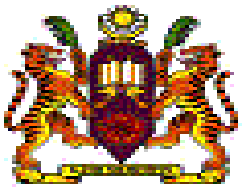
GUT MICROFLORA: ASSOCIATION AND RISK OF SIGMOID COLON AND RECTAL CANCER

DR FAIZAH MOHAMED SIKANDAR

MD (USM)

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE (GENERAL SURGERY)



UNIVERSITI SAINS MALAYSIA

APEX

2015

TABLE OF CONTENTS **PAGE**

1. ACKNOWLEDGEMENT

This dissertation is a proved of hardworking and sacrifice of many people especially my lecturers and my family. The unbearable obstacles and hard times were not over without their guidance and support to me in completing this dissertation. A special compliment goes to my former head of department, Dr Mohd Nor Gohar, my supervisor as well as my new head of department, Dr Zaidi Zakaria, my Co-Supervisors, Dr Siti Rahmah Hashim Isa Merican, Dr Ikhwan Sani Mohamad and Dr Siti Asma Hassan (from microbiology department) for their guidance, advice and the time spent to complete up this dissertation.

A million thanks also I rewarded to Dr Syed Hassan Syed Abdul Aziz, Dr Andee Dzulkarnean Zakaria, Dr Wan Zainira Wan Zain, Dr Nizam Mohd Hashim, Dr Rosnelifaizur Ramely and all the endoscopy room staffs for their commitment in assisting me to complete my study samples.

I would like to thank Dr Ismail Ibrahim from the community medicine department, Dr Saiful Bahari from department of Public Health and Puan Anis Kausar (statistician) for statistical advice. Without their support, this dissertation would not have been complete.

A great appreciation also I dedicated to Puan Fazilah Ahmad, the senior microbiology lab staff who had spent a lot of time patiently in helping me to analyse the study samples and without her effort, I could not achieve this until this stage.

I would like to convey my deepest love and thanks to my beloved husband, my children and my parents as well as my family who continuously supported me physically and emotionally in completing this dissertation. Last but not least, to all my fellow colleagues and to others who are not mentioned by name, thousands of thanks I forwarded for their advice and guidance

II. ABBREVIATION

Gastrointestinal	GI
Superior Mesenteric Artery	SMA
Colorectal Cancer	CRC
Gastrointestinal Stromal Tumour	GIST
Familial Adenomatous Polyposis	FAP
Hereditary Non Polyposis Colonic Carcinoma	HNPCC
Inflammatory Bowel Syndrome	IBD
Ulcerative Colitis	UC
Short Chain fatty acids	SCFA
Lactic acid producing bacteria	LAPB
Polycyclic Aromatic Hydrocarbon	PAH
Interleukin-8	IL-8
Mitogen Activated Protein Kinases	MAPKs
Surgical Outpatient Daily Clinic	SOPD
Hospital Universiti Sains Malaysia	HUSM
Brain Heart Infusion	BHI
Degree Celsius	°C
Benign prostatic hyperplasia	BPH

Chronic obstructive airway disease	COAD
Carcino-embryogenic antigen	CEA
Computed Tomography Scan	CT scan
Magnetic Resonance Imaging	MRI
Aerobic Gram Negative Bacilli	AGNB
T-*bet*$^{-/-}$ x *Rag*2$^{-/-}$ Ulcerative Colitis	TRUC
Sulfate Reducing Bacteria	SRB
Gram Negative	GN
Gram positive	GP
Anerobic colony	ANC

III. LIST OF FIGURES

IV. LIST OF TABLES

V. ABSTRACT (ENGLISH)

BACKGROUND: Based on published data that few bacterial species (normal flora) of the Gastrointestinal (GI) tract can promote carcinogenesis of the large bowel, a study was carried out to identify types of bacteria that present in tumour of the sigmoid colon and rectum in comparison with mucosa of the normal sigmoid colon and rectum and its association with the malignancy of the sigmoid colon and rectum.

METHOD: Total of 66 patients, who presented with bowel symptoms, underwent colonoscopy procedures and was divided into two groups (normal and cancer group). Two biopsies were taken at the sigmoid colon and rectum (one biopsy each part) from 33 patients with normal colonoscopy results. One biopsy was taken from the tumour site, either at the sigmoid colon or rectum from the other 33 patients with positive colonoscopy findings. These biopsied samples were sent to the microbiology lab for cultivation and bacterial identification.

RESULTS: More than 30 types of bacteria were isolated from the mucosa of the normal sigmoid colon and rectum with few differences in the species. The bacterial colonization appeared to be more in the rectum compared to the sigmoid colon. *Proteus mirabilis, Beta haemolytic streptococcus, Enterococcus avium, Clostridium bifermentans, Morganella morganii, Streptococcus mutans, Parvimonas micra, Eggenthella lenta, Clostridium subterminale* and *Finegoldia magna* are significantly associated with sigmoid colon carcinoma with (p<0.05). *Proteus mirabilis* and *Morganella morgana* were significantly associated with rectal carcinoma (p<0.05).

CONCLUSION: Our study suggested that there is a difference in the type of bacterial species in different part of the bowel (between sigmoid colon and rectum) with more bacterial

colonies in the rectum compared to sigmoid colon and there was an association of certain bacteria with an increased risk of developing carcinoma.

VI. ABSTRAK (BAHASA MELAYU)

LATAR BELAKANG: Berdasarkan data yang diterbitkan mengenai beberapa spesies bakteria (flora normal) daripada saluran gastrousus (GI) boleh menggalakkan karsinogenesis usus besar, satu kajian telah dijalankan untuk mengenal pasti jenis bakteria yang hadir dalam kanser kolon sigmoid dan rektum berbanding dengan mukosa kolon sigmoid normal dan rektum dan kaitannya dengan kanser kolon sigmoid dan rektum.

KAEDAH: Seramai 66 orang pesakit yang mempunyai symptom / gejala usus, menjalani prosedur kolonoskopi dan dibahagikan kepada dua kumpulan iaitu kumpulan normal dan kumpulan cancer. Dua biopsi diambil di kolon sigmoid dan rektum (satu biopsy setiap bahagian) daripada 33 pesakit yang mendapat keputusan kolonoskopi normal. Satu biopsi diambil dari kedudukan tumor, sama ada di kolon sigmoid atau rektum daripada 33 pesakit lain yang mempunyai kolonoskopi positif. Sampel biopsi ini telah dihantar ke makmal mikrobiologi bagi pemeliharaan/pembiakan/penamaan dan pengenalan bakteria.

KEPUTUSAN: Lebih daripada 30 jenis bakteria telah diasingkan daripada mukosa kolon sigmoid normal dan rektum yang mempunyai beberapa perbezaan pada spesies. Proses pengkolonian bakteria kelihatan lebih dalam rektum berbanding kolon sigmoid. *Proteus mirabilis, Beta haemolytic streptococcus, Enterococcus avium, Clostridium bifermentans, Morganella morganii, Streptococcus mutans, Parvimonas micra, Eggenthella lenta, Clostridium subterminale* dan *Finegoldia magna* adalah signifikan berkaitan dengan kanser kolon sigmoid dengan (p<0.05). *Proteus mirabilis* dan *Morganella morgana* adalah signifikan dikaitan dengan kanser rektum (p<0.05).

KESIMPULAN: Kajian kami menunjukkan bahawa terdapat perbezaan pada jenis bakteria di bahagian yang berbeza di usus (antara kolon sigmoid dan rektum) dengan lebih koloni bakteria di dalam rectum berbanding kolon sigmoid dan terdapat perkaitan pada bakteria tertentu dengan peningkatan risiko untuk perkembangan kanser.

1. INTRODUCTION

1.1 ANATOMY OF THE LARGE INTESTINE

The large bowel begins from the ileo-caecal valve and extends to the anus. It has a variable diameter about 150cm in length and divided anatomically and functionally into the colon, rectum and anal canal. The colon consists of a caecum, ascending colon, transverse colon, descending colon and sigmoid colon. The caecum represents the proximal part of the colon with an average length of 10cm. The ascending colon, about 15cm in length runs upward toward the liver on the right side and it is fixed against the retro-peritoneum posteriorly, whereas the lateral and anterior surfaces are true intra-peritoneal structures. The transverse colon is about 45cm in length and is hanging between the fixed positions of the hepatic and splenic flexures. It is completely covered by the visceral peritoneum. The descending colon lies ventral to the left kidney and extends downward from the splenic flexure for about 25cm. It is smaller in diameter compare to ascending colon but share the same character where it is fixed posteriorly against the retro-peritoneum. The sigmoid colon varies in length from 15 to 50cm and is very mobile. Although it is usually located in the left lower quadrant, its redundancy and mobility can result in a portion of the sigmoid colon residing in the right lower quadrant **(Courtney M. Townsend, 2012).**

The rectum is about 12 to 15cm in length and occupies the curve of the sacrum in the true pelvis and the posterior surface is almost completely extra-peritoneal as it is adhere to the pre-sacral soft tissues and thus is outside the peritoneal cavity. Anal canal begins at the ano-rectal junction and terminate at the anal verge. It measures 2 to 4 cm in length and is generally longer in men than in women.

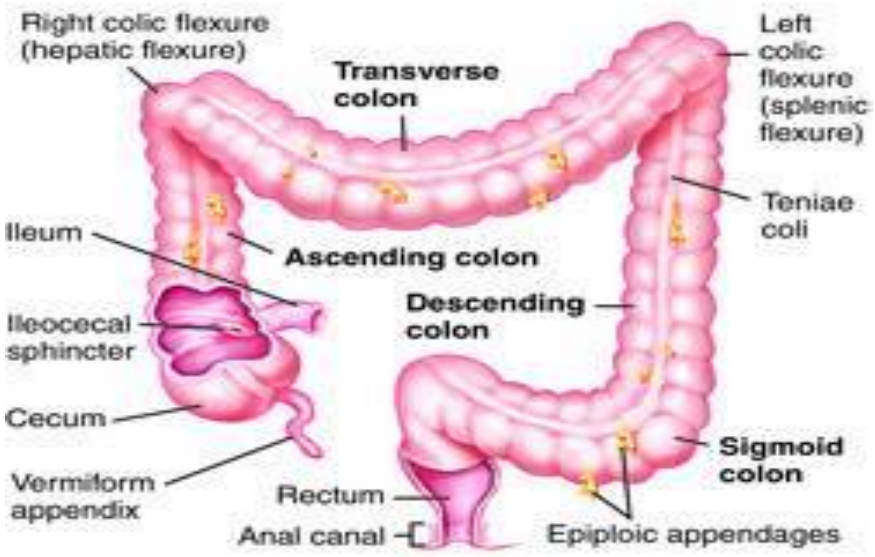


FIGURE 1: Different parts of large bowels

(Diagram taken with permission from Mosby's Medical Dictionary, 8th edition, 2009)

In general, the superior mesenteric artery (SMA) branches into the ileo-colic, right colic, and middle colic artery. The ileo-colic artery will supply the terminal ileum and proximal ascending colon, right colic artery supplying the ascending colon and the middle colic artery will supply the transverse colon. The inferior mesenteric artery branches into left colic artery which supplies the descending colon, several sigmoidal branches, which supply the sigmoid colon and superior rectal artery, which supplies the proximal rectum. The remaining part of the rectum receives their blood supply from the middle rectal artery which arises from the internal iliac and the inferior rectal artery which arises from the internal pudendal artery (branch of the internal iliac artery). These rich networks of blood supply make the rectum relatively resistant to ischemia **(F. Charles Brunicardi, 2007).** Anal canal as a continuation of the rectum also received the arterial supply from the superior rectal artery (upper part) while lower anal canal received an arterial supply from the inferior rectal artery.

The veins of the colon and rectum follow their corresponding arteries and bear the same terminology except for the inferior mesenteric artery. It ascends in the retroperitoneal plane over the psoas muscle and continues posterior to the pancreas to join the splenic vein and drains into portal vein. The superior rectal vein drains into the portal system via the inferior mesenteric vein, the middle rectal vein drains into the internal iliac vein and the inferior rectal vein drains into the internal pudendal vein, and subsequently into the internal iliac vein.

1.2 BACTERIA ASSOCIATED WITH CANCER / MALIGNANCY

On March 24, 1882 Robert Koch presented his famous lecture at the Physiological Society in Berlin, suggesting that tuberculosis is caused by a bacterium. It was probably the surprising discovery of the infectious aetiology of tuberculosis – a disease which until then was not suspected to be caused by infectious agent. This fact had turned the interest of microbiologists at the end of the nineteenth century towards a possible infectious cause of other chronic conditions, such as cancer. Later in the twentieth century was indeed the century of Modern Medicine with tremendous strides made in understanding and control of infectious disease, as well as introduction of life saving antibiotics and vaccines. Unfortunately, along with this advances came the perils of genetic engineering, the increasing threat of newly emerging viruses, bio-warfare and bioterrorism. Despite these scientific achievements, the cause of cancer remains a mystery. Scientists suspect genetic susceptibility, possible cancer-causing viruses, and environmental factors might play a role in some cancers, but none of these factors explained why millions of people die yearly from a varies of malignancies **(Jr., 1990)**

It was in 1863, when Rudolf Virchow suggested the association between inflammation and cancer after he noted the presence of leukocytes in neoplastic tissues. He finally make a conclusion that this findings was a mirrored of tumour infiltration at sites of chronic inflammation **(Mairi E. Hope, 2005).** Since then, there was an increasing number of evidence supporting the involvement of microflora in the chronic inflammation of the colon and leading to development of colorectal cancer. After the year of 1980s, there has been a dramatic increase in research of infections and cancer. It has been reported in 2002 that infectious agents accounted for 18% of all cancer worldwide and this estimation was based

on the burden of disease associated with cancers that have known infectious aetiologies such as cervical, liver and gastric cancer **(Andrea N. Burnett-Hartman, 2008), (Parkin, 2006).**

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 **(Kyotaro Kanazawa, 1996).** 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 **(Moore, 1995)**.

This study was conducted to identify types of bacteria that present in both the mucosa of normal sigmoid colon and rectum and types of bacteria that present in the sigmoid colon and rectal tumour. This study was also carried out to see whether there is any association or predominance of certain bacterial with rectal cancer.

2. LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF SIGMOID COLON AND RECTAL CARCINOMA

For long time, colorectal cancer has been considered a disease of western developed countries. With its high mortality and incidence, colorectal cancer (CRC) constitutes a health burden in most industrialized countries. Worldwide, Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women and contributed for the third most common cause of cancer-related deaths **(D. Max Parkin, 2005).** There were so many previous studies have reported regarding the rapid increases in colorectal cancer incidence rates, particularly in developing countries in many parts of the world, and these increases are thought to reflect changing dietary and physical activity patterns. Due to the high incidence and mortality in Western populations, CRC has been progressively studied in these countries.

The highest rates of CRC have been reported in developed countries, including the United States, Canada, Australia, and north-western Europe. Comparatively, less number of cases is observed in Asian, African, and South American countries although incidence rates are increasing in countries that were previously considered low incidence due to increased in health awareness and early detection of the disease. **(Pourhoseingholi, 2012)**. This However, there is an increase in colorectal cancer incidence rates in women compared with men worldwide which may reflect the slower adoption of certain risk behaviours associated with colorectal cancer. This includes, changes in smoking behaviour, whereby traditionally lags several decades in women compared with men, with peak prevalence occurring at a much lower rate **(Melissa M. Center, 2009a; Melissa M. Center, 2009b)**. Other risk factors for colorectal cancer include obesity, a diet low in fruits and vegetables, physical inactivity, and smoking.

In Asia, the incidence and mortality of colorectal cancer (CRC) is rising rapidly with CRC is the third most common malignant disease in both men and women. Data from the International Agency for Research on Cancer shows that the incidence in many affluent Asian countries is similar to that in the West. Among ethnic groups in Asia, Chinese has higher incidence of CRC compared to other races and according to the Chinese National Cancer Database of 2003, CRC together with lung and female breast cancer had a rapid increasing incidence in the country between 1991 and 2005 **(Pourhoseingholi 2012)**. Leung at al. in his study regarding colonoscopic survey in patients who presented with bowel symptoms also found that, 42.3% out of 1634 patients who have endoscopic polyps or tumor during the colonoscopic procedure were Japanese. Only 8.5% of them were Indians **(Wai K. Leung, 2006)**. It seems that ethnicity has an important etiological role but the incidence, anatomical distribution and mortality of CRC among Asian populations however are not different from those in Western countries.

In Malaysia, the first report of the National Cancer Registry which covers the period of one year from 1st January 2002 to 31st December 2002 showed a total of 26,089 cancers were diagnosed among all residents in Peninsular Malaysia in the year 2002, with 11,815 involving the males and 14,274 females. In the regional cancer registry survey in 2002, CRC was the tenth leading cancer among males The top cancer in males was lung cancer, followed by nasopharynx, stomach, urinary bladder, rectum, non-Hodgkin's lymphoma, larynx, liver, colon and oesophageal cancer. In females, the commonest malignancies were cervix, breast, ovary, lung, nasopharynx, oesophagus, thyroid, colon, rectum and non-Hodgkin's lymphoma **(Lim, 2002).** However, in the latest Malaysian Cancer Registry 2007, colorectal has become the second leading cancer in male after lung cancer and female after breast cancer **(Zainal Ariffin Omar, 2011)**

Cancer of the rectum accounted for the fifth commonest cancer in males and the eighth in females which involved 6.4% of males and 3.4% females. Chinese ethnicity had the highest incidence followed by Indians and Malays, whereby the cumulative lifetime risk for Chinese males was 1 in 48, for Indian males was 1 in 71, and for Malay males was 1 in 91. The difference in incidence between these populations the possible bring the possibility of the genetic factors play in the aetiology of colorectal cancer**. (Melissa M. Center 2009), (K.L. Goh, 2005).** The difference in incidence between genders in rectal cancers has been observed to be age related. Below the age of 60 years, the disease occurred almost equally among males and females. Thereafter the frequency in males rose more rapidly. The incidence of rectal cancer increased exponentially with age **(G. C. C. Lim, 2003).** A study conducted by Hospital University Sains Malaysia in 2010 to identify the prognostic factor for patients with colorectal cancer in 10 years' time (from 1996-2005) found that, the overall 5-years survival rate for colorectal patients in HUSM was 34.3%. This results showed this rate was lower compared to the overall 5 years survival rate in developed countries such as Australia where it reached up to 50% or more **(Anis Kausar Ghazali, 2010).**

2.2 RISK FACTOR FOR SIGMOID COLON AND RECTAL CANCER

The commonest type of colorectal cancer was adenocarcinoma where it constitutes 70% of all malignancy arising from the large bowel. Other types of tumour that can occur in the large bowel include carcinoid (neuroendocrine) tumour, gastrointestinal stromal tumour (GIST) and lymphoma.

There are many factors controlled the risk of colorectal cancer; some of these factors are modifiable and others are not. The non-modifiable risk factors that have been identified include a personal or family history of colorectal cancer or adenomatous polyps, and a personal history of chronic inflammatory bowel disease. The American Cancer Society and other organizations recommended that some people have the risk for colorectal cancer because of these conditions have been screening at an earlier age. Modifiable risk factors which have been associated with an increased risk of colorectal cancer include physical inactivity, obesity, and high consumption of red or processed meats, smoking, and moderate-to-heavy alcohol consumption. A recent published data has found that about one-quarter of colorectal cancer cases could be avoided by following a healthy lifestyle, eating a healthy diet, not smoking, and not drinking excessive amounts of alcohol (**Rick Alteri, 2011)**

1. Diet

Many epidemiological studies have shown than westernized diet, which is high in fat and low in fibre is associated with CRC and had suggested that limited consumption of red and processed meats, taking a variety of vegetables, fruits and non-processing grains will help reduce the risk of developing colorectal cancer **(Yuk Kei Yee, 2009).** Fibres increases the stool bulk and speeds transit of food through the colon, thus diluting the gut contents and perhaps reducing the absorption of carcinogens by the colonic mucosa **(Timothy J Key,**

2004). Epidemiological studies also have shown a positive association between alcohol intake and the development of hyperplastic and adenomatous polyps in the large intestine. In addition, heavy alcohol consumption will increased the risk of colorectal cancer **(T. Nosova, 1996).**

2. Familial risk factor

People with a first-degree relative (parent, sibling, or offspring) who has had colorectal cancer have 2 to 3 times risk of developing the disease compared to individuals with no family history. The risk is increased 3 to 6 times higher if the relative was diagnosed in younger age group or if there is more than one affected relatives. Charles et al in his study found that there is an increased risk of colorectal cancer among people with two or more affected relatives and an increased risk among people with a family history who were younger than 50 years of age **(Charles S. Fuchs, 1994).** Familial adenomatous polyposis (FAP) is a well-characterized genetic condition that has an inevitable risk of developing colorectal cancer if not treated. It is autosomal dominant inheritance and was first reported in the literature in 1861 and the hallmark of FAP is the propensity to develop hundreds to thousands of adenomatous colon polyps **(Jerome D. Waye, 2007).**

Hereditary non polyposis colonic carcinoma (HNPCC) is a more common but less obvious than FAP and is autosomal dominant inheritance. It is clinically similar to the sporadic type of CRC cases but more common in younger age group. In 1991, Lynch had classified HNPCC into 2 subtypes: Lynch 1 (is site specific colon cancer where individuals of a family are susceptible to colonic cancer but no cancers of other organs). Lynch 2: is cancer family syndrome where female members of the family are prone to breast and uterine cancer as well as colonic cancer. The cancer occurs predominantly on the right side of the colon and is low malignancy in contrast of sporadic cases.

3. Personal medical history

Study has shown that people who have had colorectal cancer before, are more likely to develop new cancers in other parts of the colon and rectum, even if the first cancer was completely removed. The risk of a second cancer is much greater if the first cancer is diagnosed at age 60 or younger. Many studies have found an association between diabetes and increased risk of colorectal cancer. Although type 2 diabetes (the most common type) and colorectal cancer share similar risk factors, including physical inactivity and obesity, a positive association between diabetes and colorectal cancer has been found after accounting for physical activity, body mass index, and waist circumference. A recent study suggests that the association may be stronger in men than in women **(Rick Alteri 2011)**

4. Physical inactivity

One of the most consistently reported relationships between colon cancer risk and behaviour is the protective effect of physical activity. Scientific evidence has shown that physical activity may reduce the risk of few types of cancer including cancer of the breast and colon, and can provide other important health benefits. For colon cancer, the movement of food through the intestine, will be accelerated by the physical activity and thus reducing the length time that the bowel lining is exposed to mutagens **(Tim Byers, 2002)**

5. Smoking

Early studies in 1950s and 60s investigating the association between smoking and colorectal cancer risk were generally null. With extended follow up, however, some studies appeared showing an increased risk among smokers. The Nurses' Health Study, for instance, found that cigarette smoking was unrelated to colorectal cancer when the uptake of smoking was less than 35 years ago, but 35-39 years after initiation of smokers of at least 10 cigarettes per day

has a 50% increase risk compared with non-smokers. Furthermore, risk of colorectal cancer depends on the intensity and duration of use, and the Cancer Prevention II cohort study showed the risk may fall with time since smoking cessation **(H. Kupper, 2000)**

6. Inflammatory Bowel Disease (IBD)

IBD consist of ulcerative colitis (UC) and Crohn's disease. The increase risk of CRC in patients with IBD mainly UC has long been recognized and it affects younger people more compared to sporadic type of CRC. People with ulcerative colitis or Crohn's disease may develop chronic inflammation of the large intestine, which increases the risk of colon cancer up to five times more than general populations depending on the site, extent and duration of the disease **(Tjalsma, 2012).**

2.3 NORMAL FLORA OF THE GASTROINTESTINAL TRACT

Human colon contains a unique microbial ecosystem composed by a large variety of bacteria mainly strict anaerobes. The large intestine is the site most heavily colonized by micro-organisms in the GI tract. It is estimated that the human body contains as many as 1014 bacteria cells and gastrointestinal tract is the most heavily colonized organ which contain over 70% of all the microbes. This vast number of microorganism collection that lives in peaceful coexistence with their hosts has been referred to as the microbiota, microflora or normal flora **(Sekirov I, 2010)**. Colonization of the GI tract occur during the first two years of life and became stable with average composition throughout the adulthood life **(Tjalsma, 2012).** Mucosal bacterial communities in the upper gastrointestinal (GI) tract and large bowel are difficult to study in healthy people, and until relatively recently, whereby more study have been conducted to find their composition, structure and function on the human colon.

Colonization of the GI tract started within a few days after delivery of the new born infants and the pattern of anaerobic colonization had been influence by the type of delivery. Within one week after vaginal delivery, the gut of full term infants was colonized by 61% of *Bacteroides fragilis* in comparison with only 9% in the infants delivered by caesarean section These findings showed that significant contamination had occurred during passage through the birth canal **(Gary L. Simon, 1986)**, **(Duerden, 1981)**. Most of oral bacteria are destroyed by the gastric acid and usually, less than 10^3 colonies are left and the commonest bacterial found are gram positive, anaerobic organism such as streptococci, staphylococci, lactobacilli and fungi. Towards the proximal small bowel, the bacterial concentrations start to increase with predominant species of *streptococci, staphylococci* and l*actobacilli* with small number of *Veillonellae* and *Actinomycos* species **(Finegold, 1969)**, **(B. S. Drasar, 1969)**. In the distal ileum, gram negative bacteria begins to outweigh the number of gram positive organisms

with anaerobic bacteria such as *Bacteriodes, Bifidobacterium, Fusobacterium* and *Clostridium* predominates. In the large bowel, starting from the caecum, the bacterial concentration increased sharply with anaerobic bacterial predominates the aerobes by 1000 fold and the commonest organisms are *Bacteroides, Bifidobacterium, Eubacterium* and few species of *Clostridium, enterococci* and *Enterobacteriaceae* **(Holdeman, 1975), (Shiner, 1969).**

There are many studies conducted to identify the bacterial that resides in the human bowels as the GI microflora research is very dynamic .More than hundred years ago, 239 novel of GI tract species have been described confirming the earlier nation that majority of the GI microorganisms are cultivable but not yet cultured **(Vos, 2014)** and faecal sample was cultured to identify the bacterial species that colonized the human gastrointestinal tract. Drasar et al. in his study found that non spores forming, rod-shaped anaerobic organisms predominates more than 99% of the human faecal flora. The commonest species present were *Bacteroides fragilis, Bifidobacterium adoloscentis* and *Eubacterium aerofaciens* while the remaining organisms are mainly *E.coli, Streptococcus viridans, Streptococcus salivarus* and *lactobacilli* **(Drasar, 1975)**. George et al. in his study comparing the microflora in humans and animals found that more than 60 different bacterial species have been isolated from the intestinal tract of humans as well as animals and the most common bacterial species was *Bacteroides*, enterobacteria, streptococci, Lactobacilli, clostridia, pseudomonads and bacilli **(Jr, 1965).**

Lactobacilli form part of the mucosal flora of the gastrointestinal tract and reside from the mouth to the rectum. In the large intestine, *Lactobacilli* are found in equal numbers to *E. coli* and higher than enterococci. The Lactobacillus species which dominate the oral cavity as well as the intestinal mucosa are *L. plantarum, L. rhamnosus* and *L. paracasei* species which have been isolated from 52%, 26% and 17% of healthy individuals,