Clinical Studies on the Qualitative and Quantitative Nature of Colonoscope Bioburden After PEG/Electrolyte Cleansing of the Large Intestine

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Disinfection/sterilization efficacy procedures are available for fiber-optic colonoscopes. Such procedures describe the need to show germicidal activity against standard laboratory strains and reductions in count against clinical strains.

Little clinical data are available on the number and identity of microorganisms present on colonoscopes prior to germicidal treatment.

We have studied the flora of colonoscopes after removal from PEG/electrolyte-cleansed outpatients, so as to gain a better understanding of the clinically relevant challenge. The organisms include gram positive and gram negative bacteria, yeast, RNA viruses and the proportion of vegetative cells to bacterial endospores. The direct *in vitro* effect of the PEG/electrolyte or Phospho Soda solutions on the bowel flora will also be discussed.

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Objective

Gastrointestinal endoscopy bears an infection risk which may result from **exogenous factors**, such as incomplete disinfection or from **endogenous factors**, such as injuries to the intestinal mucosa allowing component of the normal flora to gain access to the blood stream.

The purpose of the study was to gain a better idea of the types and numbers of microorganisms present on and in clinical colonoscopes immediately prior to disinfection.

Observation on the antimicrobial properties of the pre-surgical irrigating solutions are also presented.

Colonoscopy is a valuable tool for the diagnosis and treatment of many diseases of the large intestine. Abnormalities suspected by x-ray can be confirmed and studied in detail. Even when x-rays are negative, the cause of symptoms such as rectal bleeding or change in bowel habits may be found by colonoscopy. It is useful for the diagnosis and follow-up of patients with inflammatory bowel disease as well.

Colonoscopy's greatest impact is probably in its contribution to the control of colon cancer by polyp removal. Before colonoscopy became available major abdominal surgery was the only way to remove colon polyps to determine if they were benign or malignant. Now most polyps can be removed easily and safely without surgery.

Periodic colonoscopy is a valuable tool for follow up of patients considered to have an increased risk of developing colon cancer such as patients with previous polyps, colon cancer, or a family history of colon, breast, uterine, ovarian and some other cancers. Irrigation of the bowel by ingestion of electrolyte solutions and/or enemas is a necessary pre-operative step.

Bacteriology

Method

Colonoscopes were provided by Somerset Surgical Associates, New York, NY (Dr. Norman Sohn). Irrigation of the colonoscopes to collect the clinical bioburden was performed as follows.

External Bioburden

The colonoscope was removed from the biohazard bag. The distal tip (approximately 80 cm) was aseptically placed into a sterile stomacher bag containing 100 mL sterile physiological saline. The bag was then manually agitated for five minutes to dislodge the micro-organisms. The physiological saline was then aseptically transferred to a 16-ounce sterile glass jar.

Internal Bioburden

The colonoscope was aseptically connected to the all-channel irrigator. The distal tip of the colonoscope, previously eluted for external bioburden, was placed into a sterile 16-ounce glass jar. 100 mL of sterile physiological saline was then flushed through the scope using the all-channel irrigator. The bioburden-laden suspension was collected in the sterile jar.

Aerobic Microorganisms were enumerated as follows:

The organisms contained in the 16-ounce jars from above steps were quantified by membrane filtration using sterile 0.45 µm membrane filters. The saline portions were serially diluted ten fold into 9 mL sterile saline and 1 mL from each dilution was quantified by membrane filtration using sterile 0.45 µm membrane filters.

After the bioburden portions of saline had passed through the filters, the membranes were rinsed with 100 mL sterile 0.1% peptone water and aseptically placed onto Trypticase Soy Agar (TSA) plates as well as blood agar plates for the determination of hemolysis. The TSA plates were incubated aerobically at 37 ± 1C for 48 hours, then observed for colonial growth.

Anaerobic Microorganisms were enumerated as follows.

The organisms were quantified by membrane filtration using sterile 0.45 µm membrane filters. The saline portions were serially diluted ten fold into 9 mL sterile saline and 1 mL from each dilution was quantified by membrane filtration using sterile 0.45 µm membrane filters.

After the bioburden portions of saline had passed through the filters, the membranes were rinsed with 100 mL sterile 0.1% peptone water and aseptically placed onto TSA plates. The TSA plates were incubated anaerobically in an anaerobe jar at 37 ± 1C for 48 hours, then observed for colonial growth. The microorganism colonies were counted using a Quebec colony-counter. To determine the presence of obligate anaerobes the colonies produced, if any, were then streaked onto TSA plates, then incubated aerobically at 37 ± 1C for 48 hours, then observed for growth to determine presence of any obligate anaerobes present.

Gram negative microorganisms were enumerated as follows.
The organisms were quantified by surface streaking onto MacConkey's (Mac) agar plates. The saline portions were serially diluted ten fold into 9 mL sterile saline and 0.1 mL from each dilution was quantified by surface streaking onto MacConkey's agar plates.

The plates were incubated aerobically at 37 ± 1C for 48 hours, then observed for differential growth.

Yeast and Mold Microorganisms
The organisms contained in the 16-ounce jars from above steps were quantified by pour plate method using Sabouraud's Dextrose agar (SDA) plates containing 150 mcg/mL of Tetracyclines. The saline portions were serially diluted ten fold into 9 mL sterile saline and 1.0 mL from each dilution was poured with SDA.

The plates were incubated aerobically at 20 to 25C for 5 to 7 days, then observed for growth.

Spore Forming Microorganisms
The 15 mL portions of the saline from the 16-ounce jars from above steps were quantified for spore forming microorganisms by heat shocking the saline portions at 80 to 85C for 20 minutes. 10 mL, 1.0 mL and 0.1 mL were quantified by membrane filtration using sterile 0.45 µm membrane filters. The membranes were rinsed with 100 mL sterile 0.1% peptone water and aseptically placed onto Trypticase Soy Agar (TSA) plates. The TSA plates were incubated aerobically at 37 ± 1C for 48 hours, then observed for colonial growth.

Identifications
The morphologically different isolates were subcultured onto TSA plates for isolation and purification. Bacterial identifications were performed according to standard bacteriological procedures including the use of selective and differential agars, hemolysis, Gram stain and cytochrome oxidase test, as appropriate. In addition, multisubstrate biochemical profiles were obtained by inoculation of API-20 strips or Vitek diagnostic cards. Yeast and mold identifications were rendered by colonial morphology, spore location, mycelial structure, and other morphological characteristics.

Viruses
To determine the presence of enteroviruses in bioburden eluates, the external and internal bioburden elutes were membrane filtered through sterile 0.45 micron membrane filters. The filtrates were inoculated onto HEp-2 (human epithelioma carcinoma) and MA104 (embryonic Rhesis monkey kidney) cells. T-25 tissue culture flasks of the cells were fed with tissues culture medium RPMI $1640_{95}CS_5$, supplemented with Penicillin G 100 Units/mL; Streptomycin sulfate 100 mcg/mL and Gentamicin sulfate 50 mcg/mL and incubated at 37 ± 1C, 5% CO_2. The cells were observed daily for signs of cytopathic effect (CPE) typical of enteroviruses infection.

Scope #	Aerobic	Anaerobic	Spores	Enterococci	Yeast/Mold	Non-fermentor	Fermentors	Enterovirus (Preliminary)
1	5.3×10^6	<10	<10	<10	<10	X	X	-
2	1.3×10^6	<10	<10	<10	<10	X	0	-
3	1.1×10^4	<10	<10	<10	<10	X	0	-
4	5.5×10^3	<10	<10	<10	<10	X	X	-
5	3.5×10^5	<10	<10	<10	<10	X	0	-
6	1.6×10^6	<10	<10	<10	<10	1.1×10^5	<10	0
7	1.3×10^6	<10	<10	<10	<10	6.1×10^5	<10	0
8	1.5×10^4	<10	<10	<10	1.1×10^5	7.0×10^3	1.0×10^4	0
9	2.9×10^5	<10	<10	<10	<10	7.0×10^3	2.6×10^5	0
10	3.0×10^4	<10	<10	<10	<10	2.9×10^4	6.0×10^3	0
Average = 1.0×10^6 Range = 10^3 to 10^6								

X = found 0 = not found

Conclusion: The internal bioburden consisted essentially of gram negative bacillus. Spores, anerobes and enterococci were not detected. Internal bioburden was higher then the external bioburden.

Table 2: External Bioburden cfu/scope

Scope #	Aerobic	Anaerobic	Spores	Enterococci	Yeast/Mold	Non-fermentor	Fermentors	Enterovirus
1	1.2×10^5	<10	<10	<10	<10	X	X	-
2	4.5×10^6	<10	<10	<10	<10	X	X	-
3	5.0×10^2	<10	<10	<10	<10	X	0	-
4	3.5×10^3	<10	<10	<10	<10	X	X	-
5	7.8×10^5	<10	<10	<10	<10	X	X	-
6	1.5×10^4	-	-	-	-	-	-	-
7	1.1×10^4	-	-	-	-	-	-	-
8	2.0×10^4	<10	<10	<10	<10	<10	6.0×10^3	0
9	3.0×10^3	<10	<10	<10	<10	<10	<10	0
10	8.0×10^3	<10	<10	<10	<10	<10	<10	0
Average = 8.0×10^3 Range = 10^2 to 10^6								

X = found 0 = not found - = not done

Conclusion: The external bioburden consisted essentially of gram negative bacillus and was lower that the internal bioburden. Spores, anerobes and enterococci were not detected. External bioburden was lower on average then internal bioburden.

Table 3: Identifications

Internal

Achromobacter xylosoxidans
Agrobacterium tumefaciens (Agrobacter radiobacter)
Bacillus sp.
Flavobacterium indologenes
Pseudomonas aeruginosa
Pseudomonas cepacia
Pseudomonas fluorscenes
Xanthomonas maltophilia (Stenotrophomonas maltophilia)
Staphylococcus simulans
Shewanella putrefaciens (Alteromonas putrefaciens)
Candida albicans
Enterobacter/Klebsiella group

External

Agrobacterium tumefaciens (Agrobacter radiobacter)
Bacillus cereus
Escherichia coli
Micrococcus sp.
Pseudomonas maltophilia
Pseudomonas aeruginosa
Staphylococcus epidermidis
Pseudomonas putida
Enterobacter/Klebsiella group

Note: The predominant organisms were members of the non-fermenting bacillus (NFB) group.

Table 4: Antimicrobial Properties of Colonic Irrigation Solutions (USP Antimicrobial Effectiveness Test) (% Reduction)

Exposure Time at Room Temperature	Solution I			Solution II		
	E. coli	*Enterococcus faecium*	*Enterococcus faecalis*	*E. coli*	*Enterococcus faecium*	*Enterococcus faecalis*
0 Time	4.1×10^6	8.1×10^5	2.2×10^6	4.1×10^6	8.1×10^4	2.2×10^6
24 Hour	1.3×10^6 (68%)	4.6×10^2 (99.9%)	2.2×10^5 (90%)	4.6×10^5 (88%)	4.9×10^4 (40%)	1.6×10^5 (93%)

Conclusion: A slight to moderate antimicrobial effect was presumptively noted suggesting that one colonoscopic bowel irrigation may produce a shift in the flora.

Solution I = Golytely PEG-Electrolyte Solution
Solution II = Phosphosoda Fleet Irrigation Solution