rates lower the chance of false-negative results, improving the safety of patients and employees [2,14].

However, assessing bacterial load through culturing is often impractical for many endoscopy centres due to limited access to microbiology laboratories and long incubation period to obtain results. Instead, currently available methods enable rapid evaluation of residual protein and organic matter within endoscope channels, providing results within seconds for rapid cleanliness validation.

To mitigate concerns regarding patient safety and significantly reduce endoscopy-related infections, we attempted to determine the best method for microbiological surveillance of reprocessed endoscopes by comparing two different guidelines and the use of commercially available protein test.

Materials and Methods

Ethical approval

This study has been reviewed by faculty of postgraduate childhood studies ethical committee – Ain Shams University. Approval no.: FRGCS_ASUREC/RHDIRB2020110401/MSDFC_2

Experimental material

Endoscopes

Different types of endoscopes were randomly selected from February 2023 to December 2023 from the endoscopy centre of a hospital in Cairo, Egypt.

The endoscopes tested included gastroscopes, colonoscopes, bronchoscopes, and duodenoscopes.

To compare between two different methods of sampling

Two approaches were used in this experiment. Samples were taken by the CFSM as followed by the CFSM guidelines and by the FBFSM in accordance with the USA guidelines.

All endoscopes were sampled at least 6 hours after undergoing HLD

Sampling method

35 ml of sterile water was flushed through the biopsy port; the elute was then collected in a sterile bottle, from which 10 ml was extracted by syringe and centrifuged for 15 minutes at 3000 rpm, then decanted to 1ml and 0.2 ml of deposit of supernatant in centrifuged samples cultured on blood agar and MacConkey's agar (0.1 ml on each plate) (CFSM)[7].

The remaining 25 ml was kept. And a sterile disposable brush was then inserted into the biopsy channel, and the channel was brushed until the brush completely exited the endoscope channel. The upper part of the brush was cut off (2 cm) using sterile scissors and then added to the same bottle for testing. Another 25 ml of sterile water was injected into the instrument port, and the collected sample was mixed with an equal amount of neutralizing broth. Then, the final sample was divided into two portions. Each half was filtered through 0.45-µm membrane filters and filter paper was placed on blood agar and MacConkey's agar media according to FBFSM [8]. The incubation was carried out at 35–37 °C for 72 hours **(Figure 1).**

- In the case of the bronchoscope, the total volume was 30 ml of sterile water; 20 ml before brushing and 10 ml after brushing. As ideally the sample collection volume to flush the endoscope channel is approximately three times the channel volume [8].

To compare the effect of two types of sampling brushes on the recovery of the bacteria:

- Two types of sampling brushes were used in the FBFSM (standard brush and pull through brush) **(Figure 2).**
- 25 ml of sterile water injected into the endoscope sampling channel and collected into a bottle (A) then the brush inserted and the channel brushed and cut (2cm) of brush head and inserted in bottle (B) and other 25 ml of sterile water were injected and collected in bottle (B) then 1ml of each bottle cultured on nutrient agar and MacConkey's agar media to compare results before (bottle A) and after brushing (bottle B) for each brush type.
- The total number of bacteria on both plates is recorded and identified by the automated VITEK® 2 system (bioMérieux. Marcy l'Etoile, France).

Rapid protein test

Prior to sampling using the FBFSM and pull-through brush, a total of 20 protein tests were performed.

Using (Getinge Assured Protein Test Flexible Endoscope 2.5m) (Getinge – USA) which is a qualitative test that can detect a protein residue of 1 µg in less than 10 seconds by inserting its brush into the endoscope sampling channel and then dropped into a vial containing the reagent (brown colour) if protein present it turns into blue colour. The deepness of the blue colour indicates the higher the protein presence in the endoscope.

Statistical method

IBM SPSS statistics (V. 27.0, IBM Corp., USA, 2020) was used for data analysis. The statistical methods used are Kruskal-Walis test, Mann Whitney U test and Ranked Sperman correlation test to study the possible correlation for non-parametric data.

Results

The results showed that among the endoscopes sampled with the CFSM with colony counts ranging from 0 to 30 CFU and the FBFSM with colony counts ranging from 2 to 72 CFU, the detection rate of bacteria was higher by the FBFSM than by the CFSM **(Table1).**

Different bacterial yields were obtained when the FBFSM+ (standard brush or pull through brush) was utilised. The bacterial kind was not identified; only the colonies were counted **(Table 2)**. Which were categorized into three groups (0 - 10 CFU \ 10- 20 CFU and more than 20 CFU **(Figure 4).**

Prior to sampling using the FBFSM and pull-through brush, a total of 20 protein tests was performed. The findings of these tests revealed positive results (indicating un-cleanliness) in 30% of the samples, whereas the FBFSM revealed appropriate bacterial growth.

Table1. Difference in the bacterial growth between CFSM and FBFSM.

Type	N	Mean	Min.	Max.	Median	Percentiles	
						25	**75**
CFSM	51	4.25	0	30	3	0	6
FBFSM	51	13.901	2	72	10	18	42
						p	**Sig**
						0.001	HS

Table 2. Culture results obtained by two different brushes in the FBFSM.

	No. of samples	CFU before brushing (median)	CFU after brushing (median)	MIN	MAX	*p*- value
	using the pull thru brush					
ALL	22	12	34	7	72	0.004
	using standard brush					
ALL	22	9	12	2	31	0.034

Figure 1. Sampling methods.

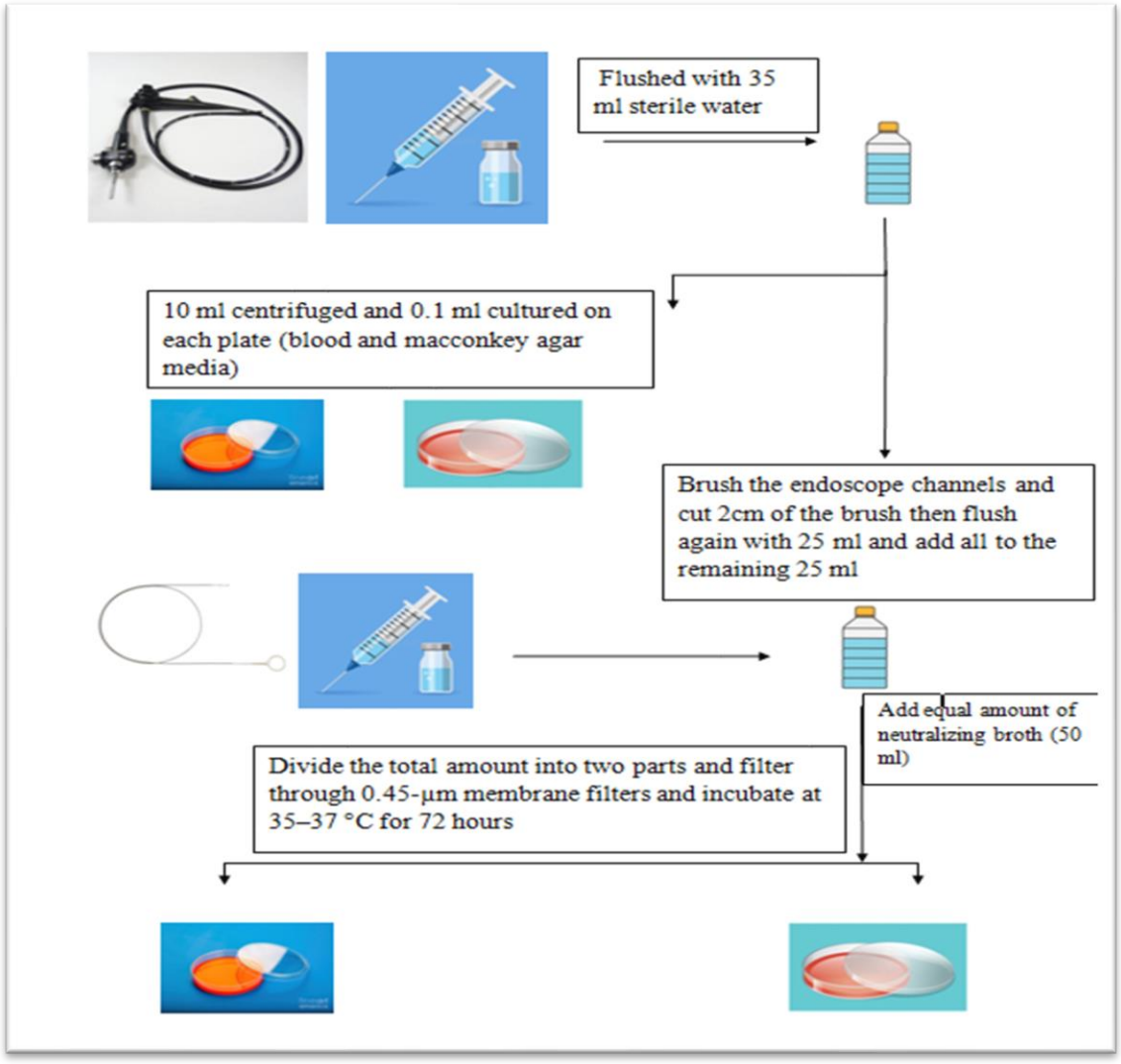


Figure 2. Pulling through brush (a) and standard brush (b).

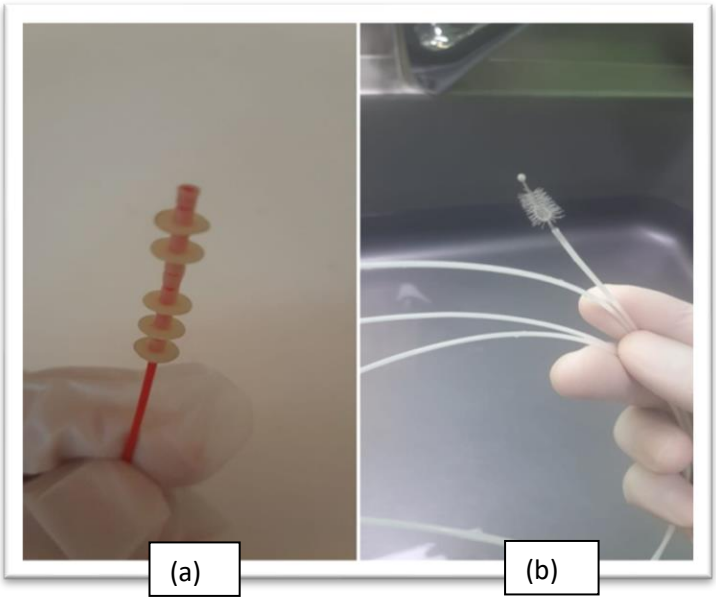


Figure 3. Percentage distribution of different bacterial isolates from two different surveillance methods.

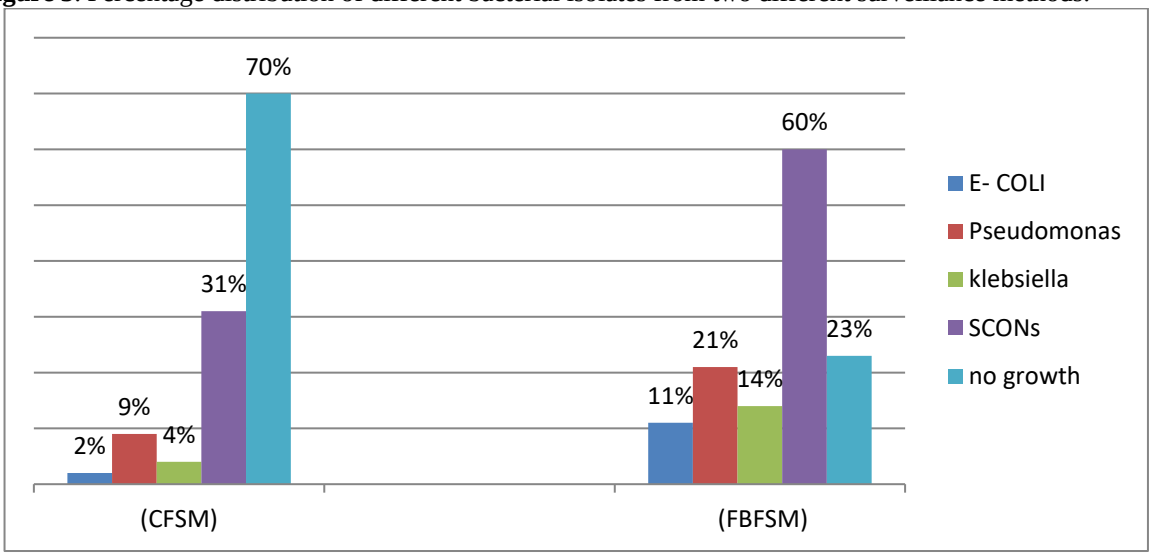
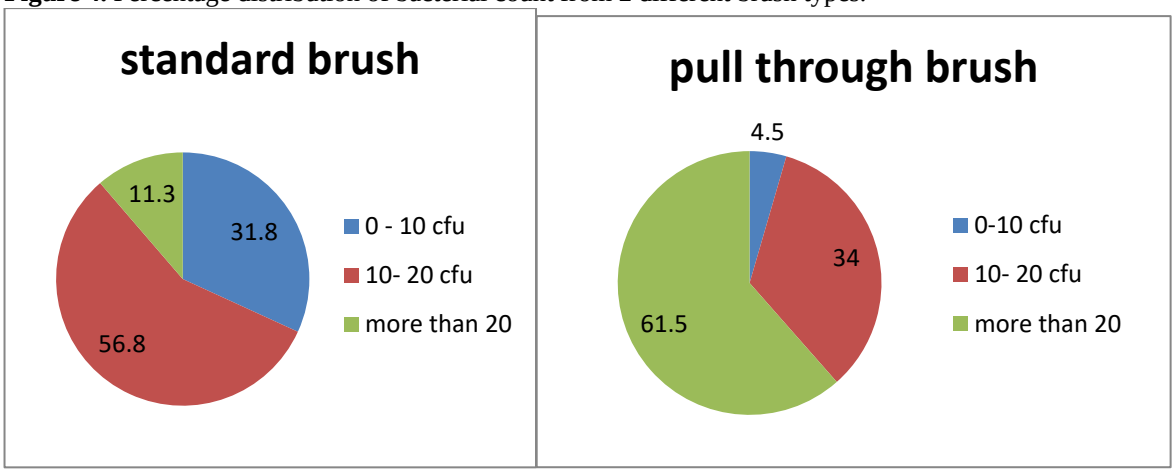


Figure 4. Percentage distribution of bacterial count from 2 different brush types.



Discussion

Endoscopes must undergo HLD prior to use, according to the Spaulding classification, because they are reusable medical devices that come into direct contact with a patient's organ mucosa [15]. Microbiological culture is a significant method for assessing the effectiveness of endoscope cleaning and disinfection to guarantee patient safety [16].

Using different sampling methods may be crucial in the recovery of microorganisms and could lead to different results of the colony count and the type of bacteria that were isolated, so that the enhanced sample technique helps to enhance the surveillance quality and accurately represent endoscope reprocessing[17, 18, 19].

So we tried two different methods (CFSM and FBFSM). When comparing the two different sampling methods used for bacterial surveillance, there was a high significant difference between the two used methods, being the highest among FBFSM and the lowest among CFSM, with detection ranges (2–72 cfu) and (0–30 cfu), respectively, and (*p* value of <0.001). **Table 1** shows that the CFSM failed to detect any bacterial growth in many cultures and showed a significantly lower bacterial count each time than the FBFSM.

This result was also compatible with [4, 15,18] confirming that FBFSM was superior to CFSM in microbial recovery efficiency.

Additionally, it was shown that the FBFSM were able to isolate more high concern bacteria than the CFSM since they included the whole sample in the examination of the elute and neutralizing broth were added to the sample, which significantly increased the bacterial yield of high concern bacteria like *Pseudomonas, E. coli, and Klebsiella***(Figure 3)**, unlike the CFSM which use only (4%) of the total collected sample and the centrifugation step which may affect the recovery of the microorganisms.

The culture results also showed important differences according to the brush used **(Table 2)**. The culture yield using FBFSM+PULL THRU BRUSH in sampling before brushing (median 12 CFU) and after brushing the yield increased by 2-3 folds with median 34 CFU ($p<.001$) for FBFSM+ STANDARD BRUSH in sampling before brushing (median 9) and after brushing the median was 12 CFU.

When dividing the obtained bacterial colonies into categories, the pull-through brush was superior to the standard brush by yielding more than 20 colonies in 61.5% of all samples, unlike the standard brush, which yields only 11.3% for the same category **(Figure 4)**, which means that a pull-through brush, when added to the sampling process, improved the culture outcomes. Which is compatible with study by Cattoir recommended the use of pull through brush with the FBFSM [4],this is explained by the fact that the pull-through brush minimises user variation in sampling techniques and maximises the sensitivity of this sampling method to evaluate the endoscope's quality following reprocessing. It does this by pulling the rubber disc through the lumen, creating a uniform cleaning action every single time.

As we find false positive results (indicating un-cleanliness) in 30% of the samples, while the microbiological testing for this sample by the FBFSM revealed accepted bacterial growth. The study demonstrated that we can't depend only on the rapid protein test as there is a higher chance of false positive result but we can use it to prove rapid cleanliness of endoscopes.

In our study, the same endoscope was used to compare between bacterial growths obtained by the two different sampling methods at the same time to reduce variability resulting from different endoscopes. However, the study had certain limitations, including a small sample size, lack of fund, and limited availability of protein tests to compare it with the FBFSM+ standard brush or CFSM or to apply the study in multiple healthcare centers.

Conclusion

Our research revealed that the FBFSM has a greater rate of bacterial positive detection than the CFSM, which indicates that it is more sensitive to the detection of bacterial growth and can be used to make safer decisions when using endoscopes after reprocessing or to guarantee the quality of such reprocessing.

Additionally, by combining the endoscopic sample procedure with the use of a pull-through brush, the microbiological count output was improved.

The rapid protein test in this study offers the advantage of providing immediate results, particularly in confirming the cleanliness of an endoscope before use. However, relying solely on this test is inadequate due to colour variations and the absence of a recognized benchmark.

Statements and Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study has been reviewed by faculty of postgraduate childhood studies ethical committee – Ain Shams University.Approval no.: FRGCS_ASUREC/RHDIRB2020110401/MSDFC_2

Consent to participate

This is an observational study and no human subjects were involved in the study.

Consent to publish

Not applicable

Data Availability

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

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