Alexandria Journal of Medicine (2015) **51**, 95–103

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Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

ORIGINAL ARTICLE

Estimation of average bioburden values on flexible gastrointestinal endoscopes after clinical use and cleaning: Assessment of the efficiency of cleaning processes

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Received 21 November 2013; accepted 7 May 2014
Available online 21 June 2014

KEYWORDS

Gastrointestinal endoscopy;
Bioburden;
Cleaning;
High level disinfection

Abstract *Background:* Endoscopy is a vital part of medical diagnostic processes. There are different kinds of flexible endoscopes used in medicine. They differ between manufacturers and even between models from the same manufacturer. However, all flexible endoscopes have the same basic components. Infections related to flexible endoscopic procedures are caused by either endogenous flora or exogenous microbes. The first major challenge of reprocessing is infection control, most episodes of infection can be traced to procedural errors in cleaning and disinfecting, the second major challenge is to protect personnel and patients from the exposure to liquid biocides used for disinfection. Because the endoscopic accessories have complex nature, attention and adherence to a validated protocol is critical for reprocessing endoscopic accessories. Bioburden is defined as the number of bacteria living on a surface that has not been sterilized. The term is most often used in the context of bioburden testing, also known as microbial limit testing, which is performed on pharmaceutical products and medical products for quality control purposes. Flexible endoscopes,

Abbreviations: cfu, colony forming units; MRI, Medical Research Institute; HLD, high level disinfection

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Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2014.05.002

by virtue of the types of body cavities they enter, acquire high levels of microbial contamination (bioburden) during each use.

Aim of the work: To detect the average bioburden values on different parts of flexible gastrointestinal endoscopes after clinical use and cleaning in order to assess the efficiency of different cleaning processes used in the endoscopy unit.

Methods: The current study included a total of 120 endoscopes randomly selected from Medical Research Institute (MRI) hospital 60 (50%) of which were from Surgical Department endoscopy unit, and 60 (50%) of which were from Internal Medicine Department endoscopy unit. The endoscopes were divided as (40) endoscopes after use (40) endoscopes after manual cleaning, and (40) endoscopes after high level disinfection. All samples were cultured for aerobic and anaerobic bacteria, and for Candida species, the number of colonies were determined as colony forming units (cfu)/ml.

Results: Microorganisms isolated immediately after use were Staphylococcus, Streptococcus, Klebsiella, *Escherichia coli*, and Bacteroides, whereas after manual cleaning the isolated strains were Staphylococcus, Streptococcus, Pseudomonas, Klebsiella, Bacteroides, and *E. coli*. The average Bioburden on endoscopy before cleaning ranged from 6×10^4 to 3.7×10^8 cfu per device (mean cfu per device 1.4×10^7), whereas after manual cleaning ranged from 2.1×10^2 to 3.5×10^3 cfu per device (mean cfu per device 4.9×10^2) and no colonies were found after sterilization. Manual cleaning resulted in a mean of $4.46 \log_{10}$ reduction in viable colony count and high level disinfection (HLD) resulted in a reduction of CFU to zero.

Conclusions: HLD is superior to manual cleaning in the process of endoscopic disinfection.

Recommendations: Microbiological screening should be undertaken for all the Endoscopy Unit personnel responsible for cleaning or if there is a clinical suspicion of cross-infection related to endoscopy. All health-care personnel in an endoscopy unit in standard infection control should be trained to reprocess endoscopes. Safe working practices in the decontamination area of each unit should be written down and understood by all staff.

1. Introduction

Endoscopes are used frequently for the diagnosis and therapy of medical disorders. Historically, the field of endoscopy has progressed from rigid endoscopy[1], flexible endoscopy invented by Kelling in 1898[1], photoendoscopy[2], video endoscopy with the first reported case done in 1956[3], to spectral endoscopy using various illumination techniques that has enhanced the visibility of features that cannot be distinguished under white light.[4–6] Recently, capsule endoscopy has been introduced for use in 2001.[7,8]

There are different kinds of flexible endoscopes used in medicine. They differ between manufacturers and even between models from the same manufacturer. However, all flexible endoscopes have two basic components[9] namely the external & internal. The external components include a light guide plug[10], an umbilical cable (cord),[11]a control section[10], and an insertion tube.[12] while the internal components are more complex & include the angulation system with its four parts (control mechanism[13], coil pipes[12], bending section[14], and suction/biopsy channel[15]), the air and water system[16,17], the image system with its four parts (lighting[18], non-video imaging[19], video imaging[20], & lens systems[21]) & finally the electrical system[22] including automatic brightness system[23] & switches.[24]

Manual cleaning is the initial key step for achieving HLD of endoscopic equipment. Cleaning dramatically reduces the bioburden on endoscopes. Several investigators have shown a mean log[10] reduction factor of 4 (99.99%) in the microbial contaminants with cleaning alone. Cleaning should be done promptly following each use of an endoscope to prevent drying of secretions, allow removal of organic material, and decrease the number of microbial pathogens.[17]

Failure to employ appropriate cleaning and disinfection/sterilization of endoscopes has been responsible for multiple nosocomial outbreaks and serious, sometimes life-threatening infections.[17]

Infection-control issues during gastrointestinal endoscopy, which are becoming increasingly important, can generally be divided into three major areas: (1) infectious complications resulting from patient's own microbial flora (autologous), (2) infections transmitted from patient to patient by way of endoscope (exogenous), and (3) infections transmitted between the patient and the health-care provider. The mean frequency of post-procedure bacteremia ranges from 0.5% for flexible sigmoidoscopy to 2.2% for colonoscopy, 4.2% for esophagogastroduodenoscopy, 8.9% for variceal ligation, 11% for endoscopic retrograde cholangiopancreatography, 15.4% for variceal sclerotherapy, and 22.8% for esophageal dilation.[25]

Although post-procedure bacteremia is not uncommon, it seldom results in infectious complications. Exogenous infections transmitted during endoscopy, which are extremely rare, generally result from failure to follow accepted guidelines for the cleaning and disinfection of gastrointestinal endoscopes, underscoring the importance of meticulous attention to endoscope reprocessing. Finally, although the risk of patient-staff transmission of infection is also rare, standard infection-control recommendations are important in protecting both patients and health-care providers.[25]

Gastrointestinal procedures have been associated with a wide range of infectious complications, including bacterial endocarditis. Although the rate of bacteremia from patient's own flora is quite high after some procedures, only a few cases of endocarditis caused by gastrointestinal instrumentation have been reported. Because of the severity of the illness, however, antibiotic prophylaxis has been recommended for patients who are categorized as high risk for some procedures. Bacteremia and other infections, such as colitis, may also originate from a contaminated endoscope.[26]

The aim of the present work was to detect the average bioburden values in different parts of flexible gastrointestinal endoscopes after clinical use and cleaning in order to assess the efficacy of different cleaning and disinfection processes used in endoscopy units.

2. Material and methods

A total of 120 endoscopes were randomly selected from two endoscopy units in Medical Research Institute, Alexandria University (Surgical Dep. endoscopy unit, with about 320 procedures/month and Internal Medicine Dep. with about 170 procedures/month).

The endoscopes were divided as follows:

- 40 endoscopes immediately after use.
- 40 endoscopes after manual cleaning, and
- 40 endoscopes after high level disinfection.

2.2. Technique of disinfection in both units

Rigorous mechanical cleaning to remove organic material from the outside and all accessible channels is done before disinfection.[27]The endoscope is then immersed in alkaline 2% glutaraldehyde preparations (e.g., Cidex, Advanced Sterilization Products, Irvine CA)[28] for 20-min at room temperature. All debris from the exterior of the endoscope is washed by brushing and wiping the instrument while submerged in the detergent solution. The endoscope is left in the detergent solution when performing all subsequent cleaning steps.[29] A small soft brush is used to clean all removable parts, including inside and under the suction valve, air/water valve, and biopsy port and openings.[30]All accessible endoscope channels are brushed, including the body, insertion tube, and the umbilical cable of the endoscope.[31] The channels are flushed with the detergent solution to remove debris.

2.3. Bioburden recovery

2.3.1. Materials

1. Sterile syringes; one for each channel to be sampled (20 cc for channels).
2. Sterile container for collecting the sample.
3. Sterile lint-free cloth wetted with 0.1% peptone water with polysorbate 80(U.S.P);Difco,Detroit,Mich).
4. Phosphate buffered saline solution as eluent.
5. Personal protective equipment (gloves, gowns, face shields), were used during sample collection.

2.3.2. Method

1. 10 mls of sterile Phosphate buffered saline (PBS) were aspirated into a sterile 20 cc syringe.
2. The syringe was attached via a piece of sterile tubing to the suction/biopsy barb of the umbilical end and 10 ml of sterile (PBS) was flushed through the channel.
3. The channel sample was collected from the distal end of the endoscope by holding the end of the insertion tube in a sterile plastic container (urine specimen container was used).
4. A syringe of air was used to flush out any residual fluid sample from the channel.
5. Two Sterile lint-free cloth wetted with 0.1% peptone water with polysorbate were used to swab the upper and lower surfaces of flexible endoscope and were labeled.
6. Once the samples have been collected, and adequately labeled, they were immediately sent to the microbiology laboratory for culture.

2.4. Sample culture

Samples were cultured for aerobic and anaerobic bacteria, including bacterial spores, and for Candida species.[32]

Samples were cultured in 5 different ways:

1. Sabouraud's dextrose broth for 48 h at 37 °C, with subsequent inoculation on Sabouraud's dextrose broth for another 48 h at 37 °C.
2. Thioglycollate broth for 48 h at 22 °C and with subsequent inoculation on blood agar for another 48 h at 22 °C.
3. Thioglycollate broth for 24 h at 37 C° with subsequent inoculation on MacConkey agar for another 24 h at 37 C°.
4. Thioglycollate broth for 48 h at 22 °C in anaerobic jar followed by inoculation on blood agar for another 48 h at 22 ° C in anaerobic jar.
5. Thioglycollate broth for 24 h at 37 °C in an anaerobic jar with subsequent inoculation on blood agar for another 24 h at 37 °C in an anaerobic jar.

2.5. Bioburden levels

If growth of organisms is detected, the number of colonies were counted and were determined as colony forming units (cfu)/ml. (cfu/ml = total number of colonies on the entire plate/0.1 mls (e.g., If 10 colonies are detected, the cfu/ml = 10/0.1 = 100 cfu/ml). and then multiplied by the recovery factor, this number represents the bioburden value.

2.5.1. Identification of bacterial isolates

The media used in identification were commercially available, as dehydrated media. They were prepared, distributed and sterilized according to the manufacturer's instruction.[33]

1. Identification of **Gram-positive cocci**.[34]
2. Identification of **Gram-positive bacilli**.

A. Isolate that formed large opaque colonies with glistening surface and were either hemolytic or non hemolytic on blood agar plates and microscopically appeared as Gram positive rod or coccobacilli and were positive in catalase test were considered as **Diphtheroids** spp.[35]
B. Isolate that formed large opaque colonies with surface appearance like ground glass, and were hemolytic or non hemolytic on blood agar plates and microscopically appeared as Gram positive spore bearing rods, and were positive in catalase test were considered as **Bacillus** spp.[35]

2.5.2. Gram-negative bacterial isolates[36]

Isolates that appeared as medium sized gray colonies, either hemolytic or non-hemolytic on blood agar, pink or pale colonies on MacConkey's agar, and microscopically appeared as Gram-negative bacilli were further differentiated and identified as follows:

1. Those that appeared as pink colonies (lactose fermenters) were considered as members of the **Enterobacteriaceae** and were further identified according to biochemical tests.
2. Those that appeared as pale colonies (non-lactose fermenters) were tested for oxidase production:
   – Oxidase positive organisms which showed no change/alkaline in reaction on TSI, were presumptively considered as *Pseudomonas aeruginosa* and were further confirmed by biochemical tests.
   – Oxidase negative organisms, which showed alkaline/acidic reaction on TSI, were tested for their urease activity. If these colonies were positive, they were suspected of being **Proteus spp**., and if negative, the colonies were suspected to be **Providencia** or **Serratia spp**. and were further differentiated according to biochemical reactions.[36]

2.5.3. Fungal isolate

All isolated colonies on Sabouraud's dextrose agar (SDA) plates were identified by their colonial morphology, microscopical characteristic and nutritional and biochemical tests according to standard microbiological testing.[37]

2.5.4. Colonial morphology

In the case of large creamy rapidly growing colonies on SDA plates with the odor of yeast and pasty nature, growth appeared by 24–48 h and microscopically had the characteristics of budding yeast cells. These were suspected to be **Candida spp**. These were further identified by germ tube test and presence of chlamydospore to identify *Candida albicans*.

4. Results

The current study included a total of 120 endoscopes randomly selected from Medical Research Institute (MRI) hospital 60 (50%) of which were from Surgical Department endoscopy unit, and 60 (50%) of which were from Internal Medicine Department endoscopy unit. The endoscopes were divided as follows:

- 40 endoscopes after use.
- 40 endoscopes after manual cleaning, and
- 40 endoscopes after high level disinfection.

Patients' data sheets were reviewed to collect data about the age and sex of patients undergoing endoscopy, the main complaints and the final diagnosis of the underlying conditions.

Also data about the result of *Helicobacter pylori* testing during endoscopy as well as HCV testing were collected (Table 1).

The study population was selected randomly. The age and sex distribution are shown in Tables 2 and 3. The age distribution ranged between 50 and 59 years (37.5%), 40 and 49 years (22.5%), 60 and 69 years (21.7%), 70 and 79 years (11.6%), 30 and 39 years (5%), and 20 and 29 (1.7%) (Table 4).

Table 2 Number and percentage of sex distribution.

Sex	Number	Percentage (%)
Male	96	80
Female	24	20
Total	120	100

Table 3 The age distribution of the studied cases.

Age	Number	Percentage (%)
20–29	2	1.7
30–39	6	5
40–49	27	22.5
50–59	45	37.5
60–69	26	21.7
70–79	14	11.6
Total	120	100

Table 1 Number and types of endoscopes used in the study.

Dept. & type of endoscope	After use	After cleaning	After HLD	Total
Surgery endoscopy unit				
Gasteroduodenoscopy	17	16	19	52
Colonoscopy	3	4	1	8
Internal medicine endoscopy unit				
Gasteroduodenoscopy	15	16	16	47
Colonoscopy	5	4	4	13
Total				120

Table 4 Number and percentage of the main patients complains.

Main complain	Number	Percentage (%)
Pain or discomfort in the upper abdomen	51	42.5
Repeated vomiting	20	16.7
Epigastric pain	13	10.8
Hematemesis	10	8.3
Melena	8	6.7
Unexplained changes in bowel habit	7	5.8
Constipation	6	5
Dysphagia	5	4.2
Total	120	100

Table 5 The final diagnosis of the studied cases.

Diagnosis	Number	Percentage (%)
Gastroduodenitis	48	40
GERD	13	10.8
Esophageal varices	18	15
Peptic ulcer	20	16.7
Cancer	15	12.5
Normal	6	5
Total	120	100

Pain and discomfort represented the main complaint in 42.5% of patients undergoing endoscopy followed by repeated vomiting in 16.7% of cases, epigastric pain in 10.8%, hematemesis in 8.3%, melena in 8%, unexplained changes in bowel habit in 5.8%, constipation in 5% and dysphagia in 4.2% of cases.

The final diagnosis of the studied cases is as shown in Table 5. *Gastroduodenitis* was diagnosed in 48 patients, peptic ulcer in 24 patients, esophageal varices in 18 patients, cancer in 15 patients, Gastroesophageal reflux disease (GERD) in 13 patients, and normal endoscope in 6 patients.

H. pylori infection tested by rapid urease production test was found positive in 18 (18%) of upper gastroendoscopy, while 81 (82%) were found negative for *H. pylori* infection. Patients data showed that within the 120 studied patients, 15 (12.5%) were positive for HCV infection, while 105 (87.5%) negative for HCV infection (Tables 6–10).

Tables 12 and 13 show that the average bioburden level in the surgical department was higher during colonoscopies than gastroduodenoscopies (1.04×10^3 versus 4.74×10^2) while Tables 14 and 15 show that the average bioburden level in the Internal Medicine Department was higher during colonoscopies than gastroduodenoscopies (7.26×10^2 versus 3.14×10^2) (Tables 16 and 17).

Table 18 shows that there was a significant difference between the 2 units as regards the range of bioburden distribution on endoscopes immediately after use. ($P = 0.038$). This could be explained by the greater number of colonoscopies & the higher bioburden level detected immediately after use of colonoscopies in the endoscopy unit of Internal Medicine Department (5 cases versus 3 cases & 5.49×10^7 versus 3.21×10^7) (Tables 9 and 11).

Table 6 Result of Helicobacter pylori testing.

H. pylori	Number	Percentage (%)
Positive	18	18
Negative	81	82
Total	99	100

Table 7 Number and percentage of HCV.

HCV	Number	Percent (%)
Positive	15	12.5
Negative	105	87.5
Total	120	100

Table 8 Bioburden distribution (cfu/ml) on flexible gastroduodenoscopes immediately after use and before pre-cleaning in surgical department endoscopy unit.

Cases	After use in surgery endoscopy unit		
	Aerobe	Anaerobe	Total
Average	1.42×10^6	1.34×10^6	2.76×10^6

Table 9 Bioburden distribution (cfu/ml) on flexible colonoscopes immediately after use and before pre-cleaning in surgical department endoscopy unit.

Cases	After use in surgery endoscopy unit		
	Aerobe	Anaerobe	Total
Average	1.51×10^7	1.70×10^7	3.21×10^7

Table 10 Bioburden distribution (cfu/ml) on flexible gastroduodenoscopes immediately after use and before pre-cleaning in internal medicine endoscopy unit.

Cases	After use in internal medicine endoscopy unit		
	Aerobe	Anaerobe	Total
Average	8.82×10^6	9.90×10^6	1.87×10^7

Table 11 Bioburden distribution (cfu/ml) on flexible colonoscopies immediately after use and before pre-cleaning in internal medicine endoscopy unit.

Cases	After use in internal medicine endoscopy unit		
	Aerobe	Anaerobe	Total
Average	2.82×10^7	2.67×10^7	5.49×10^7

Data in Table 19 show that there was no significant difference between the 2 units as regards the range of bioburden distribution on endoscopes after manual cleaning ($P = 0.246$).

The average Bioburden on endoscopy before manual cleaning ranged from 4.97×10^5 to 3.7×10^8 cfu per device (mean

Table 12 Bioburden distribution (cfu/ml) on flexible gastroduodenoscopes after cleaning in surgical department endoscopy unit.

Cases	After cleaning in surgery endoscopy unit		
	Aerobe	Anaerobe	Total
Average	2.74×10^2	2.51×10^2	4.74×10^2

Table 13 Bioburden distribution (cfu/ml) on flexible colonoscopes after cleaning in surgical department endoscopy unit.

Cases	After cleaning in surgery endoscopy unit		
Aerobe	Anaerobe	Total	
Average	5.56×10^2	4.80×10^2	1.04×10^3

Table 14 Bioburden distribution (cfu/ml) on flexible gastroduodenoscopes after cleaning in internal medicine endoscopy unit.

Cases	After Cleaning in internal medicine endoscopy unit		
Aerobe	Anaerobe	Total	
Average	1.83×10^2	1.24×10^2	3.14×10^2

Table 15 Bioburden distribution (cfu/ml) on flexible colonoscopes after cleaning in internal medicine endoscopy unit.

Cases	After cleaning in internal medicine endoscopy unit		
Aerobe	Anaerobe	Total	
Average	4.41×10^2	3.60×10^2	7.26×10^2

cfu per device 3.7×10^7), whereas after manual cleaning ranged from 5.1×10^2 to 3.42×10^3 cfu per device (mean cfu per device 1.0×10^3) and no colonies were found after sterilization. Manual cleaning resulted in a mean of 4.57 $\log_{10}$ reduction in viable colony count and HLD resulted in a reduction of CFU to zero (Table 20).

Table 18 Comparison between the 2 endoscopy units as regarding the range of bioburden distribution immediately after use.

Units	Range of CFU on endoscopes	Mean
Surgery	$(5.01 \times 10^5$–$8.7 \times 10^7)$	3.49×10^7
Internal medicine	$(4.97 \times 10^5$–$3.7 \times 10^8)$	7.6×10^7

Table 19 Comparison between the 2 endoscopy units as regards the range of bioburden distribution after cleaning.

Units	Range of CFU on endoscopes	Mean
Surgery	$(5.1 \times 10^2$–$6.5 \times 10^3)$	1.06×10^3
Internal medicine	$(5.2 \times 10^2$–$3.42 \times 10^3)$	1.04×10^3

Table 20 Summary of reduction of bioburden after manual cleaning and after sterilization.

Location	CFU on endoscopy	Mean
Pre-cleaning	4.97×10^5–3.7×10^8	3.7×10^7
After manual cleaning	5.1×10^2–3.42×10^3	1.0×10^3
After sterilization	0	0

Table 21 Frequent and percentage of microorganisms isolated immediately after use (pre-cleaning).

Microorganism	Frequent of isolation	Percentage (%)
Staphylococcus	80	100
Streptococcus	72	90
Klebsiella	26	32.5
E. coli	16	20
Bacteroides	10	12.5

Table 21 lists the main microorganisms that have been isolated from both the surface and channels of the endoscopes immediately after use. Staphylococci were the main strains isolated from all the 40 endoscopes (surface and channel)

Table 16 Summary of gastroduodenoscope bioburden immediately after use and after cleaning (Surgical department endoscopy unit).

Location	Range		Mean	
	cfu in channel	cfu on surface	cfu in channel	cfu on surface
Immediately after use	6.1×10^4–5.7×10^7	3.4×10^5–3×10^7	7.60×10^6	6.74×10^6
After cleaning	2.1×10^2–3.5×10^3	2.8×10^2–3.4×10^3	5.98×10^2	5.67×10^2

Table 17 Summary of gastroduodenoscope bioburden immediately after use and after cleaning (internal medicine endoscopy unit).

Location	Range		Mean	
	cfu in channel	cfu on surface	cfu in channel	cfu on surface
Immediately after use	1.4×10^5–3.7×10^8	6×10^4–6.6×10^7	4.40×10^7	1.15×10^7
After cleaning	2.5×10^2–3×10^3	2.6×10^2–4.2×10^2	4.81×10^2	3.12×10^2

Table 22 Frequent and percentage of microorganisms isolated after manual cleaning.

Microorganism	Frequent of isolation	Percentage (%)
Staphylococcus	80	100
Streptococcus	80	100
Pseudomonas	35	43.3
Klebsiella	22	27.5
E.coli	8	10
Bacteroides	5	6.3

followed by Streptococci 72 times (90%), Klebsiella was 26 times (32.5%), *Escherichia coli* 16 times (20%), Bacteroides 10 times (12.5%) and no fungi were isolated.

The microorganisms isolated after manual cleaning are summarized in Table 22. Staphylococci and Streptococci were the main organisms isolated from all the 40 endoscopes (surface and channel) followed by Pseudomonas 35 times (43.3%), Klebsiella 22 times (27.5%), Bacteroides 10 times (12.5%) and *E. coli* 8 times (10%). No fungi were isolated.

5. Discussion

Flexible endoscopes, by virtue of the site of use, have a high bioburden of microorganisms after use. In the present study, the bioburden found on flexible gastrointestinal endoscopes immediately after use ranged from 10^5 to 10^8 CFU/ml, with a mean of 3.7×10^7. In a similar study, Vergis et al. microbiologically assessed GI endoscopes following use and found bioburden values ranging from 10^5 to 10^{10} CFU/ml.[38]

In our study, manual cleaning alone reduces the bioburden on endoscopes by about a $\log^{10}$ reduction factor of (4.57) in the microbial contaminants. This reveals that our two studied endoscopy units follow pre-established protocols for cleaning and disinfection of endoscopes, which reflect their good compliance with national and international guidelines.

H. pylori status of our patients was identified using rapid urease testing. 18% of our cases were found to be positive for *H. pylori* by this test. *H. pylori* contamination was assessed by culturing rinsing samples from the endoscopes before and after manual cleaning and disinfection. No *H. pylori* could be detected by culturing rinsing samples after routine manual cleaning and disinfection – indicating that these cleaning and disinfection procedures are sufficient to eradicate *H. pylori* from endoscopes completely.

Given that flexible endoscopes are not stored under sterile conditions and that some sites use manual reprocessing that includes a final rinse with tap water, detection of a few colonies growing on the culture plates would not be of concern.[39] Although reprocessed endoscopes should be free of pathogenic organisms, small numbers of avirulent microbes representing environmental contamination (e.g., Bacillus species, coagulase-negative staphylococci) may persist in the lumen of reprocessed endoscopes. The presence of gram negative bacilli in endoscope channels suggests waterborne contamination.[40]

The aldehydes contain the most popular disinfectants (e.g. formaldehyde, **Glutaraldehyde** (GA) and **Ortho-Phthalaldehyde (OPA)**), which are recommended by many guidelines.[41] Full immersion of the endoscope in the high level disinfectant and complete perfusion of all the channels for the approved contact time are stressed in the disinfection process. Immersion of endoscopic equipment in 2% GA for 20 min at 20 °C is widely accepted as the major disinfection method, and seems to be adequate for the prevention of gastrointestinal endoscopy cross-infection.[32]

GA has a broad-spectrum antimicrobial activity. Several studies have shown that 2% aqueous solutions of GA, buffered to pH 7.5–8.5 with sodium bicarbonate, effectively kill vegetative bacteria in <2 min, fungi and viruses in <10 min, Mycobacterium tuberculosis in <20 min, 2% GA was still the most widely used disinfectant (88.5%). Unfortunately, GA has a prominent vapor component, which has been associated with ocular, nasal and respiratory problems. Another problem with GA is the potential to crosslink residual protein material, which causes it to be hard to remove.[42] OPA is an alternative to GA for HLD and is commercially available at an active concentration of 0.55%. Advantages of OPA are its higher efficacy compared with GA, long lifespan, and odorless property.[43]

In our survey, 2% GA was the disinfectant of choice used effectively in internal medicine endoscopy unit, while OPA was the disinfectant of choice used in surgical department endoscopy unit. Both disinfectants nearly give the same efficacy in the two studied units. Surgical department endoscopy unit took the advantage of the higher efficacy and long life span of OPA to avoid the prominent vapor component of GA.

Our results proved that aldehydes (GA, OPA) which are used as high level disinfection solution in our units, effectively kill vegetative microorganisms.

Culture of bacterial fecal flora (*E. coli*, coliform enterobacteriaceae, enterococci) was interpreted indicating failure of the manual cleaning procedure and disinfection of endoscopes. Detection of Pseudomonas spp. (especially *P. aeruginosa*) and other non-fermenting rods indicating microbially insufficient final rinsing and incomplete drying of the endoscope or a contaminated flushing equipment for the air/water-channel – pointed out endoscope recontamination during reprocessing or afterward.

In our study, microorganisms isolated immediately after manual cleaning were as follows: Staphylococcus was isolated from all the 40 endoscopes (surface and channel), i.e., 80 times (100%), Streptococcus was isolated 80 times (100%), Pseudomonas was isolated 35 times (43.3%), Klebsiella was isolated 22 times (27.5%), Bacteroides was isolated 10 times (12.5%), *E. coli* was isolated 8 times (10%). The presence of gram negative bacilli in our endoscopes after manual cleaning denotes the presence of waterborne contamination in our two studied units.

Although no statistically significant relationship was found between the growth of bacteria and fungi and the scope type or channel type, microorganisms were detected in more colonoscopies after manual cleaning than in esophagogastroduodenoscopies used in our study. A possible explanation for this is that colonoscopes are generally more longer and thus the most difficult to dry properly, and moisture residue in the channels allows the growth of environmental bacteria.

We believe that monitoring microbial levels in reprocessed channels after weekend storage is a useful quality indicator (QI) in endoscopy clinics. This QI verifies that the clinics reprocessing and storage conditions are adequate to ensure

that scopes are safe to use. HLD is superior to manual cleaning in the process of endoscopic disinfection.

Whether or not the cleaned and disinfected scopes need to be reprocessed immediately before use has been a subject of debate.[44]

Although some previous studies emphasized the value of regular microbiological monitoring of endoscopes, very few addressed what is considered non-acceptable microorganism types and counts, and a consensus regarding an acceptable bioburden count in patient-ready scopes is still lacking. The international guidelines state an acceptable bioburden count of <20 cfu/channel.[45] Heeg recommended that *Pseudomonas aeruginosa*, *E. coli*, other Enterobacteriaceae, streptococci, enterococci, *Staphylococcus aureus*, and other relevant nosocomial pathogens should not be detected in any amount.[46]

Vergis et al. microbiologically assessed patient-ready GI endoscopes daily for 2 weeks, and found that the reprocessing of clean, unused scopes is unnecessary for at least 7 days. The authors concluded that guidelines recommending more frequent reprocessing lack scientific merit and should be revisited.[38]

In another study, Osborne et al. tested 200 flexible endoscopes before use on the first case of the day and found that the scopes remained free of pathogenic organisms overnight.[45]

We conclude that every endoscopy unit should have its own protocol regarding cleaning & disinfection to ensure that scopes are safe to use.

We also recommend training all health-care personnel in the endoscopy unit in standard infection control to reprocess endoscopes. Safe working practices in the decontamination area of each unit should be written down and understood by all staff. Also, water used in the cleaning process should be free of micro-organisms. This can be achieved either by using bacteria-retaining filters or by other methods, for example the addition of biocides. The final rinse water should be tested for its microbiological quality at least weekly. The study also showed the need for further large-scale trails and cost effective analysis (viruses and especial bacteria) to assess the efficiency of the different cleaning processes used in the endoscopy unit.

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