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Effectiveness of Cleaning and Disinfection Processes on the Bioburden on Endoscopes

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Abstract:
Background & Objectives: *Awareness about infections transmitted through endoscopes is increasing. Every incident is due to a breach in cleaning and disinfection protocol. No study in India evaluated the bio burden on endoscopes and the effectiveness of cleaning and disinfection of endoscopes.*
Methods: *A cross-sectional study, 30 endoscopes (20 gastroscopes and 10 colonoscopes) were conveniently sampled at the department of gastroenterology in a tertiary care center in Coimbatore. Sterile water flushed through the suction biopsy channel - after use, post water cleaning, post manual cleaning and post disinfection - were collected for microbiological analysis.*
Results: *A mean bioburden of 3.5 x 10^4 CFU/ml, upto 4.2 x 10^5 CFU/ml was obtained from endoscopes after use with a culture positivity of 97% (29/30). After manual cleaning the bioburden on gastroscopes reduced by 2 logs and on colonoscopes by 1 log. Post disinfection, 7% (2/30) endoscopes were contaminated. Both were colonoscopes and had colony counts of 1.8 x 10^3 and 2.8 x 10^2 CFU/ml. Immediately after use, gastroscopes grew gram-negative organisms like E.coli, Klebsiella, Enterobacter and Pseudomonas. Microbial burden in 4 endoscopes after water cleaning was greater than after patient use. Two of them grew different flora in after-use and post-water-clean samples. Average time taken for manual cleaning was 3.3 minutes.*
Interpretations & Conclusions: *The reasons for inadequate cleaning and disinfection were contaminated rinse water and inadequate manual cleaning. Adherence to standard reprocessing protocols is required to avoid transmission of infection through contaminated endoscopes.*
Keywords: *Bio burden, cleaning, disinfection, endoscope reprocessing.*
**CFU/ml – Colony Forming Units / ml*

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I. Introduction

Endoscopes have revolutionized health care by providing minimally invasive techniques for the diagnosis, treatment and prevention of many gastrointestinal (GI) diseases. From rigid endoscopes, they have evolved to flexible endoscopes, which are sophisticated, complex and reusable with narrow, long internal lumens, passing through areas of the body, which are not sterile; hence posing a challenge to its cleaning and disinfection[1]. Endoscopes are increasingly used on immunocompromised patients who are at a greater risk of infection transmission and also have high bacterial load, which leads to higher contamination of endoscopes.

Endoscope is classified as a semi-critical device, as it comes in contact with mucous membranes and does not ordinarily penetrate sterile tissue. Hence, it requires high-level disinfection (HLD)[2], which kills all vegetative bacteria, mycobacteria, fungi, and viruses, except for small numbers of bacterial spores. (According to Dr. E. H. Spaulding who classified medical devices into critical, semi-critical and non-critical based on the risk of infection involved with their use). HLD is a multi-step process, beginning with manual cleaning, followed by disinfection, rinsing and drying[3].

GI endoscopes, after clinical use, have bio burden levels ranging from 10^5 to 10^{10} CFU/ml[4,5,6]. Manual cleaning removes organic debris and reduces bio burden levels by 4-6 logs (99.99%)[5,6]. Lack of cleaning or failure during the cleaning process could lead to the survival of pathogens after disinfection, increasing the risk of cross-contamination between patients. In addition, bacteria that remain after insufficient reprocessing may form a biofilm inside the instruments[7]. In order to ensure complete endoscope cleaning and disinfection, federal agencies such as FDA and CDC and professional organisations such as American Society for Gastrointestinal Endoscopy, American College of Gastroenterology have recommended standards for each element of endoscope reprocessing[8].

Inspite of these guidelines, there had been occurences of pathogen transmission related to GI endoscopy. The pathogens involved ranged from Mycobacterium tuberculosis, Hepatitis B virus, Hepatitis C virus to Pseudomonas aeruginosa, Salmonella, Escherichia coli, Klebsiella and Enterobacter[7,9,10,11]. All

published occurences of pathogen transmission had been associated with failure to follow established cleaning and disinfection/sterilization guidelines or use of defective equipment[7,11,12,13].

Many studies evaluated the effectiveness of cleaning and disinfection of endoscopes by various methods like bio burden, ATP, protein and carbohydrate estimation[4,5,6,13,14,15,16,17]. In India, two studies, in 1990 and 2000, by means of a postal survey, sent a questionnaire to selected GI endoscopy centers and evaluated the disinfection practices in India. They found that the disinfection practices are inadequate[18,19]. However, to date, no published clinical studies are available in India, which evaluated the standard of cleaning and disinfection and its effectiveness by bio burden estimation or other methods.

The growing concern on the risk of transmission of infection by endoscopes and the non-availability of data regarding the effectiveness of endoscope disinfection in India had led us to evaluate the bio burden on endoscopes and the effectiveness of cleaning and disinfection of endoscopes using 0.55% ortho-phthalaldehyde (OPA) in a tertiary care center in Coimbatore.

II. Materials and Methods

Our study got ethical approval from the Institutional Human Ethics Committee. A cross-sectional study, it was conducted between October to November 2011, at the department of gastroenterology at a tertiary care center in Coimbatore, performing 450 endoscopies per month.

30 patients undergoing endoscopy at the department of gastroenterology were included by convenient sampling after obtaining informed consent. 20 gastroscopes and 10 colonoscopes were sampled. Patient' s demographics, type of scope (gastroscope or colonoscope), time of sample collection, presenting complaints, past history including any immunocompromised state and other relevant history were noted.

- **Steps of endoscope reprocessing followed in the department of gastroenterology**

It was a 3-step process consisting of

1. Water cleaning: Immediately after patient use, tap water and air was aspirated into the endoscope through the suction channel.
2. Manual cleaning: Detergent (savlon) was aspirated into the endoscope. The exterior of the scope was wiped to remove all the debris. All accessible interior channels were brushed. The endoscope was then tested for leaks.
3. Disinfection: The endoscope was immersed in 0.55% OPA for 10 minutes. Tap water was then flushed into the scope, followed by alcohol rinse and forced-air drying.

- **Sample collection**

3 samples were collected from each endoscope:

1. Immediately after use (n = 30)
2. Post water cleaning (n = 13); Post manual cleaning (n = 17)
3. Post disinfection (n = 30)

This study was conducted in a busy gastroenterology department. Due to time constraints, all endoscopes could not be sampled for post water and post manual cleaning. Hence, 13 were sampled post water cleaning and the rest 17 were sampled post manual cleaning.

- **Sample collection method**

50ml of sterile saline was flushed through the suction valve hole with a sterile syringe. Suction channel was thoroughly rinsed with saline and the flushed material was collected at the distal end in a sterile screw-capped container (we used urine collection container)(Figure 1). Collected material was taken to the microbiology laboratory. Suction channel was sampled since it is exposed to the highest bioburden, the most difficult to clean and disinfect, and represent the greatest risk to patient safety. If results indicate that these locations were effectively reprocessed, this provides some assurance that the entire endoscope was effectively reprocessed[20]. Sterile saline is the recommended test solution[21]. All samples were collected under aseptic conditions to avoid environmental contamination.

Figure 1: Sample collection method: 50 ml sterile saline was flushed through the suction valve hole with a sterile syringe. After thorough rinsing of suction tube, flushed material was collected at the distal end in a sterile screw-capped container.

- **Microbiological analysis**

▪ Sample culture

After appropriate dilution, samples were cultured for aerobic bacteria. 125µl of the diluted sample (25 drops, each containing 5µl) was placed in 2 blood agar and 2 mac conkey agar plates. Each blood agar and mac conkey agar plate was incubated at 37°C and 25°C for 24 hours.

▪ Bio burden estimation

After 24 hours of incubation, if growth was seen, the colonies grown on the blood agar plates were counted and converted to Colony Forming Units (CFU)/ml. Since each sample was incubated at 37°C and 25°C, there were 2 values of CFU/ml for each sample. Hence, the sample that grew the greater number of colonies was taken for analysis.

▪ Organism identification

Organisms were identified by Gram' s stain and standard biochemical tests.

- Data analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) 17.0. Chi square test was used to test statistical significance. P value of <0.05 was considered statistically significant.

III. Results

30 endoscopes (20 gastroscopes and 10 colonoscopes) were sampled after use, post water cleaning, post manual cleaning and post disinfection. Males (17) and females (13) were equally distributed (p=0.465). The mean and median age of patients was 44 years (range 11-75 years). The common complaints of the patients were abdominal pain (37%), diarrhoea (20%), vomiting, melaena (10% each), constipation, heartburn and peptic ulcer (7% each). 7% (2/30) had an immunocompromised state - kidney transplantation and anal canal carcinoma.

A summary of the microbial growth after patient use, post water cleaning and post manual cleaning is given in Table I and the organisms grown in gastroscopes and colonoscopes is given in Table II.

Table I: Summary of microbial growth after use, post water cleaning and post manual cleaning

	% Positive growth (number)	Mean (CFU/ml)	Range (CFU/ml)
After use			
Total	97 (29/30)	$3.5x10^4$	$8.0x10^1$-$4.2x10^5$
Gastroscopes	95 (19/20)	$2.7x10^4$	$8.0x10^1$-$4.2x10^5$
Colonoscopes	100 (10/10)	$5.1x10^4$	$3.2x10^3$-$1.2x10^5$
Post water cleaning			
Total	70 (9/13)	$1.6x10^3$	$2.6x10^2$-$8.2x10^3$
Gastroscopes	71 (5/7)	$6.9x10^2$	$2.6x10^2$-$2.3x10^3$
Colonoscopes	67 (4/6)	$2.8x10^3$	$2.0x10^3$-$8.2x10^3$
Post manual cleaning			
Total	35 (6/17)	$4.4x10^2$	$4.2x10^1$-$2.5x10^3$
Gastroscopes	23 (3/13)	$2.2x10^2$	$4.2x10^1$-$2.5x10^3$
Colonoscopes	75 (3/4)	$1.1x10^3$	$1.4x10^2$-$2.4x10^3$

Table II: Summary of organisms grown after use, post water cleaning and post manual cleaning

	After use		Post water cleaning		Post manual cleaning	
	G* (n=19)	C* (n=10)	G (n=5)	C (n=4)	G (n=3)	C (n=3)
Staphylococcus	26(5)#	-	-	-	33(1)	-
Streptococcus	58(11)	-	40(2)	-	33(1)	-
E.coli	11(2)	100(10)	40(2)	-	-	67(2)
Klebsiella	26(5)	70(7)	40(2)	75(3)	-	100(3)
Enterobacter	16(3)	60(6)	20(1)	25(1)	-	33(1)
Enterococcus	-	60(6)	-	50(2)	-	33(1)
Pseudomonas	11(2)	30(3)	-	50(2)	33(1)	-
Micrococci	21(4)	-	-	-	-	-
Acinetobacter	-	10(1)	-	-	-	-
Branhamella	5(1)	-	-	-	-	-
Neisseria	5(1)	-	-	-	-	-
Unidentified	-	-	20(1)	-	-	-

*G=Gastroscope, C=Colonoscope; #Percentage of samples that grew the particular organism out of n samples (number of samples that grew the organism out of n samples);

Immediately after patient use:

97% (29/30) of the endoscopes were contaminated. One gastroscope had no growth. Mean bioburden of colonoscopes 5.1 x 10^4 CFU/ml was found to be greater than gastroscopes 2.7 x 10^4 CFU/ml (Table I). Gastroscopes were contaminated with gram-positive organisms and gram-negative organisms such as E.coli, Klebsiella, Pseudomonas, Branhamella and Neisseria. But Colonoscopes grew only gram-negative organisms. E.coli was isolated from all the 10 colonscopes. (Table II).

Post water cleaning:

Percentage of contamination of gastroscopes and colonoscopes were equal (70% and 71% respectively). Total mean microbial growth was 1.6 x 10^3 CFU/ml. However, average growth from colonoscopes (2.8 x 10^3 CFU/ml) was greater than gastroscopes (6.9 x 10^2 CFU/ml) (Table I). Again, Gastroscopes were contaminated with both gram positive and gram-negative organisms but colonoscopes grew only gram-negative organisms (Table II).

Post manual cleaning:

23% (3/13) of the gastroscopes and 75% (3/4) of the colonoscopes were contaminated. Manual cleaning had reduced the mean bioburden on gastroscopes by 2 logs (2.2 x 10^2 from 2.7 x 10^4 CFU/ml) . However, the mean bioburden on colonoscopes reduced by only 1 log (1.1 x 10^3 from 5.1 x 10^4 CFU/ml). (Table I).

The total mean bioburden reduced by 1 log after each step of reprocessing (from 3.5 x 10^4 to 1.6 x 10^3 to 4.4 x 10^2 CFU/ml) (Table I). Statistical significance of the reduction in bio burden was not calculated since endoscope cleaning and disinfection involves meeting the standard of reduction in bio burden rather than a significant reduction in bioburden.

Post disinfection:

After disinfection 7% (2/30) endoscopes were contaminated. Both were colonoscopes. One colonoscope was contaminated with 2.0 x 10^4 CFU/ml immediately after use, reducing to 2.4 x 10^3 CFU/ml post water cleaning and a small reduction to 1.8 x 10^3 CFU/ml post disinfection. After use sample grew E.coli and Acinetobacter whereas both post water cleaning and post disinfection samples grew Klebsiella and Enterococcus. The other sample had a microbial contamination of 1.2 x 10^5 CFU/ml after use, no growth post

water cleaning and 2.8 x 10^2 CFU/ml post disinfection. Organisms grown after use and after disinfection were different except Enterobacter (Table III).

Table III: Summary of bioburden and organisms grown in samples which were contaminated post disinfection

	Sample 23		Sample 30	
	Bio burden (CFU/ml)	Organisms grown	Bio burden (CFU/ml)	Organisms grown
After use	$2.0x10^4$	E.coli, Acinetobacter	$1.2x10^5$	E.coli, Enterobacter, Enterococcus, Pseudomonas
Post water cleaning	$2.4x10^3$	Klebsiella, Enterococcus	-	-
Post disinfection	$1.8x10^3$	Klebsiella, Enterococcus	$2.8x10^2$	Klebsiella, Enterobacter

The microbial growth in 4 samples post water cleaning was found to be greater than their respective after use samples. 1 out of the 4 samples had no growth after use but had growth after cleaning. 2 samples grew gram-positive organisms after use whereas gram-negative organisms grew after water cleaning. All samples were from gastroscopes and were collected on the same day (Table IV).

Table IV: Samples with post water cleaning CFU greater than after use CFU and the organisms grown in them

Sample number	Bio burden (CFU/ml)		Organisms grown	
	After use	Post water cleaning	After use	Post water cleaning
15	$4.0x10^2$	$8x10^2$	Micrococci	E.Coli++, Klebsiella+, Streptococcus
16	$1.6x10^3$	$2.3x10^3$	Streptococcus	E.coli, Klebsiella
18	-	$2.6x10^2$	No growth	Unidentified
20	$2.4x10^2$	$8x10^2$	Enterobacter	Enterobacter

The average time taken for manual cleaning of endoscopes was 3.3 minutes. It was completed in 2, 3 and 5 minutes in 53%, 7% and 40% of endoscopes respectively. All the endoscopes were disinfected in the OPA solution for a minimum of 10 minutes, which is adequate.

IV. Discussion

Factors that affect the efficacy of disinfection are:[3,22]

1. Level of microbial contamination
2. Prior cleaning of the object
3. Disinfection
4. Water quality
5. Drying and storage
6. Physical nature of the object (e.g., crevices, hinges, and lumens).

1. Level of microbial contamination:

In our study, the average bioburden in gastroscopes was found to be 2.7 x 10^4 CFU/ml and 5.1 x 10^4 CFU/ml in colonoscopes. This value is less than previous studies where average colony counts of 10^5 to 10^{10} were noted[4,5,6]. This indicates a lesser microbial contamination level of the endoscopes.

2. Prior cleaning of the object:

Appropriate manual cleaning can reduce the bio burden levels by 4 logs (10^4 times)[5,6]. A minimum of 19 minutes is required for optimal cleaning of endoscopes, which will reduce the bio burden 10^4 times[23]. The time taken for manual cleaning in India was estimated to be 0.8 minutes which is 4% of the optimal cleaning efforts[24]. In our study, it was 3.3 minutes which is 17% of the optimal cleaning efforts. However, only 10^1 to 10^2 times reduction in bioburden was noted. Even though all the steps of manual cleaning were followed, only 2 logs reduction in bioburden may be due to the less time taken for manual cleaning.

3. Disinfection:

Endoscopes have to be placed in the OPA solution for minimum of 5 minutes at 25°C to achieve HLD[3]. In our study, all the endoscopes were placed in OPA for a minimum of 10 minutes, which is adequate.

The acceptable microbial count after disinfection is <20 CFU/ml[21]. However, since HLD involves killing of all microorganisms except bacterial spores, no growth should be present post disinfection[2]. In our study, 2/10 of colonoscopes were contaminated with $1.8x10^3$ and $2.8x10^2$ CFU/ml. This is beyond acceptable limits indicating

inadequate reprocessing inspite of adequate exposure to the disinfectant. In addition, both post water cleaning and post disinfection samples were contaminated with Klebsiella and Enterococcus in sample 23(Table III). Growth of Enterobacteriaceae means insufficient cleaning and/or disinfection procedures[21].

4. Water quality:

We noted absence of microbial growth post cleaning in sample 30 as shown in table III. However, the post disinfection sample was contaminated with 2.8×10^2 CFU/ml indicating contamination in final rinse water. Microbial growth from gastroscopes immediately after patient use should be the normal flora of the oropharynx. However, we found growth of gram-negative organisms like E.coli, Klebsiella, and Pseudomonas in gastroscopes after patient use. This indicates that the endoscope was contaminated prior to patient use. The contamination is probably from the final rinse water, which was flushed into the endoscope after disinfection. Any contamination of the rinse water will inevitably lead to contamination of the endoscope regardless of the potency, strength, or effectiveness of the preceding cleaning process or of the disinfectant, automated endoscope reprocessor or automated processing system. Use of sterile or bacteria-free water is recommended to minimize the risk of infection transmission[22].

Adequate cleaning of endoscopes leads to reduction in bioburden with every step of reprocessing. However, in 4 of the samples, ' post-water cleaning-colony-count' was greater than ' after-use-colony-count' . This indicates that the water used for cleaning was contaminated. This is further substantiated by the following findings:

- Growth of different flora in after use and post water cleaning samples
- Absence of microbial growth after use but presence of growth after water cleaning.

5. Drying and storage:

Drying the endoscopes prior to reuse or storage is a step frequently overlooked[22]. In our study, post processing, every endoscope was dried by flushing 70% alcohol and forced air and it was hung vertically for storage. The importance of drying cannot be overemphasized where a serious outbreak of Pseudomonas was found to be due to inadequate drying. No additional infections were reported following drying with 70% alcohol and forced air[25].

6. Physical nature of the object:

Martin Favero, director of scientific and clinical affairs for Advanced Sterilization Products, referred to the endoscope as "the device from hell" when discussing the difficulties in effectively cleaning and disinfecting endoscopes[26].

Endoscopes have a complex physical arrangement of various channels and valve systems[1]. Most gastrointestinal endoscopes have three major channels: air, water, and biopsy-suction channel and valve housings such as biopsy, air and water port. The air and water channels, which are approximately 1.0 to 1.2 mm in internal diameter, cannot be cleaned by physical means and require flushing with liquids or air. Within the endoscope, lumens, crevices, joints, pores, and loosely mated or occluded surfaces provide areas that may collect patient material[10]. Hence the emphasis on meticulous manual cleaning of all channels, valves, connectors and all detachable parts.

Reasons for inadequate disinfection:

The major reason for inadequate cleaning and disinfection was found to be contaminated water. Water used for rinsing after cleaning and also after disinfection, were both contaminated. Discarding rinse water after each use greatly minimizes the risk of contamination. Also, hygienic surroundings, separate room for reprocessing are other recommendations. Also, time taken for manual cleaning was inadequate. More time needs to be spent for brushing the internal channels, which carry the highest microbial burden and pose the greatest challenge to disinfection.

When all recommended reprocessing standards are followed, the risk of disease transmission from an endoscope is virtually non-existent. All reported cases have been associated with a breach of these protocols or defective equipment[27]. Brief steps of endoscope cleaning and disinfection after leak testing are as follows[3,18]:

- Step 1: Manual cleaning: Clean all debris. Immerse endoscope completely in detergent, clean and brush all accessible surfaces and channels followed by rinse with water. Rinsing solution should not be reused.
- Step 2: Disinfection: Immerse endoscope completely in disinfectant and fill all channels with disinfectant. (Time: 10 min for OPA, 20 min for 2% gluteraldehyde).
- Step 3: Rinsing: Use atleast " drinking water quality" water. Discard water after each use.
- Step 4: Drying: Rinse with alcohol and forced air.
- Step 5: Store: Hang the endoscope vertically.

The manual component of reprocessing appears most prone to error[28]. Periodic surveillance may potentially help reduce such errors by reinforcing adherence to the many steps in reprocessing. The ESGE-ESGENA (European Society for Gastrointestinal Endoscopy - European Society of Gastroenterology and Endoscopy Nurses and Associates committee) recommends surveillance cultures of reprocessed endoscopes at intervals of not more than 3 months[21]. The maximal total microbiological count should be less than 20 CFU/ml for fluid collected after flushing the endoscope channels with 20 mL of sterile saline solution with placing of 1 mL of the fluid on each agar plate[21].

This study was conducted in a single center with a small sample size. We recommend multi center studies with larger sample size. Other parameters such as protein, carbohydrate and ATP levels need to be assessed.

V. Conclusion

Endoscope cleaning and disinfection practices are highly suboptimal. It is high time that we take necessary amendments to ensure standarised protocols, regular microbiological surveillance and most importantly patient safety. Let us all make sure this boon does not become a curse because of inadequate disinfection.

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