

Micro-organisms play roles in colorectal cancer, is it true?

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Background: Normal gut bacterial floras play a direct or indirect role in the initiation and progression of tumour by inducing chronic inflammation and producing toxins and metabolites. However, data on the types of bacteria that are significant in the sigmoid colon and rectal tumour are still lacking. This study determined the types of bacteria that are associated with the risk of developing sigmoid colon and rectal cancer.

Methods: This case control study involved patients with bowel symptoms and had been subjected to elective colonoscopy procedure from December 2013 to September 2014. Standard bowel preparation was carried out two days prior to colonoscopy either at home or in ward. Biopsy samples were sent to microbiology laboratory for cultivation. The mean of bacterial colonies in the mucosa of sigmoid colon tumour and rectal tumour were compared with the mucosa of normal sigmoid colon and rectum, respectively.

Results: For normal colonoscopy results, 66 biopsies were taken from bowel mucosa (33 at sigmoid colon and 33 at rectum). Eight patients had sigmoid colon tumour and 25 patients had rectal tumour. The results showed that *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were only present in sigmoid colon tumour and were significantly associated with sigmoid colon cancer ($p = 0.041$). More than 15 types of bacteria were isolated in the rectal tumour compared to the normal rectal mucosa, however, only *Proteus mirabilis* ($p = 0.040$) and *Morganella morganii* ($p = 0.046$) were significant in promoting rectal cancer.

Conclusion: *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were associated with the risk of developing sigmoid colon cancer while *Proteus mirabilis* and *Morganella morganii* were associated with rectal cancer.

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Worldwide, colorectal cancer contributed for the third most common cause of cancer-related deaths.¹ The highest rates of colorectal cancer have been reported in developed countries, as compared to less developed ones.² In Malaysia, the first report in the National Cancer Registry which covers period from 1st January 2002 to 31st December 2002 showed a total of 26,089 cancers diagnosed among all residents in Peninsular Malaysia, with 11,815 involving males and 14,274 females.³ Chinese ethnicity had the highest incidence followed by Indians and Malays.³ The differences in incidence between these populations bring the possibility of genetic factors contribution in the aetiology of colorectal cancer.^{4,5} A previous study showed that incidence of rectal cancer in gender are age related.⁶ A study conducted by Hospital Pakar Universiti Sains Malaysia (HPUSM) in 2010 observed a lower overall 5-years survival rate for colorectal patient in HPUSM as compared to developed countries, such as Australia.⁷

The large intestine is the site most heavily colonised by microorganisms in the gastrointestinal tract. This vast number of microorganism collection that lives in coexistence with their hosts has been referred to as the microbiota, microflora or normal flora.⁸ Colonisation of the GI tract occurs during the first two years of life and became stable with average composition throughout the adulthood life.⁹ Recently, the concept that normal bacterial flora is essential for development of inflammation induced carcinoma has emerged from studies of well-known colonic bacterial floras.^{10,11}

The relationship between various environmental factors and cancer of the colon has received much attention since epidemiological studies correlated the incidence of the disease with the degree of economic development. In conjunction with food, the colonic bacteria play an important role in promoting carcinogenesis of the large bowel by inducing chronic bowel inflammation and alteration of metabolites product.^{12,13} Therefore, identifying the gut bacterial species involved could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

In 1970s, a study on association between gut floras and colorectal carcinoma was conducted. However, due to technical difficulties in characterizing the faecal microflora, it was not completed until 1995. Stool from three different parts of the world with

different diet intake were studied and the results showed different in diet intake will carries different risk of developing colonic cancer.¹⁴

This study was conducted to identify types of bacteria present in both the mucosa of normal sigmoid colon and rectum and types of bacteria that are significant in the sigmoid colon and rectal tumour.

METHODS

Study design and setting

This is a case control study of patients with bowel symptoms and had been subjected to elective colonoscopy procedure due to variety of bowel symptoms at the Hospital Pakar Universiti Sains Malaysia (HPUSM), Kubang Kerian, Kelantan, Malaysia. The study was conducted in the Endoscopy Unit, Department of Surgery and the Microbiology Laboratory of HPUSM. HPUSM is an academic, tertiary care center, and one of the largest hospitals with colorectal unit in Kelantan.

Participants

All patients who presented with bowel symptoms and had been subjected to elective colonoscopy procedure due to variety of bowel symptoms at the HPUSM between December 2013 and September 2014 were recruited. All patients with age more than 18 years old and above, and patients with any medical illness whose diagnoses are confirmed were included.

Patients whose colonoscopy revealed a mass in the rectum or sigmoid colon, but whose histopathological examination (HPE) indicated only inflammatory changes, were excluded from the study. Additionally, patients with a mass or tumour at a site other than the rectum or sigmoid colon were not included. Written informed consent was obtained from all participating patients.

For the reference population, we selected patients who had normal colonoscopy findings, with no pathology identified serving as control patients. The source population, on the other hand, consisted of patients with positive results for a tumour in either the sigmoid colon or the rectum.

Sample size for this study was calculated using the PS: Power and Sample Size Calculation Version 3.0.12

with the significance level (alpha) 0.05, and the power of study ($1 - \beta$) of 80% based on the parameter estimate (true probability of exposure among cases = 0.48) obtained from Moore and Moore.¹⁴ The final targeted sample size was determined by considering 10% drop-out rate. The estimated sample size for this study was 66 samples (33 patients for control patients and 33 patients with tumour).

Colonoscopy and biopsy samples collection

Standard bowel preparation was carried out two days prior to colonoscopy either at home or in ward. This ensured that all patients followed the same dietary regimen for at least three days prior to the procedure: a low-residual diet for one day, only clear fluids for one day, and administration of a laxative (oral fleet) one day before the procedure. Additionally, patients were kept nil by mouth for six hours before colonoscopy.

Colonoscopy was performed by experienced endoscopists according to the current practice at the Endoscopy Unit, HPUSM using Olympus system. For patients with normal colonoscopy findings, indicating the absence of any visible pathological changes, we obtained biopsy specimens from two specific sites of the bowel mucosa: the sigmoid colon and the rectum. These locations were selected due to their representativeness and their common use in the evaluation of colonic microbiota in healthy individuals.

In cases where the colonoscopy yielded positive results, indicating the presence of tumours, biopsy samples were taken directly from the tumour sites. This targeted approach was designed to explore the potential correlation between specific microbial populations and tumourigenesis, in line with our hypothesis that certain organisms may contribute to tumour occurrence and exhibit higher concentrations at the site of pathology.

All biopsies were taken using the biopsy forceps (alligator jaw) with diameter of 2.8 mm. These biopsies were sent to the Microbiology Laboratory, HPUSM for cultivation.

Bacterial counts in biopsy samples

For cultivation (i.e., on either aerobic blood agar, anaerobic blood agar, Mc-Conkey agar), five quadrant

streak method was used. Growths were inspected and followed by purification to enable identification procedure (gram stain). Subsequently, identification using Biomerium Vitek 2 systems took place. The test reactions from Vitek machine were transmitted to optical system for interpretation of test reactions using different wavelengths in visible spectrum.

After calculations of raw data had been completely performed, test reaction results would appear as "+", "-", "(-)" or "(+)". Reactions that appear in parentheses are indicative of weak reactions that are too close to test threshold. If a unique identification pattern was not recognized, a list of possible organisms was given, or the strain was determined to be outside database scope.

Ethical approval

An ethical clearance was obtained from the Human Research Ethics Committee, Universiti Sains Malaysia (USM) in Kelantan, Malaysia (No. USM/JEPeM/274.3.(6)).

Data analysis

Descriptive statistics were used to summarise the demographics and clinical characteristics of subjects. Numerical data were presented as means (standard deviation, SD). Categorical data were presented as frequency (percentage). The mean of bacterial colonies present in the mucosa of sigmoid colon tumour and rectal tumour were compared with the mucosa of normal sigmoid colon and rectum, respectively using the independent samples t-tests. Statistical analysis was carried out using IBM SPSS Statistics for Windows ver. 22.0 (IBM Corp., Armonk, NY, USA). The limit of significance was set at 0.05.

RESULTS

Patient demographics and clinical characteristics

A total of 66 patients were recruited in this study after completed the colonoscopy procedure. Thirty-three patients were selected after the colonoscopy showed normal findings (no pathological causes of the symptoms) and another 33 patients were selected after the colonoscopy showed either sigmoid or rectal growth or mass. Overall, the

youngest patient who underwent the colonoscopy was 23 years old and the oldest patient was 88 years old with the mean age of 57.3 years and standard deviation of 14 years.

Majority of the patients were Malay (95%) and only 5% were Chinese. This is consistent with demographic data of Kelantan, Malaysia population where more than 90% of populations are Malay. More male patients presented with the bowel symptoms (n = 39) and 19 patients having positive results of tumour. Out of 27 female patients, 14 patients turn out to have malignancy (Table 1).

Bacterial colonies present in the mucosa of normal sigmoid colon and rectum

Supplemental Figure 1 shows mean of bacterial colonies present in the mucosa of normal sigmoid colon and rectum. About 40 types of bacteria were isolated from both normal sigmoid colon and rectum, and almost all bacteria were anaerobic. The commonest bacteria that were isolated in normal sigmoid colon include *Escherichia coli* (*E. coli*), *Klebsiella pneumonia*, *Collinsella aerofaciens*, *Bacteriodes caccae* and *Fusobacterium mortiferum*. Meanwhile, the commonest bacteria that

were isolated in the mucosa of normal rectum were *E. coli*, *Prevotella ovalis*, *Streptococcus sanguinis*, *Bacteriodes caccae*, *Bacteriodes thetaiotaomicron* and *Enterococcus cloacae*.

There were a few types of bacteria that were isolated only in the normal sigmoid colon or rectum. The bacteria that were identified only in the sigmoid colon were *Bifidobacterium* species, *Plesiomonas shigellois*, *Leuconostoc pseudomesenteroides* and *Comamonas testosteroni*. Types of bacteria that were isolated only from normal rectum include *Staphylococcus hominis*, *Eggerthella lenta*, *Clostridium subterminale*, *Enterococcus hirae*, *Lactobacillus* species, *Peptoniphilus asaccharolyticus* and *Streptococcus sanguinis*.

This result also showed that there was no significant difference in terms of types of bacteria that were present in the mucosa of both normal sigmoid colon and rectum. *Prevotella intermedia*, *Aeromonas hydrophila*, *Streptococcus salivarius*, *Streptococcus parasanguinis*, *Proteus vulgaris*, *Actinomyces naeslundii*, *Fusobacterium varium*, *Enterococcus raffinosus* and *Bacteriodes stercoris* were present in equal number in both normal sigmoid colon and rectum.

Other bacteria appeared to be more in rectum than sigmoid colon. These include non-fermentative *E. coli*, *Klebsiella pneumonia*, *E. coli*, *Streptococcus gallolyticus*, *Streptococcus agalactiae*, *Enterococcus faecium*, *Streptococcus mitis*, *Prevotella oralis*, *Fusobacterium nucleatum*, *Bacteriodes thetaiotaomicron*, *Enterococcus cloacae*, *Parabacteriodes distasonis* and *Burkholderia cepacia*. In overall however, bacterial colonisation or community appeared to be more in rectum than sigmoid colon.

Comparison of bacterial colonies present in the mucosa of sigmoid colon tumour and normal sigmoid colon

Supplemental Figure 2 shows the mean of bacterial colonies present in the mucosa of sigmoid colon tumour and normal sigmoid colon. From the result, there were certain bacterial species that were present only in sigmoid colon tumour or normal sigmoid colon. *Clostridium subterminale*, *Fingoldia magna*, *Eggerthella lenta*, *Parvimonas micra*, *Streptococcus mutans*, *Morganella morganii*, *Clostridium bifermentans*, *Enterococcus avium* and

Table 1 Demographic data of the two groups (control patients, n = 33 and patients with tumour, n = 33).

	Control	Patients with tumour
n	33	33
Mean age, years (SD)	52.58 (13.75)	61.94 (12.72)
Age range, years	23 - 81	26 - 88
Male, n (%)	20 (60.6)	19 (57.6)
Symptoms of presentation, n (%)		
Abdominal pain	8 (24.2)	15 (45.5)
Abdominal distension	3 (9.1)	8 (24.2)
Vomiting	1 (3.0)	9 (27.3)
Per-rectal bleeding	16 (48.5)	22 (66.7)
Altered bowel habit	23 (69.9)	27 (81.8)
Tenesmus	8 (24.2)	24 (72.7)
Mucus	8 (24.2)	22 (66.7)
Constitutional symptoms	9 (27.3)	21 (63.6)

beta haemolytic streptococcus were present only in sigmoid colon tumour.

There were several bacteria which appeared to be more predominant in sigmoid colon tumour compared to normal sigmoid colon. These bacteria include *Enterococcus gallinarum*, *Streptococcus mitis*, *Prevotella intermedia*, *Enterococcus faecalis*, *Bacteriodes fragilis*, non-fermentative *E. coli*, *Bifidobacterium* species and *Proteus mirabilis*.

Aeromonas hydrophila, *Klebsiella pneumonia*, *E. coli* and *Collinsella aeroficiens* were present in both mucosa of sigmoid colon but more dominant in normal sigmoid colon. The rest of the bacteria were only present in the mucosa of normal sigmoid colon.

Table 2 shows a comparison of the mean of bacterial colonies present in the normal sigmoid colon and sigmoid colon tumour. The results showed that *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were only present in sigmoid colon tumour and were significantly associated with sigmoid colon cancer ($p = 0.041$).

Comparison of bacterial colonies present in the mucosa of rectal tumour and normal rectum

Supplemental Figure 3 shows the mean of bacterial colonies that have been identified in the mucosa of rectal tumour in comparison with normal rectum. Almost all bacteria were present in both mucosa of rectum but more predominant in normal rectum. However, there are a few species which highly colonised rectal tumour. These include *Comamonas testosteroni*, *Staphylococcus hominis*, *Streptococcus parasanguinis*, *Enterococcus gallinarum*, *Streptococcus mitis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Streptococcus dysgalactiae*, *Prevotella intermedia*, *Morganella morganii*, *Actinomyces meyeri*, *Enterococcus faecalis*, *Clostridium bifermentans*, *Enterococcus avium*, *Bifidobacterium* species, *Clostridium perfringens*, beta-haemolytic streptococcus, *Aeromonas sobria* and *Proteus mirabilis*.

Table 3 shows a comparison of the mean of bacterial colonies present in the normal rectum and rectal tumour. Even though, more than 15 types of bacteria

Table 2 Comparison of the mean of bacterial colonies present in the mucosa of sigmoid colon cancer and normal sigmoid colon

Bacteria	Sigmoid colon	Mean (SD)	t-statistic	p-value*
<i>Clostridium subterminale</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.63 (1.768)		
<i>Finegoldia magna</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.63 (1.768)		
<i>Enterococcus avium</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.63 (1.768)		
<i>Eggerthella lenta</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.38 (1.061)		
<i>Parvimonas micra</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.38 (1.061)		
<i>Clostridium bifermentans</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.38 (1.061)		
beta-hemolytic streptococcus	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.38 (1.061)		
<i>Streptococcus mutans</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.25 (0.707)		
<i>Morganella morganii</i>	Normal	0.00 (0.000)	-2.118	0.041
	Tumour	0.13 (0.354)		

*The independent samples t-test was applied.

Table 3 Comparison of the mean of bacterial colonies present in the mucosa of rectal cancer and normal rectum

Bacteria	Rectum	Mean (SD)	t-statistic	p-value*
<i>Proteus mirabilis</i>	Normal	0.18 (0.635)	-2.102	0.040
	Tumour	0.80 (1.528)		
<i>Morganella morganii</i>	Normal	0.00 (0.000)	-2.038	0.046
	Tumour	0.28 (0.792)		

*The independent samples t-test was applied.

were isolated more in the rectal tumour in comparison with the normal rectum, only two bacteria were significant in promoting rectal cancer which were *Proteus mirabilis* ($p = 0.040$) and *Morganella morganii* ($p = 0.046$).

DISCUSSION

The usage of bowel cleanser might have changed the composition of bacterial populations^{15,16} or may not have any effects¹⁷, and bacteria which still present are termed mucosal-associated bacteria.¹⁸⁻²⁰ In addition to that, different bacteria location may have different bacteria amount, i.e., between bacteria in faeces and mucosa of large bowel.¹⁹

From the results, we can see that *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were present in mucosa of both normal sigmoid colon and rectum with the highest frequency of *E. coli*. Among all aerobic gram-negative bacilli (AGNB), *E. coli* is the only bacteria that reside in gut of healthy people. It has been postulated that AGNB receptor was expressed on healthy mucosa lining of bowel but are covered by protective fibronectin layer.²¹ This postulation maybe true as in this study, we can see that even though biopsy was taken from mucosa of normal bowel, patient might have an underlying acute or chronic illness as presented with bowel symptoms which render them for colonoscopy procedure.

From this study, almost all bacteria isolated from sigmoid colon and rectum were anaerobes such as *Bacteroides*, anaerobic *Streptococcus* and *Enterococcus* species. These show that Asians still harbour anaerobic bacteria as the commonest commensal flora compared to Western populations^{22,23} and this may result from dietary intake and lifestyle of Asians.²⁴

Previous study found anaerobic bacteria to be predominant in human colonic wall^{25,26} with the commonest bacteria present in rectal population.^{27,28} From the current study, more bacteria populations are found in rectum compared to sigmoid colon mucosa with several types of bacteria only present in rectum. These findings are supported by past researches^{19,29,30}, however there are also researches that contradict these findings.³¹ This research show that rectal bacterial populations were more common than sigmoid colon and this can be postulated that it

may be a mixture of both bacteria from faecal remnant inside the rectum and also mucosa-associated bacteria. Previous study had highlighted the association of *Streptococcus bovis* with an increased risk of developing colorectal cancer with 94% of *S. gallolyticus* subspecies *gallolyticus* (biotype 1) bacteraemia have been shown to be associated with colorectal cancer while 18% was associated with *S. bovis* biotype 2.^{32,33}

A large variety of plant derived substances are present in human diet such as lignans and flavonoids which belong to polyphenols group. Few species of bacteria have been identified which can catalyse conversion of isoflavonoids and ligands into active metabolites which includes *Bacteroides*, *Clostridium*, *Eggerthella*, *Eubacterium* and *Ruminococcus*³⁴. This finding in reverse is contradicting with our study in which, we found out that nine types of bacteria were only present in sigmoid colon tumour compared to normal sigmoid colon mucosa and was highly significant in causing colorectal cancer. These include *Clostridium subterminale*, *Fingoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bif fermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii*. Meanwhile, *Enterococcus gallinarum*, *Streptococcus mitis*, *Prevotella intermedia*, *Enterococcus faecalis*, *Bacteriodes fragilis*, non-fermentative *E. coli*, *Bifidobacterium* species and *Proteus mirabilis* were present in both groups but more predominant in sigmoid colon tumour. Based on this result, we can postulate that even though dietary substances may have a chemo protective effect, there are still more metabolites produced by gut bacteria which can induced the carcinogenesis process.

Bile acids are the end products of cholesterol metabolism. Cholic acid and chenodeoxycholic acid which constitute primary bile acids will be dehydroxylated and converted into secondary bile acids, deoxycholic acid and lithocolic acid in large bowel by colonic bacteria.³⁵ Few bacteria which have the ability to conjugate bile acid include *Bacteriodes* species, *Veillonella* species, *Clostridium* species, *Staphylococcus aureus*, *Streptococcus faecalis* and *Bifidobacteria*. These bacteria with different degrees of conjugation ability, through cholate-7-dehydroxylase enzyme, will breakdown 7-OH group from cholic acid thus producing deoxycholate.^{36,37}

Few studies have been reported regarding association of bile acids with risk of developing rectal cancer in which the cytotoxic metabolites lead to induction of colorectal cancer.³⁸ With this finding, our study has been supported with *Clostridium* species have been isolated and significantly associated with sigmoid colon tumour.

Compared to sigmoid colon cancer, only two types of bacteria have been identified to be significantly associated with rectal cancer which were *Proteus mirabilis* and *Morganella morganii*. Even though no direct association between *Proteus mirabilis* and human colorectal cancer has been published, an animal study conducted found that *Proteus mirabilis* and *Klebsiella pneumoniae* were present in *T-bet*^{-/-} x *Rag2*^{-/-} ulcerative colitis (TRUC) mice and was also found in faeces of the TRUC mice compared to faeces of normal mice. Our concern is on inflammatory bowel disease as one of the risk factors of developing colorectal cancer and with this data, we can postulate that *Proteus mirabilis* and *Klebsiella pneumoniae* appeared to be more predominant in mucosa of inflammation bowel and indirectly associated with colorectal cancer.³⁹

Morganella morgani has been identified as one of Sulfate-reducing bacteria (SRB) in which it conducted sulphate reduction process thus producing a great quantity of sulphide to obtain energy. This process in turn would produce the end metabolites, hydrogen sulphide which is a cytotoxic compound that will inhibit oxidation of butyrate. Butyrate is the main energy supplier for colonocytes, and this inhibition will impair intestinal epithelial cell functions that will lead to cell death and finally chronic inflammation.⁴⁰ This fact showed *Morganella morganii* also inhibit colonic mucosa of inflammatory bowel which in turn predisposed to development of colorectal cancer.

There were certain limitations within this current study. Firstly, as this study was only comparing the type of bacteria in sigmoid colon and rectal mucosa, the difference of bacterial colonies diversity was not well seen especially in the sigmoid colon tumour compared to rectal tumour biopsy samples. This is because only eight samples of sigmoid colon tumour were collected in this study compared to 25 samples of rectal tumour. This study also only focused on the

SUMMARY BOX

What is known about the subject

- Normal gut bacterial flora play a direct or indirect role in the initiation and progression of tumour by inducing chronic inflammation and producing toxins and metabolites.
- Identifying the gut bacterial species involved could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

New findings

- *Clostridium subterminale*, *Fingoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta-hemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were associated with the risk of developing sigmoid colon cancer while *Proteus mirabilis* and *Morganella morganii* were associated with rectal cancer.
- This study broadens our knowledge on the gut bacterial species involved in colorectal cancer which could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

left side of the large bowel which most probably have the similar types of bacterial residing the mucosa and only involved samples from the normal mucosa in comparison with tumour tissues. While we implemented standard bowel preparation protocols for all participants to minimize dietary confounding, other potential confounders such as medication use and comorbidities were not comprehensively adjusted for. Future research should aim to incorporate more rigorous adjustments for these factors to better isolate the relationship between gut microbiota and colorectal cancer risk.

As a recommendation, biopsy of the colonic mucosa should be routinely taken during the colonoscopic procedure and send for cultivation. Our study followed the standard cultivation practices used in our hospital for the analysis of bacterial species from

biopsy samples. The use of molecular techniques, such as 16S rRNA sequencing, would provide a more comprehensive bacterial profiling. Even though no pathology was found during this procedure, identification of certain bacterial which significantly associated with sigmoid colon and rectal cancer can gives us a clue that the patient may have some underlying diseases which predisposed them to the development of malignancy later in life and more frequent colonoscopic screening can be applied to detect the cancer at an early stage. There shall be a study conducted for longer duration with more study samples and to compare the types of bacterial residing both the left and right side of the large bowel. This should include identifying the type of bacterial that present in the tumour involving the right and left colon and to see the difference in total number of bacteria as well as in the diseases that significantly associated with colorectal cancer such as patients with polyps and inflammatory bowel diseases.

From the results of the present study, we can conclude that there were a significant number of

bacterial species which can be isolated from mucosa of the large bowel. There were more bacteria present in mucosal of rectum compared to sigmoid colon mucosa with no significant difference in terms of type of bacteria present. There was a slight difference on bacterial type present in normal mucosa of the sigmoid colon and rectum in comparison with the type of bacteria present in sigmoid colon and rectal tumour. *Clostridium subterminale*, *Fingoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* has been identified as being significantly associated with sigmoid colon cancer while *Proteus mirabilis* and *Morganella morganii* were significantly associated with rectal cancer.

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