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ORIGINAL ARTICLE

Mechanical bowel preparation does not affect the intramucosal bacterial colony count

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Abstract
Purpose The aim of this study was to determine if mechanical bowel preparation (MBP) influences the intramucosal bacterial colony count in the colon.
Materials and methods Macroscopically normal colon mucosa was collected from 37 patients (20 with and 17 without MBP) who were undergoing elective colorectal surgery at three hospitals. The biopsies were processed and cultured in the same laboratory. Colony counts of the common pathogens *Escherichia coli* and *Bacteroides* as well as of total bacteria were conducted. The study groups were comparable with regard to age, gender, antibiotics use, diagnosis and type of resection.
Results MBP did not influence the median colony count of *E. coli*, *Bacteroides* or total bacteria in our study.
Conclusions MBP did not affect the intramucosal bacterial count in this study. Further studies are suggested to confirm these findings.

Keywords Colorectal surgery · Bacteria · Colon mucosa · Bowel preparation

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Introduction

In the twentieth century, mechanical bowel preparation (MBP) was used widely to minimise intraluminal mass for the purpose of reducing the risk of anastomotic leakage and infectious complications. However, in recent years, data from two large randomised trials and several smaller studies failed to support this practice [1–10]. The microflora in the colon is known to have important functions in normal physiology and is suggested to have a role in the aetiology of pathological states, such as inflammatory bowel disease and colorectal cancer [11–14]. The intraluminal and intramucosal bacterial compartments in the colon are distinct entities separated by a mucous layer that protects the healthy mucosa [11]. It is known that the intraluminal bacterial count in humans is not reduced by MBP [15]. However, to our knowledge, no information is available concerning possible effects of MBP on the intramucosal bacterial count, which is the issue we aim to address in the present study.

Materials and methods

We collected macroscopically normal colon mucosa from 37 patients (20 with and 17 without MBP) who were undergoing elective colorectal surgery. As shown in Table 1,

Table 1 Demographic data, diagnosis, type of antibiotics and participating hospital

	MBP	No-MBP	*p* values
Male/female	8/12	5/12	0.73a
Mean age (years)	72.1	68.6	0.82b
Cancer (%)	17 (85.0)	13 (76.5)	0.68a
Resection			0.52a
Right colon	9	10	
Left colon or rectum	11	7	
Hospital			0.84a
1	9	7	
2	7	5	
3	4	5	
Preoperative antibiotics			0.72a
IV cephalosporin+metronidazole	7	5	
Oral sulfamethoxazole–trimethoprim+metronidazole	13	12	

[a] Fisher's exact test

[b] Mann–Whitney *U* test

the two groups were balanced with regard to age, gender, antibiotics used, diagnosis and type of resection. Three hospitals participated in this study. Twenty-three of the patients were included in a multicentre study that compared the postoperative outcome (30-day morbidity and mortality) after elective large bowel surgery with or without MBP [2]. All patients received preoperative prophylactic antibiotics according to each hospital's routine; 25 received oral sulfamethoxazole–trimethoprim+metronidazole, and 12 received intravenous cephalosporin+metronidazole. The bowel preparation for those who received MBP was sodium phosphate (Phosphoral®; Ferring Pharmaceuticals, Limhamn, Sweden) in nine patients and polyethylene glycol (Laxabon®; AstraZeneca, Oslo, Sweden) in 11 patients.

Immediately after division of the colon and with the bowel still in the abdomen, a small full-thickness biopsy was taken from the bowel wall. The biopsy was placed in an Eppendorf tube containing 0.5 mL peptone–yeast–cystein–glycerol broth, pH 7.0, and stored at −20°C pending analysis.

All biopsies were processed in the same laboratory. After thawing at room temperature, the biopsy was pestled in its broth, and 0.1 mL of the suspension was plated on aerobic and anaerobic blood agar plates and incubated at 35°C for 48 h. Colonies were counted and identified by standard methods [16]. The biopsies weighed a median 2.0 g (interquartile range 1.7–2.1).

All identified colonies were registered (*Escherichia coli*, *Klebsiella*/*Enterobacter*, diphtheroids, *Enterococci*, alpha-haemolytic streptococci, *Staphylococci*, *Clostridium*, *Bacteroides* and *Propionibacteria*). For comparison between the two groups, the common pathogens *E. coli* and *Bacteroides* were chosen, as well as the total number of bacterial colonies.

We used Fisher's exact test for categorical variables and the Mann–Whitney *U* test for differences between continuous variables. Two-tailed *p* values<0.05 were considered significant.

Results

Between June 2004 and February 2005, we collected biopsies from the large bowel of 37 patients. Twenty patients had MBP and 17 did not. There was no significant difference in the median total bacterial colony count between the MBP and no-MBP groups: 80 (range 0–1,500) versus 113 (1–2,000), respectively ($p=0.46$). Likewise, no differences were found between groups in the median colony count of *E. coli* [13 (0–500) and 3 (0–500), $p=0.75$] or *Bacteroides* [0 (0–500) and 0 (0–1), $p=0.33$]. *E. coli* colonies were found in 12/20 biopsies in the MBP group compared with 8/17 biopsies in the no-MBP group ($p=0.72$). The *E. coli* colony counts are listed in Table 2. The distribution of total bacterial colony counts per gramme tissue in the two study groups is shown in Fig. 1.

When the two types of antibiotics used in the study (oral sulfamethoxazole–trimethoprim+metronidazole, $n=25$, and intravenous cephalosporin+metronidazole, $n=12$) were compared, we found that biopsies from patients receiving oral antibiotic prophylaxis had a significantly higher *E. coli* count ($p=0.02$). No corresponding difference was seen in

Table 2 Colony count of *E. coli* in biopsies

	MBP ($n=20$)	No-MBP ($n=17$)
No colonies	8	9
1–100	8	5
>100 colonies	4	3

Relative risk for growing >100 colonies per biopsy=0.93 (95% Confidence Interval, 0.45–1.92)

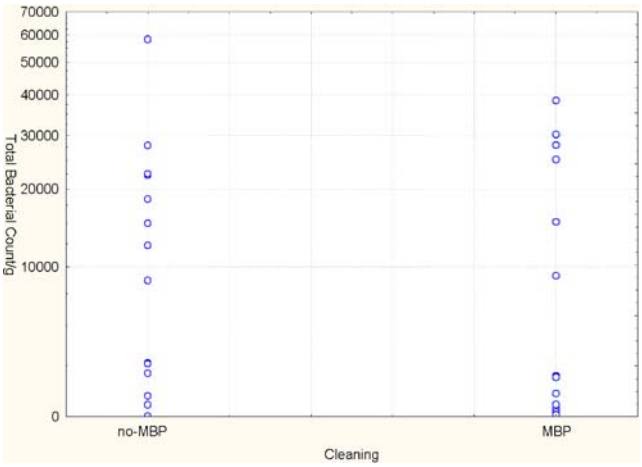

Fig. 1 Scatterplot of total bacterial count per gramme of tissue in the two study groups (MBP or no-MBP)

total bacterial count (Table 3). There was no significant difference in the studied bacterial count between the right colon and the left colon/rectum (data not shown).

Discussion

Few studies have investigated the influence of MBP on viable bacterial counts in colonic mucosa. The present study shows that the bacterial count in colorectal biopsies was unaffected by MBP and that *E. coli* growth in the biopsies was more pronounced in patients receiving oral sulfamethoxazole–trimethoprim+metronidazole compared with patients given intravenous cephalosporin+metronidazole.

The present study was small and involved three different hospitals. To reduce any variability in the handling of biopsies, only one surgeon at each hospital harvested the biopsies. The biopsies were stored at a standard temperature (−20°C) until they were processed at the same laboratory. The majority (23/37) of patients were recruited from a randomised trial that compared the outcome after elective colon surgery with or without MBP [2]. There was no difference in background data between the two groups in the present study.

The biopsies were harvested from different sites of the colon and rectum. Previous studies revealed differences in intramucosal bacterial growth in the proximal and distal colon in animals [17] but not in humans [18]; therefore, the site of the colon biopsies was not standardised. However, because there are conflicting data concerning this issue, we tested the distribution of right colon versus left colon/rectal biopsies in the studied groups (MBP and no-MBP) and found no inter-group difference. No difference in the total bacterial count in the right colon versus the left colon/rectum was observed, irrespective of MBP status.

Intramucosal growth of *E. coli* is seen in pathologic conditions such as colorectal cancer [12] and inflammatory bowel disease [17, 19], but it is unknown whether intramucosal bacterial growth influences the aetiology of these conditions. Because the majority of patients in the present study were diagnosed with colorectal cancer, it is not surprising that we found positive *E. coli* cultures. However, there was no difference in the number of positive *E. coli* cultures between patients receiving MBP or not.

22In accordance with the participating hospitals' routines, two different antibiotic prophylactic regimens were used (oral or intravenous). The two regimens were distributed evenly between the groups of patients with or without MBP. Surveillance data from Swedish microbiological laboratories for the time period of this study (www.srga.org) indicate that the resistance of *E. coli* to cephadroxil and trimethoprim was 1% and 15%, respectively. Data for sulphametoxazole and cotrimoxazole were unavailable. These data might account for the higher *E. coli* counts in the oral sulfamethoxazole–trimethoprim+metronidazole group. To evaluate the possible clinical importance of a higher *E. coli* count for prophylactic oral sulfamethoxazole–trimethoprim+metronidazole treatment, we analysed data from the bowel preparation trial [2] and found no significant difference between the two prophylaxis regimens with regard to postoperative general septic (7.0% oral sulfamethoxazole–trimethoprim+metronidazole and 7.4% intravenous cephalosporin+metronidazole) or surgical site (15.3% oral sulfamethoxazole–trimethoprim+metronidazole and 15.1% intravenous cephalosporin+metronidazole) complications (unpublished data). Thus, the clinical significance of the difference in *E. coli* counts following oral sulfamethoxazole–trimethoprim+metronidazole and intravenous cephalosporin+metronidazole prophylaxis is unclear.

Biopsies were full-thickness ones from the large bowel wall and were not washed before storage. This is a methodological weakness, since the biopsies might have been contaminated with bacteria from the intraluminal compartment. However, the median colony counts of bacteria were lower than expected, if there had been a

Table 3 Bacterial colony count according to prophylactic antibiotic regimen

	Intravenous antibiotics (n=12)	Oral antibiotics (n=25)	p value
E. coli	0 (0–500)	23 (0–500)	0.02
Bacteroides	0 (0–500)	0 (0–0)	0.34
Total bacterial colony count	100 (0–2000)	67.5 (0–1500)	0.58

Figures are shown as median colony count (range)

p values calculated with Mann–Whitney U test

significant contamination from intraluminal bacteria. Furthermore, the handling of biopsies was similar in both study groups, which allows us to make the comparison between the groups concerning the effect of MBP on mucosa-associated bacteria.

There are several techniques to identify microorganisms in tissues, depending on the purpose [20]. We chose bacterial identification by standard bacterial culture techniques used in clinical practice in Sweden, which are capable of identifying the two most common pathogens found in cultures from infected sites, *E. coli* and *Bacteroides* [21]. A DNA-detection technique possibly could identify a greater variety of bacteria in the colon mucosa, but that was not the aim of the present study. Intramucosal bacterial growth as studied in cultures from homogenised colon biopsies reflects invasive, adhesive and crypt bacterial growth. It is unclear whether a positive bacterial culture in these different intramucosal compartments has a specific influence on postoperative pathology. New techniques can separate these compartments and demonstrate the spatial organisation of bacteria in the colon wall. Such studies may shed light on the mechanisms of bacterial translocation and anastomotic dehiscence.

In conclusion, mechanical bowel preparation did not significantly affect the counts of viable pathogenic bacteria in this study. Further studies are suggested to confirm these findings.

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