

Survey of endoscopes reprocessing and evaluation of outcomes using bacteriologic cultures for optimization of strategies

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ABSTRACT

Background: Flexible endoscopes are complex equipment that are expensive and reuse deems necessary thorough reprocessing composed of several important steps. This study aims to look into the practices used for endoscope reprocessing at a tertiary care unit and help evaluate outcomes using bacteriologic cultures at the end of High-level disinfection (HLD).

Material and Methods: The study was conducted at the endoscopy suite of Indus Hospital & Healthcare Network Karachi which is a tertiary care unit from November 2025 till February 2026. It was a hospital based cross sectional study with an embedded clinical audit component of the current endoscopic reprocessing practices using CDC Audit tool. Sixty-two endoscopes including bronchoscopes, endoscopes, colonoscopes and duodenoscopes were cultured for bacteriologic and Acid-fast bacillus (AFB) growth after HLD to serve as a measure of efficacy of disinfection.

Results: We found bacterial growth in 14 out of 62 endoscopes rendering a culture positivity rate of 23%. The highest proportion of contamination was found in colonoscopes amongst all endoscopes. Total 17 bacterial pathogens were isolated, 8 were identified as *Pseudomonas* species. About 74% samples were collected 12-18hrs after disinfection The mean time duration between sample collection and sample processing was 2.85 hrs.

Conclusion: Current practices displayed suboptimal adherence to recommended protocols for disinfection of endoscopes translating into positive cultures. Strategic steps were taken for formal training of the personnel, provision of recommended infrastructure and proposition of an appropriately bioengineered endoscopy suite to optimize care.

Keywords: Centers for Disease Control and Prevention, U.S., Disinfection, Endoscopes.

BACKGROUND

Endoscope reprocessing is one essential element of safe endoscopy practice.¹ Flexible endoscopes are architecturally complex, including narrow lumens, and deep crevices.²

They are exposed heavily to secretions, blood, and mucus and may carry 10 million-10 billion (10^{7-10}) micro-organisms prior to processing, rendering them semi-critical instruments & making their reprocessing critically important.³

Reprocessing of endoscopes before next patient use is usually comprised of: precleaning, manual cleaning, high-level disinfection (HLD), rinsing after HLD, and drying and storage.⁴

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Many studies conducted in past have demonstrated lack of compliance to scientifically designed guidelines for disinfection leading to infectious outbreaks and patient exposure.⁵

The endoscopic contamination rates range from 6% to 72% after HLD in most of the studies conducted in real time.⁶ The largest in region was conducted in Taiwan in 2019 which found a contamination rate of 33% in duodenoscopes after HLD.⁷ However, the effectiveness of endoscope decontamination in resource-limited settings is usually unexplored.

To the best of our knowledge, such study has been conducted in Pakistan to date.

Hereby we analyzed the current endoscopic reprocessing practices in a tertiary care unit by doing a clinical audit followed by endoscope culture collection after HLD. This will help improve disinfection strategies so that risk of cross-infection is minimized.

MATERIAL AND METHODS

It was a hospital based cross sectional study with an embedded clinical audit component. It was conducted at the endoscopy suite of Indus Hospital and Healthcare Network IHHN Karachi from November 2025 to

February 2026. The sample size was calculated using OpenEpi for a single population proportion. A previous study Goyal et al reported an endoscope contamination rate of 19.98%⁸. Assuming a 95% confidence level and a 10% margin of error, the minimum required sample size was calculated to be 62. A margin of error of 10% was chosen to provide a reasonable level of precision while maintaining a feasible sample size for an audit conducted within the available time and resource constraints. The sample included all 10 functional endoscopes being used in endoscopy suite during the study period: 4 gastroscopes (2 x Olympus GIF-Q180, 2 x Olympus GIF-H190), 1 colonoscope (Olympus CF-H190L), 2 bronchoscopes (1 x Olympus BF-Q180, 1x Olympus BF-Q190), and 3 duodenoscopes (2 x Pentax ED34-i10T, 1 x Olympus TJF-160VR). A non-probability consecutive sampling technique was used whereby all the above stated endoscopes undergoing reprocessing were cultured in 62 endoscopic procedures done during the study period. The study was approved by Institution Review Board IRB of IHHN, approval number IHHN_IRB_2025_06_023.

Audit of Current Practices: Firstly, a comprehensive audit tool was devised using Healthcare Infection Control Practices Advisory Committee HICPAC Sample Audit Tool designed by CDC for healthcare facilities to verify competency of flexible endoscope reprocessing. An impromptu visit was made on a working day to endoscopy suite after seeking permission from the unit's head. All the observations were made & recorded to identify gaps at every step of reprocessing. (Table-I)

Microbiological Sample Collection Protocol: A Standard Operating Protocol of sample collection & processing was established in collaboration with Microbiology Department using Scientific Society Guidelines keeping into account human & infrastructural limitations. Quality control was taken into account at every step. An eligibility criterion was made for sample acceptance/ rejection in the laboratory and strictly adhered to.

Samples were collected as swabs with transport media & sterile saline liquid samples. After endoscopes were reprocessed post patient use and dried & hung after HLD, sample collection was conducted in endoscopy suite. Liquid samples were taken from working biopsy channel, suction and air/water channels. (Table-II). Using aseptic technique 80ml normal saline was flushed

through working channels and collected in one sterile plastic leak proof culture bottle. For Bronchoscopes, 2 separate liquid samples were taken 40ml each, one for routine bacterial organisms & the other for mycobacterial growth. Swabs were first soaked in sterile saline and then sample obtained separately from each channel (biopsy channel, air/water channel, suction channel,) and for duodenoscopes additional sample was taken from distal elevator recess. Each swab sample was labelled separately referenced from the name of the channel it was collected from.

No neutralizing agents were used due to cost limitations. For the same reason, some of the CDC recommended steps to enhance microbial yield were not undertaken which have been discussed in detail under Discussion part. Lastly, samples were labeled using 2 different identifiers, made sure they were sealed well & transported to Hospital's Lab within 3 hours of collection at room temperature.

Timing of Sample Collection: Owing to the personnel's unvarying work hours, our protocol included sample collection either 4-6 h after reprocessing or 12-18 h, which makes either at the end of the same day of endoscope use after reprocessing or just before patient use the next day.

Microbiological Specimen Processing: Liquid samples were cultured onto sheep blood agar (SBA) except for bronchoscope liquid samples which were first inoculated into brain heart infusion (BHI) broth and subsequently sub-cultured onto chocolate agar after 24 hours. Swab samples were directly inoculated onto SBA, and for bronchoscopes, onto chocolate agar using quadrant streaking to obtain isolated colonies. All cultures were incubated at $35 \pm 2^\circ\text{C}$ in a CO_2 incubator and examined daily for growth up to 72 hours. AFB cultures were inoculated into BBL™ MGIT™ tubes (500 μL) and Löwenstein–Jensen (LJ) media (200 μL) for up to 42 days and 56 days at 37°C respectively. Quality control for each step of sample processing was performed according to standard operating procedures. Data was entered and analyzed using SPSS version 27.0. Descriptive statistics were applied to summarize the characteristics of the samples and study variables. Categorical variables, including type of sample (liquid and swab), types of endoscopes, and bacterial pathogens identified, were presented as frequencies and percentages. Continuous variables, such as the time duration between sample collection and processing,

were expressed as mean \pm standard deviation. Inferential statistics were employed to assess associations between variables using the Chi-square test.

RESULTS

Upon observation of practices of endoscope reprocessing using HICPAC Sample Audit Tool designed by CDC, we found out the use of Virusolve (Bis 3-aminopropyl dodecyl-amine and Didecyl dimethyl ammonium chloride) as High level disinfectant which is in fact a quaternary ammonium compound agent, labelled a Low-level disinfectant by FDA and not approved for use in endoscopes. There was no supply of Reverse Osmosis water neither was thermoregulation done rendering surfactant use inefficient. No test strips were used to check minimum effective concentration / potency of disinfectant before use. The design of endoscopy suite didn't meet the standards such that unidirectional flow for clean and dirty instruments wasn't maintained, inappropriately sized sinks & tubs were installed leading to incomplete submersion of endoscopes during reprocessing, use of wooden cabinets for storage contributing to damp environment and further microbial growth and fewer air-exchanges & essentially no negative pressure controls hindering use of glutaraldehyde if made available in future. Amongst other shortcomings, the most notable were no leak testing before washing and unavailability of air guns for drying at the end of reprocessing (Table-I).

After that, we intervened 62 endoscopic procedures (20 bronchoscopes, 25 gastroscopes, 14 colonoscopes & 3 duodenoscopes) from November 2025 till February 2026, by culturing the endoscopes after they are reprocessed (after High level disinfection) after patient-use. 14 out of 62 harbored growth making a total culture positivity rate of 23%. We took a total of 168 swab samples and 68 liquid samples from 62 endoscopes as mentioned in table 1. Among liquid samples, 14 out of 68 cultures were found positive showing a positivity rate of 17%, whereas 1 out of 168 swabs was found positive making a positivity rate of 0.5%. Total 17 bacterial pathogens were isolated out of which 8 were identified as *Pseudomonas* species. (Figure-I) Out of all 14 colonoscopes, 4 (28%) were found contaminated reaching highest proportion amongst all endoscopes (Figure-II) however the association between the type of endoscope and culture positivity when evaluated using the Chi-square test did not demonstrate a statistically significant relationship ($p = 0.14$). The mean time duration between sample collection and sample processing was $2.85 \text{ hrs} \pm 0.73$. However, relationship between time duration from sample collection to processing and culture positivity was not found to be statistically significant ($p = 0.08$). Similarly, 74% of samples were collected 12-18hrs after disinfection whereas 26% were collected 4-6hrs after disinfection. Amongst the endoscopes which flagged microbial growth, 85% were cultured 12hrs after disinfection while the rest of 15% were cultured 4-6hrs after disinfection.

Table-I: CDC-derived Audit tool for endoscope reprocessing showing observations & recommendations.

Audit Item	Yes	No	Comment/Action
Pre-cleaning			
Endoscopes undergo prompt bed-side pre-cleaning immediately after use	Yes		
Uses lint free cloth for pre-cleaning	Yes		
Cleaning materials (cloth and solution) are discarded after single use	Yes		
Transporting			
Contaminated endoscopes and accessories are promptly transferred following use	Yes		
Transporting carts are appropriately labeled with biohazard signage.		No	The used endoscope is transported in bare hands from point of use to cleaning area
Workspace			
Is designed to have separate exit & entry for used & ready-to-use scopes		No	Single entrance is used for both clean & used scopes.
Has recommended Air exchanges per hour (ACH)	Yes (for GI endoscopy)	No (for bronchoscopy)	6 ACH are recommended during GI endoscopy & 12 for bronchoscopy while the suite has 9.88 ACH currently

Wash basins/tubs are designed according to recommendations		No	Only one washbasin is installed which doesn't meet standards neither has demarcations for volume specification, the tubs being used for manual cleaning & HLD submersion are small & undemarcated.
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PPE

Staff wears PPE as recommended	Yes		Wears gloves, gown & goggles
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Leak-Testing

Leak testing is carried out prior to manual cleaning.		No	Need to perform leak test
Air manometer is used for leak detection		No	Need to use it

Manual Cleaning

Fresh cleaning solution is made for each cycle without topping up	Yes		
Checks temperature of detergent-water solution		No	Thermometer isn't available
Uses Reverse Osmosis water for cleaning		No	RO water supply point isn't available
Checks hardness of water (if RO water uses not feasible)		No	Hardness sticks are unavailable
Dilutes according to manufacturer guidelines	Yes		
Completely submerges the endoscope and accessories.		No	The endoscopes are not completely submerged
Cleans exterior surfaces of the endoscope with a soft, lint-free cloth or sponge.		No	Sponges aren't being used
Discards sponge after use		No	
Channels and distal ends are brushed using manufacturer-recommended brushes	Yes		
Elevator mechanism if present is manipulated during cleaning	Yes		
Brushing continues till no visible debris.	Yes		
Brushes 3 times each channel	Yes		
Do not brush back & forth	Yes		
Channels are flushed thoroughly with cleaning solution	Yes		
Valves are manually actuated during cleaning	Yes		
Endoscopes are rinsed with water multiple times (minimum 3-5) to remove detergent debris		No	Endoscopes aren't rinsed in water after manually cleaned by enzymatic solution
External surfaces are dried using air.		No	Air gun is unavailable
Reuseable components are reprocessed according to manufacturer guidelines		No	Buttons are neither autoclaved nor sent to plasma sterilizers, instead reused after HLD against manufacturer guidelines
Single use items are properly discarded after use	Yes		

Inspection

Inspects and evaluates endoscopes and accessories for			
Cleanliness			
missing parts	Yes		
clarity of lenses	Yes		
integrity of seals and gaskets	Yes		
physical or chemical damage	Yes		
Moisture			
Function			

High Level disinfection

Uses AER for HLD		No	
Uses mechanical methods for HLD	Yes		
Uses FDA approved HLD for flexible endoscopes		No	Virusolve (Bis 3-aminopopyl dodecylamine and Didecylidimethyl ammonium chloride) is being used for HLD which

is a quaternary ammonium & not FDA approved

Expiry date of disinfectant is checked prior to use.	Yes		
Disinfectant potency is verified using appropriate test strips		No	Test strips are unavailable.
All channels and lumens are filled with disinfectant	Yes		
Endoscopes are completely immersed in for recommended contact time		No	The endoscope isn't completely submerged due to small size of sinks, however time is being followed.
Post disinfection rinsing is performed using recommended quality water.		No	Filtered water with UV light is being used.
Drying			
Flushes lumens using 70% to 90% ethyl or isopropyl alcohol according to the endoscope manufacturer's IFU	Yes		
Dries exterior surfaces and removable parts of the endoscope and purges all channels with air.		No	Air gun isn't available
Storage			
Clean gloves are used while handling endoscopes for storage		No	Don't change gloves for transportation
Storage cabinets are made of stainless steel		No	Wooden cabinets are currently used for storage
Endoscopes are stored without coiling and do not contact cabinet surfaces.	Yes		
Valves are left open and detachable parts are removed during storage	Yes		
Sterile accessories are stored in designated sterile area		No	No separate storage area for other sterile accessories of endoscopes

Table-II: The type and number of samples collected as determined by the type and model of each endoscope.

	Bronchoscope	Gastroscope	Colonoscope	Duodenoscope
Liquid sample				
Biopsy channel	X	X	X	X
Suction channel	X	X	X	X
Air channel		X	X	X
Swab sample				
Biopsy channel	X	X	X	X
Suction channel	X	X	X	X
Air channel		X	X	X
Distal elevator recess				X
Model of scope	<i>Olympus BF-Q180, Olympus BF-Q190</i>	<i>Olympus GIF-Q180 x2, Olympus GIF-H190 x2</i>	<i>Olympus CF-H190L, Pentax EC-3490LI</i>	<i>Olympus TJF-160 VR, Pentax ED34-I10T</i>

Table-III: Overview of current endoscope processing guidelines (✓indicates recommendation)

Processing step	AAMI ST91 2021	Multi society 2021	AORN 2023	SGNA 2023
Does bedside prompt pre-cleaning after use	✓	✓	✓	✓
Has unidirectional flow of work	✓	✓	✓	✓
Decontamination is carried at different area	✓	✓	✓	--
Leak detection is checked prior to disinfection	✓	✓	✓	✓
Manual cleaning is performed before disinfection	✓	✓	✓	✓
Conduct cleaning verification tests	✓	--	✓	✓
Thorough inspection with light magnification is done before further processing	✓	✓	✓	✓
Performs HLD	✓	✓	✓	✓
Automated endoscope reprocessor is used if available	✓	✓	✓	--

Processing step	AAMI ST91 2021	Multi society 2021	AORN 2023	SGNA 2023
Minimum effective concentration is checked to affirm potency	✓	✓	✓	✓
Critical items are sterilized	✓	✓	✓	✓
Endoscopes are dried with ≥ 10 min of forced air	✓	✓	✓	✓
Endoscopes are stored in well-ventilated clean cabinets	✓	✓	✓	✓

Proportion of Bacterial Pathogens Identified (n=17)

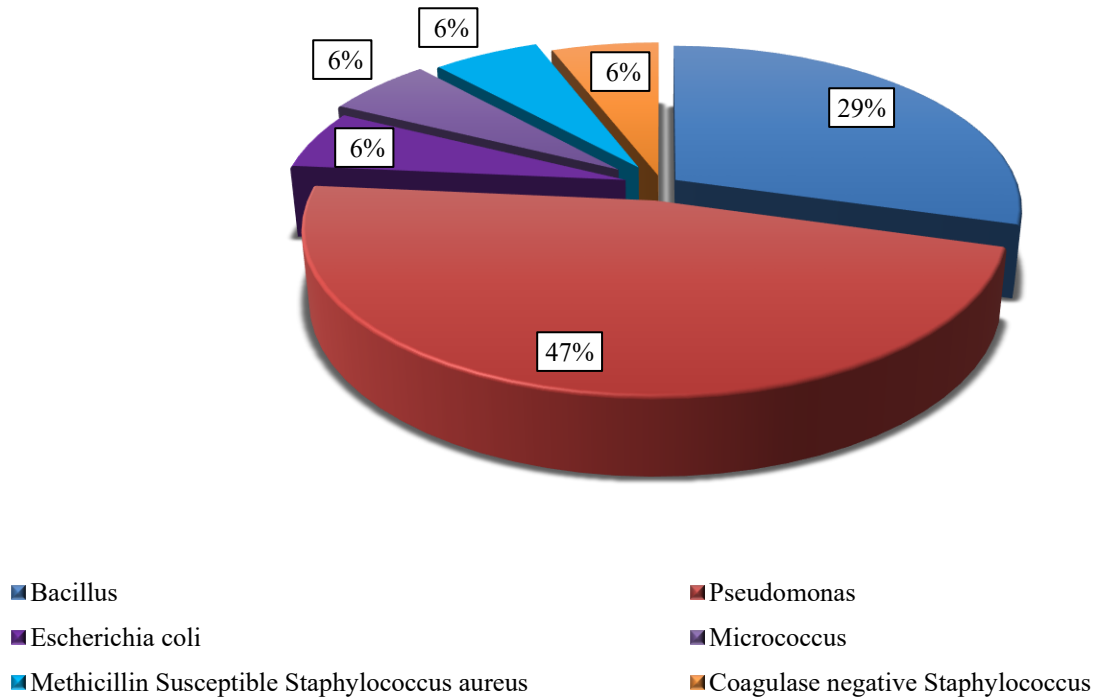


Figure-I: Proportion of bacterial pathogens identified in endoscopes included in study.

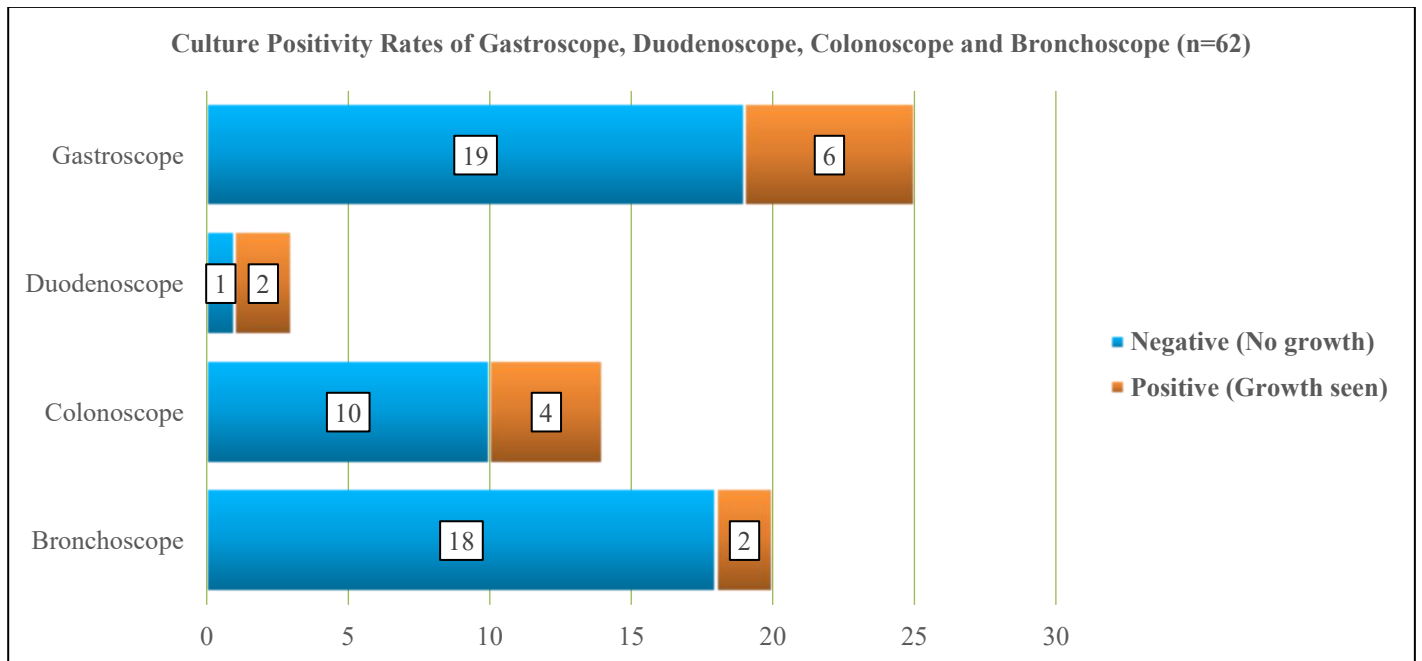


Figure-II: Culture positivity rate of endoscopes.

DISCUSSION

Many professional associations have published guidelines and standards for reprocessing of endoscopes since 2020. Cori et al precisely summarized the joint recommendations by most notable societies providing guidance in Infection Control in the table below.⁹

American National Standards Institute and the Association for the Advancement of Medical Instrumentation (ANSI/AAMI) in ST91 2021 Flexible and Semi-rigid Endoscope Processing in Health Care Facilities,¹⁰ the 2021 Multi society Guideline on Reprocessing Flexible GI Endoscopes and Accessories,¹¹ the Association for peri Operative Registered Nurses (AORN) 2023 Guidelines for Perioperative Practice Guideline for Processing Flexible Endoscopes,² and the Society of Gastroenterology Nurses and Associates (SGNA) 2023 Standards of Infection Prevention in Reprocessing Flexible Gastrointestinal Endoscopes¹²

Despite the emphasis on the aforementioned steps, there have been lapses reported in the endoscope decontamination worldwide. Hereby in our study we also found lack of compliance in practices when compared to standards. Our study revealed substandard practices - with respect to lack of clean & dirty workplaces, improperly constructed sinks & storage cabinets, ill ventilated suite, failure to use High-level disinfectant, lack of RO water & its thermoregulation and unavailability of MEC sticks to check disinfectant concentration, all translating into threats for microbial contamination of endoscopes and cross-contamination. In an audit of endoscopy practices, Kurtz et al surveyed 16 clinics in Florida to trace High-level disinfection; unidirectional workflow wasn't seen for clean & contaminated equipment. Most didn't follow point of use cleaning. Appropriate storage cabinet was observed only in one unit. Lack of compliance was seen mostly with manual reprocessing when compared to AER (67% vs 29% respectively).¹³ Similarly, in 2019, Tuvo conducted an audit of Bronchoscopy Unit at a large tertiary care hospital in Italy and found that precleaning wasn't performed, reusable brushes were used instead of disposable ones during manual cleaning, drying was inappropriate and so was storage of bronchoscopes after HLD. After improved practices and adoption of recommendations, improved results via conformity rate, highlighted role of adherence to guidelines to prevent cross infection.¹⁴

Additionally, the endoscopic contamination rates range from 6% to 72% after HLD in most of the studies conducted in real time. Okamoto et al conducted a prospective study across 16 centers in USA, assessed 859 duodenoscopes and detected an overall High concern organisms HCO contamination rate of 5.3% in non-outbreak settings. High-concern (HC) organisms were those highly associated with disease, including gram-negative rods, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, β -hemolytic *Streptococcus*, *Enterococcus* spp, and yeasts.⁶ Likewise, a systematic review and meta-analysis conducted by Sara et al included 20 studies conducted across USA, Europe & Asia including 1,059 positive cultures from 7,903 samples taken from all kinds of gastrointestinal endoscopes including echoendoscopes, gastroscopes, and colonoscopes. The total contamination rate detected was 19.98%±0.024.¹⁵ Similarly, a study in Iran showed contamination in distal tip of duodenoscopes which was comparable to bile samples of patients with hepatobiliary diseases. *Pseudomonas aeruginosa* was detected in 38.2% of the elevator channels' and 26.6% of the bile samples raising serious concerns for suboptimal disinfection of endoscopes.¹⁶ Tuvo et al described contamination in (59%) of bronchoscopes: 18% as HCO and 36.4% as high microbial count (≥ 100 CFU/all channels) and HCO in a large Bronchoscopy unit in Italy.¹⁴ Realizing endoscopic decontamination as a real challenge, FDA changed recommendation historically in 2015 for semi-critical instruments to undergo enhanced methods for reprocessing, including additional cycles of HLD, use of ethylene oxide or liquid-chemical germicide for sterilization. To check its effectiveness, Mark et al conducted a study in 2020 and detected microbes proportionately similar in samples even after having undergone additional cycles of HLD.¹⁷

Translating into disease, there have been infectious outbreaks reported in the past due to residual endoscopic decontamination after HLD, as described above. A Michigan facility reported 9 cases of phylogenetically similar Metallo-beta-lactamase producing *E Coli* using Whole Genomic Sequencing analysis with less than 26 single nucleotide polymorphism (SNP) differences detected between isolates. Epidemiologic tracing suggested (89%) patients had a duodenoscope and/or gastroscopes. Cultures harbored many microorganisms but *E coli* was not found. The outbreak ended when the

culprit endoscopes were removed from service.¹⁸ Similarly, in an acute care hospital at New Jersey, over a twelve-month period in 2024, nine patients were identified with *NDM-KP* infection or colonization found to be similar on WGS, seven of them had gastrointestinal endoscopic procedures. Although none of the endoscopic cultures grew the microbe, the epidemiological tracing and genomic relatedness made the endoscopic mode of transmission most likely highlighting the importance of processing quality.¹⁹ Similarly in our study we found significant culture positivity and the number should be taken into account with caution. We believe this is an underestimate as being resource limited we didn't have the privilege to take all necessary procedures to improve microbial yield. We couldn't use CDC recommended flush-brush-flush techniques using friction to help disrupt biofilm to increase microbial recovery during sampling. Also, we couldn't use the CDC recommended eluent buffered solution during sampling instead of sterile water to neutralize effect of residual high-level disinfectant so as to effectively harbor microorganisms from internal channels. Being resource limited we didn't use concentrating samples using filtration nor did we incubate samples for ≥ 72 hours for fastidious organisms.²⁰ Additionally, the timing of culture collection after reprocessing was another limitation. It is recommended by Italian Multi society position paper to sample endoscopes after a minimum of 6 to 12 h after storage so as the bacterial yield is increased due to biofilm formation. To the contrary, in our study around one-third of cultures were taken within 4-6 hrs of disinfection due to fixed work hours of personnel underestimating the total culture positivity we had.²¹ It is important to recognize that culture-based methods may underestimate contamination due to presence of viable but noncultureable organisms and biofilm associated microbes. There is high likelihood to underrate processing failure due to sole reliability on culture positivity as gold standard. Michelle et al described using a simulated-use buildup biofilm (BBF) experimental model that cross-infections may be caused by conditionally viable organisms.²² Conversely, misdiagnoses and pseudo-outbreaks may happen due to ineffective handling also. Jin et al found that the rate of detection of *Tuberculosis* (TB) nucleic acid residue on reusable bronchoscopes cleaned via standard processing techniques was statistically higher than those more

intensively processed whereas all TB cultures were negative causing false positives in Xpert MTB/RIF, thus implying unnecessary use of antimicrobials.²³ Keeping in view the aforementioned, a robust response was initiated by the Infection Control Practitioners. The results were discussed in the quarterly Infection Control Committee meeting with the hospital administration, and considerations made regarding rectification of gaps. An appropriately bioengineered Endoscopy suite having recommended air exchanges, unidirectional workflow for clean and dirty equipment, RO water supply with thermoregulation, appropriately sized & structured sinks & moisture-free storage cabinets was proposed. Immediately 0.55% ortho-phthalaldehyde by the trade name of CIDEX was arranged for use as High level disinfectant as recommended by CDC and test strips were ordered to check its concentration before use every day with maintenance of a diary for the former. Air guns to ensure drying before storage were also ordered. A formal training of personnel working in the suite for reprocessing of endoscopes was conducted on the essential steps of the process with emphasis on utility of leak testing, use of sponges and thorough washing with water after enzymatic cleaning. A protocol for 6-monthly microbiological surveillance of endoscopes was made so as after corrective measures are assimilated fundamentally, the effect can be seen translated as reduction of culture positivity of endoscopes post HLD.

CONCLUSION

We conclude that we identified gaps in the disinfection of endoscope reprocessing and deviation from standards in our study which translated into significant microbial contamination of endoscopes after high level disinfection. Given the evidence that current practices are suboptimal and may lead to cross infection, meticulous multidisciplinary efforts were made immediately after communicating with hospital administration to improve the infrastructure and rectify human errors so as to upscale the quality of processing and patient safety. This is an important aspect of Hospital's Infection Control & Prevention and needs to be addressed with active efforts through standardized IC practices. Using microbiological surveillance protocol, a subsequent study may be conducted to quantify effects of the strategic improvements in terms of reduction in culture positivity of reprocessed endoscopes.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Hira Nadeem: Conceptualized, conducted the study, did analysis and wrote manuscript, final approval, accountable for every aspects of research

Samreen Sarfaraz: Devised the project, the main conceptual ideas and proof outline, final approval, accountable for every aspects of research

Ayesha Akram: Headed and carried out collection and data analysis, final approval, accountable for every aspects of research

Bakhat Ali: Carried out sample collection, helped in formulating the collection protocol, final approval, accountable for every aspects of research

Muhammad Umar: Proof reading and helped in discussion. final approval, accountable for every aspects of research

Muhammad Bilal: Carried out statistical analysis, final approval, accountable for every aspects of research

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