East African Medical Journal Vol. 102 No. 6 June 2025
DIVERSITY OF MUCOSAL ASSOCIATED GUT MICROBIOTA IN COLORECTAL AND NON-COLORECTAL CANCER TISSUES FROM PATIENTS IN NAIROBI, KENYA
Millicent Ogutu (MSc-Tutorial Fellow), Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi., Kenya. Andrew Nyerere (PhD-Senior Lecturer), Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi, Kenya. Mourine Kangogo (PhD-Lecturer), Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi., Kenya. Shahin Sayed (MBChB, M.MED, PhD-Pathologist), Department of Pathology, Aga Khan University Hospital, Nairobi, P.O.BOX 30270-00100 Nairobi, Kenya.

Corresponding author: Millicent Ogutu, Department of Medical Laboratory Sciences Jomo Kenyatta University of Agriculture and Technology. E-mail: mogutu@jkuat.ac.ke

DIVERSITY OF MUCOSAL ASSOCIATED GUT MICROBIOTA IN COLORECTAL AND NON-COLORECTAL CANCER TISSUES FROM PATIENTS IN NAIROBI, KENYA

M. Ogutu, A. Nyerere, M. Kangogo and S. Sayed

ABSTRACT

Background: **There is growing evidence that gut microbiota could significantly influence the development of colorectal cancer (CRC) a malignancy that currently ranks among the top causes of cancer-related illness and death globally. This study was purposed to examine the microbial diversity found in both malignant and non-malignant colon tissues.**

Objective: **To determine the diversity of the colon bacterial flora in malignant and non-malignant tissues from the colon / rectum of patients attending the Aga Khan Cancer Clinic in Nairobi, Kenya.**

Design: **A retrospective case-control study**

Setting: **Aga Khan University Hospital and Jomo Kenyatta University of Agriculture & Technology**

Materials and methods: **A total of sixty tissue blocks comprising those with cancer (thirty) and those without (thirty) were examined. The main outcome measures were ; age, sex, colorectal malignancy, Colorectal non-malignancy, Cancer stage, Cancer grade, and subtype and type of microbiota. In addition history of changes in bowel habits, rectal bleeding, and abdominal pain were documented.**

Results: **The males were 18 (60%), while the females were 12 (40%) of the total (30).** ***Oscillospiraceae bacterium, Clostridales bacterium, Acetivibro sp, Eubacterium, Texcoconibacilllus texcoconensis*** **and** ***Staphylococcus sp*** **were found in malignant (CRC) tissues.**

Invasive adenocarcinoma was the most dominant subtype, followed by mucinous adenocarcinoma. Distribution by stage; stage I had three, stage II sixteen and stage III were eleven tissues.

***Conclusion*: This study shows that colorectal cancer patients have different patterns of mucosal-associated gut microbiota. These varied with sex and age. Further studies should be done to support the potential use of microbial markers for risk assessment and early CRC detection.**

INTRODUCTION

Colorectal cancer (CRC) presents a global health problem of tremendous proportions. It represents one of the top three cancers found among the world population and is a principal cause of cancer-related deaths[1]. Its reach extends well beyond high-income countries, where it has typically found a fertile incidence soil, to anchorage areas in less affluent parts of the globe, including several countries in Africa, where it has become an urban disease. Yet even this alarming picture deserves qualification. In Kenya, for instance, the World Health Organization's African Cancer Registry reports that CRC has become the country's second most common cancer (following that of the cervical uterus), with an incidence that is fast approaching what is found in many developed countries[2,3]. Death rates from CRC, meanwhile, have been rising steadily. At the same time, the gut microbiota (especially the mucosal-associated microbiome) appears to play an increasingly important role in the cancer's very pathology[4,5].

The human gut is home to trillions of microorganisms, which form a complex, largely uncharted ecosystem that controls metabolism, immunological responses, and inflammation[6]. Colorectal cancer (CRC) disrupts this ecosystem. Along with CRC, dysbiosis, an imbalance of microbial communities in the gut may take place prior to overt carcinogenesis and may impact the course of the disease[7,8]. The mucosal microbiome has emerged as a critical factor in colorectal cancer pathogenesis, with specific bacterial species shown to directly influence tumor initiation and progression[7,8]. In CRC, microbial community composition and colonic disease proximity appear to be major gut microbiome composition drivers [9,10]. In spite of the advancements, the majority of microbiome research in CRC has been carried out on cohorts from Western and Asian nations, with very few studies focusing on populations from Africa. This is particularly relevant since the composition of gut microbiota has been shown to vary widely based on geography, diet, and ethnicity [11,12,13,14]. When the dynamics of these microbes are not studied in populations that are largely understudied, the findings risk being non-generalizable. Nairobi, Kenya, is a particularly interesting site to study CRC and gut microbiota.

This research aimed to describe the diversity and composition of the mucosal gut microbiota in colorectal cancer compared to non-cancer patients in Nairobi.

MATERIALS AND METHODS

Design

A retrospective case-control study was conducted on Formalin Fixed Paraffin Embedded (FFPE) tissues collected from subjects with colorectal tumor.

Study site

The study was conducted at Aga Khan University Hospital (AKU) and the Jomo

Kenyatta University of Agriculture and Technology (JKUAT). AKU cancer clinic serves a diverse patient demographic from across Kenya and East Africa. It provides access to CRC patients across different stages, subtypes and age groups, which is essential for microbiota diversity studies. The 16SrRNA profiling and bioinformatics for microbial diversity analysis was carried out at the Pan African University Laboratory in JKUAT.

Using archived formalin-fixed paraffin-embedded colonic tissue samples and a retrospective case-control design, we attempted to associate microbial signatures with colorectal cancer in this understudied population.

Selection criteria

Confirmed diagnosis of CRC based on histopathology reports formed the cases, while tissues from non-CRC sites from the same patient were considered as the control group. Fleiss formula for proportions was used to calculate the number of cases to be sampled[15].

Ethical considerations

The study was approved by the AKUH Institutional Ethics and Review Committee (ref: 2019IERC-111(v3)) and to National Commission for Science and Technology (NACOSTI-License No: NACOSTI/P//24/37704) for use of medical records to obtain demographic information and for retrieval of FFPE blocks of the identified CRC blocks from the archive. Confidentiality of the information obtained was maintained as the identity of the patients was concealed.

Quality assurance

The lesion detection was done by the colonoscopist. The biopsies were obtained from suspicious lesions using standard procedure during colonoscopy. The specimens were labeled and the clinical information recorded on the request form. Histological examination was performed by the qualified pathologists who used standardized WHO criteria for classification of tumors. There was adequate tissue sampling and fixation. There was standardized reporting by use of synoptic pathology reports for consistency on the type of tumor, grade, stage, margin status, lymph vascular invasion and pathology subtype. Each confirmed case was reviewed in a multidisciplinary team setting which included oncologist, surgeon, pathologist and radiologist.

Data management

Data accrual

The demographic features were extracted from the pathology reports linked to the tissues. The records included Age at diagnosis, gender, residence, clinical diagnosis, cancer stage and histopathology subtype. Each block was uniquely coded using sample identification (ID). The ID linked the sample to the corresponding patient's clinical data stored in the hospital database. Direct identifiers such as patients name were not stored with the sample to maintain confidentiality. Samples from patients with histologically confirmed CRC formed the cases. Non-cancerous colon adjacent tissue form the control group. Each block was assigned to case or control groups based on confirmed pathology and clinical diagnosis recorded at the time of sample collection. Ten percent of the sample was used to pre-test the check list for collection of data including; age, gender, grade of tumour, pathology sub type. The information obtained was analysed to get an idea of how the data will appear for the whole study

Histological processing and imaging of colorectal tissue sections

The Formalin-Fixed Paraffin Embedded (FFPE) Sections were cut in to 3µm, processed and stained with Hematoxylene-Eosine. Imaging of the histopathology sections were

done using light microscope at 10x magnification. Representative images were captured from sample to demonstrate wide spread cancer of the colorectal and relatively normal colon histology in the controls.

Microbial profiling of CRC and adjacent normal tissue

To compare the microbial community in the colon of colorectal cancer and non-malignant region, Nucleic acid was extracted from the FFPE sections from the malignant and non-malignant regions. The product was amplified and sequenced using Illumina protocol. The sequences were searched against the NCBI 16S Microbial database using Basic Local Alignment Search Tool (blastn) that compares a nucleotide querry sequence against a database of nucleotide sequences to identify the microbial community present.

Nucleic acid extraction

Sections of 5 μm of the thirty CRC and thirty adjacent normal FFPE samples were used for DNA analysis. Sterile gloves, scalpel and forceps were used to minimize contamination. Blank reagents were used as control in the analysis to check the possibility of contamination from the reagents. Further contamination minimization were achieved by using nuclease-free water that was filter-sterilized and UV-treated. The quality of water was tested by using PCR using microbial DNA primers before use. The work surfaces were decontaminated by washing with 10% chlorine to hydrolyze possible DNA contaminants. Nucleic acid free reagents and aerosol resistant pipette tips were used. All sample racks and reusable equipment were also washed in 10% chlorine and autoclaved after use. 10% chlorine was used to spray pipettor and working areas then placed in UV chamber for at least 30 minutes after and before use to destroy DNA.

Pre-amplification procedure of mixing and aliquoting reagents was done on the bench top of the UV cabinet. Total DNA was isolated from samples using AllPrep DNA/RNA FFPE Kit (Qiagen) following the manufacturer's protocol[16]. The extracted DNA was quantified using the NanoDrop Lite spectrophotometer (ThermoScientific) [17] which ranged from 30-98ng/μl. The availability of microbial DNA was verified by PCR amplification using 16S rRNA specific primers.

16S rRNA Sequencing

FFPE samples derived from 30 CRC and 30 normal tissues were subjected to two-step process of the Illumina protocol. Exactly 500 base pair of 16S rRNA genes were amplified with the universal primers using high-fidelity AB-gene DNA polymerase (ThermoScientific) using (95^{o}c for 3min, 30 cycles (95^{o}c for 30s, 55^{o}c for 30s, 72^{o}c for 30s), 72^{o}c for 5 min then finally held at 4^{o}c) as the PCR condition.

Data management

The demographic features were imported into R. version 4.2.2 (2022-10-31 ucrt) for CRC frequency distribution analyses. The Microbial 16S Sequences were classified into Operational Taxonomic Units (OTUs). The Sequences were assigned a putative taxonomy using BLAST[18]. The most abundant unique sequence was searched against the NCBI 16S Microbial database using blastn, with the argument -max_target_seqs 20. Resulting hits were sorted first by *e*-value, then bitscore and the taxonomy of the highest scoring sequence was reported.

The results were presented in tables and graphs.

RESULTS

The study enlisted a total of 30 participants and evaluated their colorectal cancer (CRC).

The results of different stages were as follows:

Stage I, 3 patients (10%), Stage II, 16 patients (53.3%), and Stage III, 11 patients (36.7%). There were no participants in Stage 0 or Stage IV.

Table 1

Sex and age groups

Characteristic	**Stage I**, N = 3[1]	**Stage II**, N = 16[1]	**Stage III**, N = 11[1]
Age group (years)			
38-47	0 (0%)	1 (6.2%)	0 (0%)
48-57	1 (33%)	4 (25%)	4 (36%)
≥58	2 (67%)	11 (69%)	7 (64%)
Gender			
Female	2 (67%)	6 (38%)	4 (36%)
Male	1 (33%)	10 (62%)	7 (64%)

[1] n (%)

Key

The age grouping was informed by the natural clustering in the study population as most subjects were older 58, followed by those aged 48-57. There were few cases in the age 38-47 years. The gut microbiota composition evolves with age and some bacteria associated with CRC are more prevalent in older adults

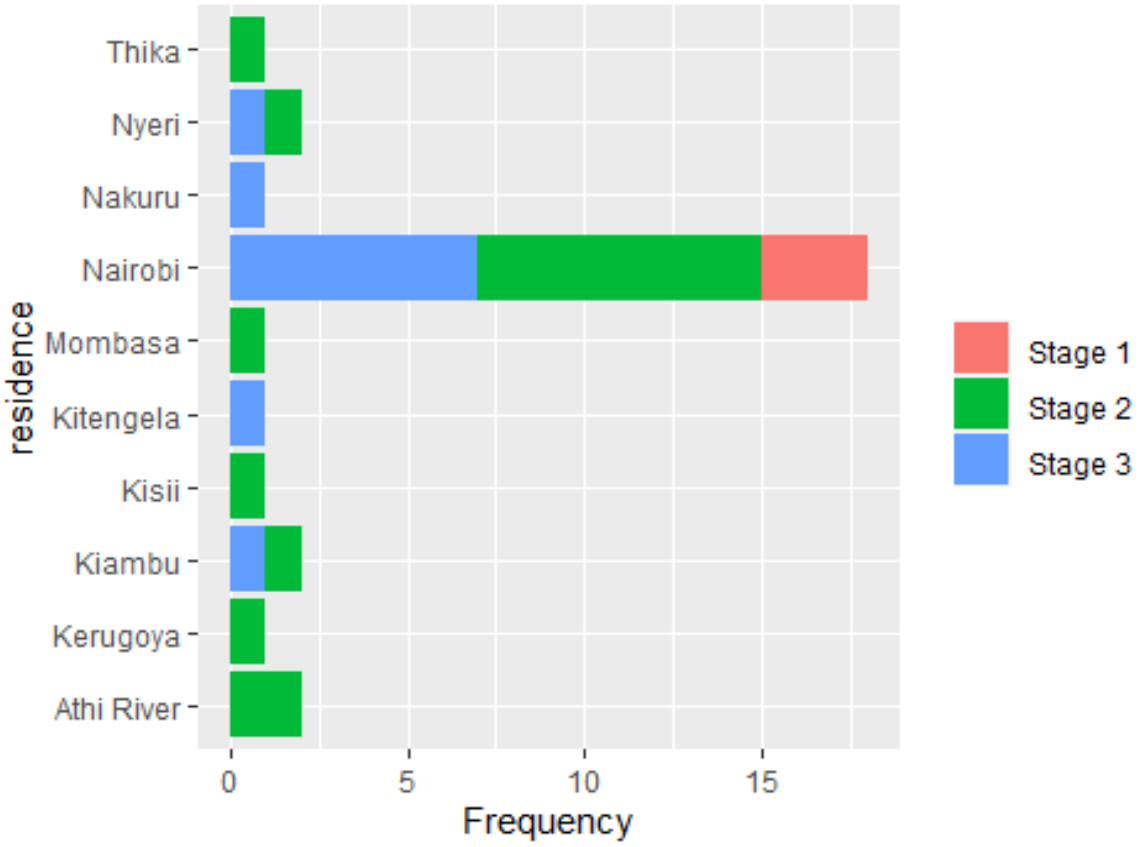

Figure 1 Distribution of subjects by residence and cancer stage

Figure 1 illustrates CRC distribution by residence and stage. It supports targeted interventions, informs policy decisions and builds a comprehensive approach to cancer care and prevention within this studied population.

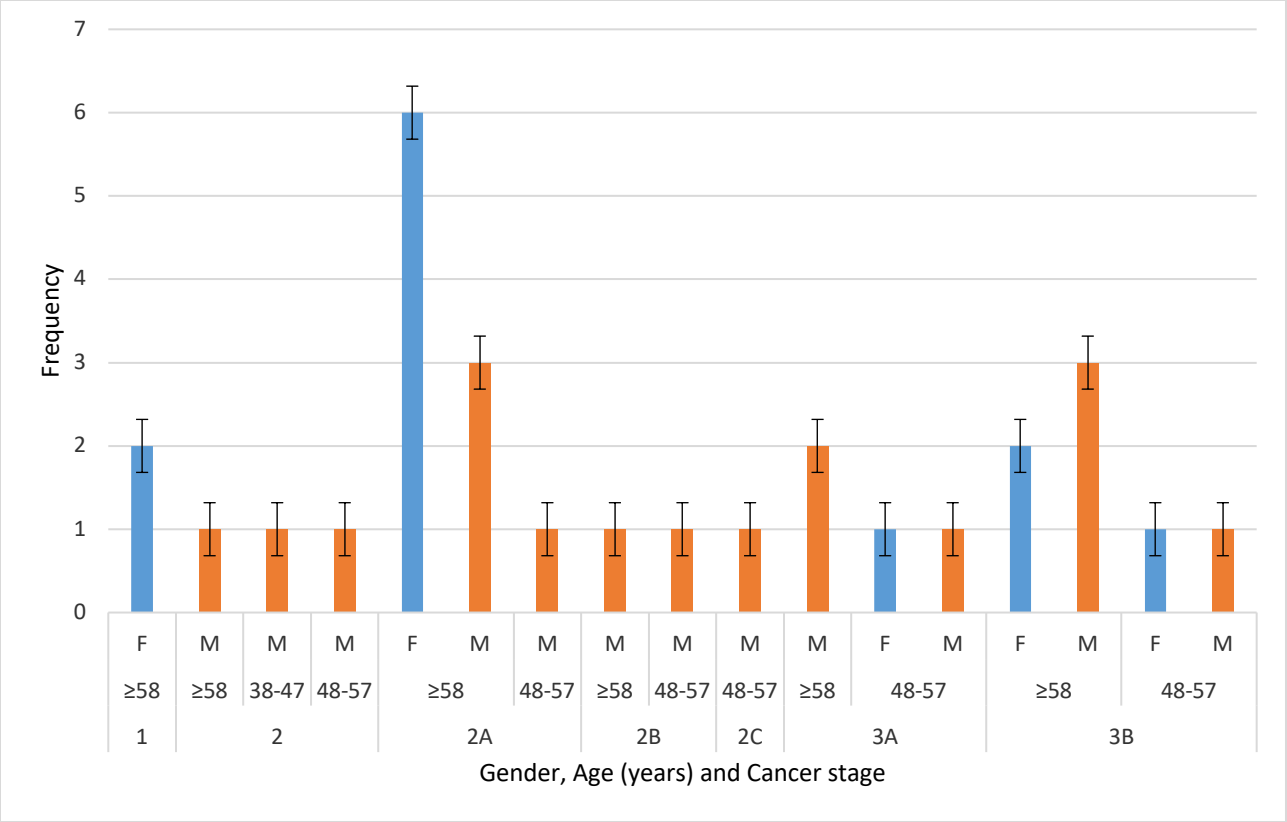

Figure 2 Distribution of stage by gender (M=males, F=females) and age (in years) among the participants

Among the most common cancer stage, subjects of age group 58 years and above were the most affected. They dominated stage IIA, IIIB and stage I followed by age group 48-57 in stage IIIA and IIIB. Among the most common cancer grades, participants of age group 58 and above were the most affected (Fig. 2)

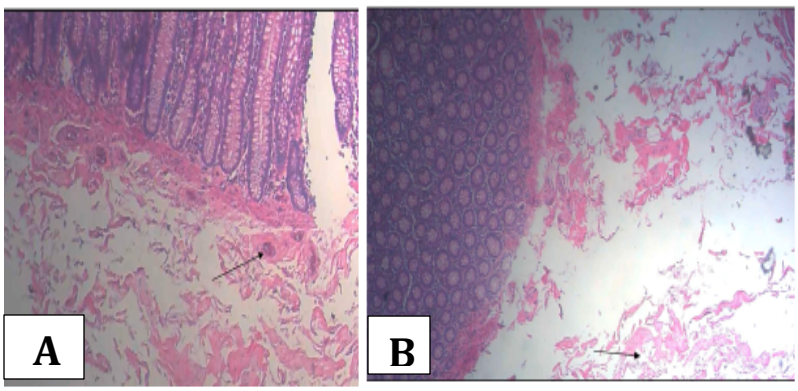

Figure 3A- *Invasive adenocarcinoma (x10),* 3B - *mucinous adenocarcinoma (x10)*

Among the assessed variables only cancer grade was significantly associated the residence of the subjects but not on cancer stage and pathology subtype of the colorectal cancer (P=0.021).

Pathology subtypes

Of the sub-type of invasive adenocarcinoma constituted 43%, and mucinous adenocarcinoma 57% (Fig. 3). Invasive adenocarcinoma was the most common subtype among the females aged 58 years and above. Among age 48-57, mucinous adenocarcinoma was found to be high (fig. 4). Twenty-six (87%) participants were found to be in grade 2, three (10%) in grade 1 and one (3%) in grade 3.

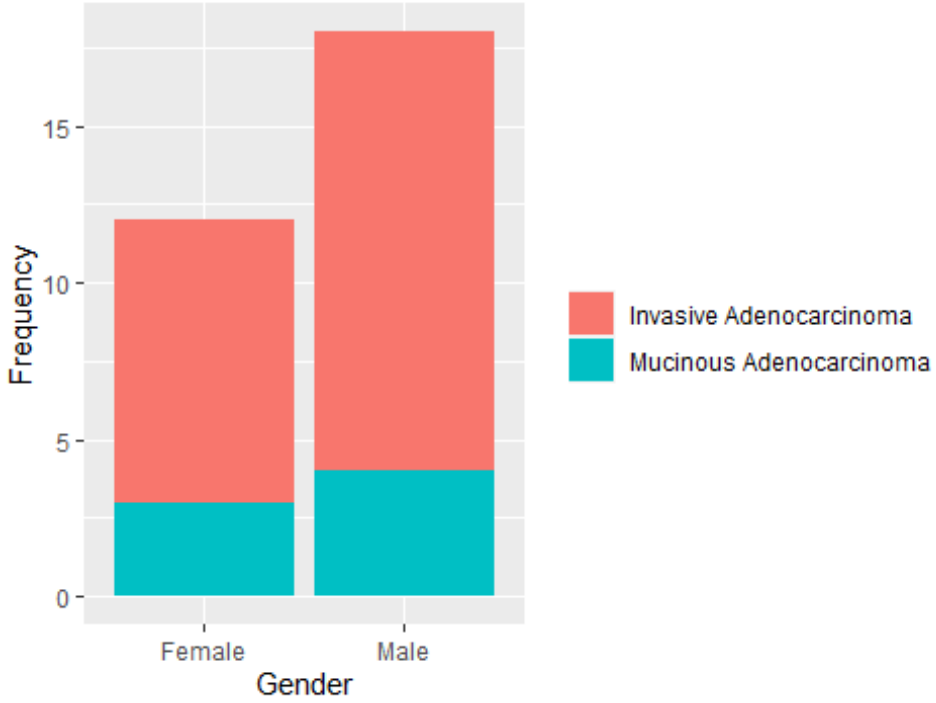

Figure 4 Pathology subtype among the subjects

Microbial community in malignant and non-malignant tissues

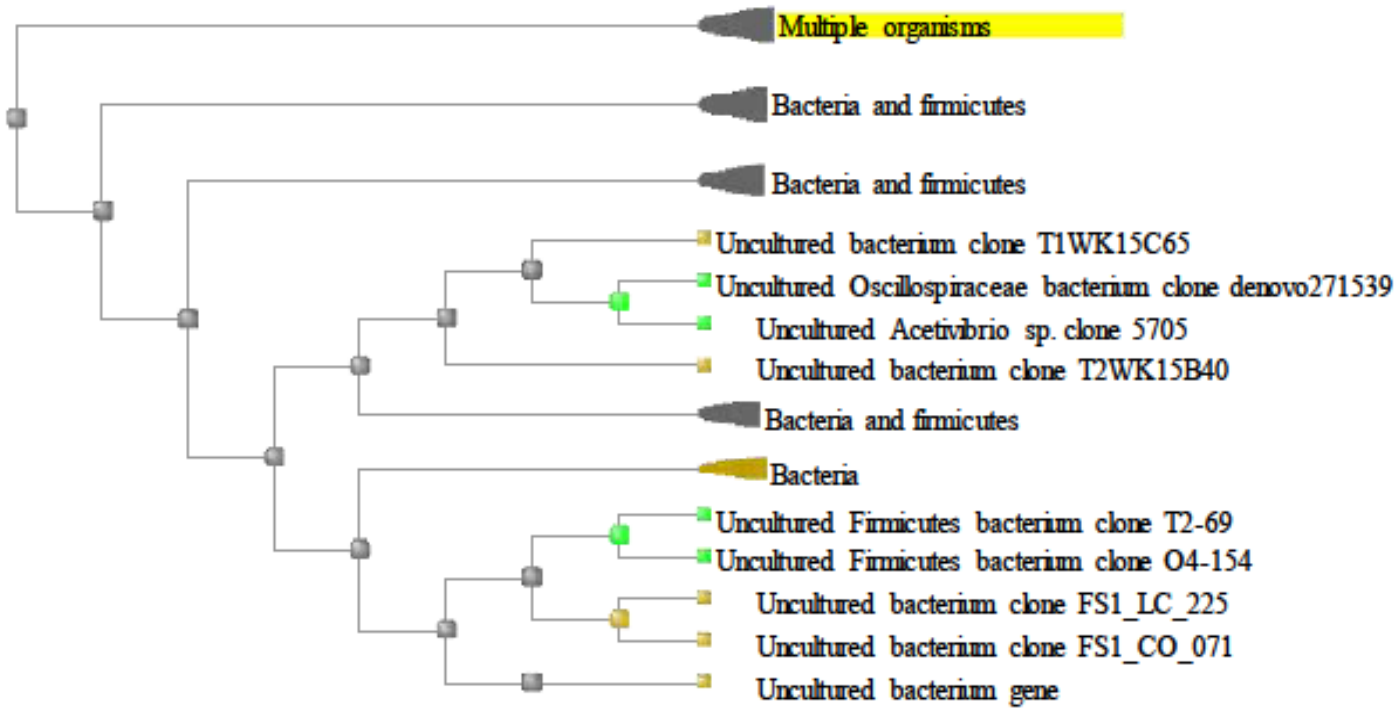

Figure 5a Cladogram of the organism identified in malignant tumors

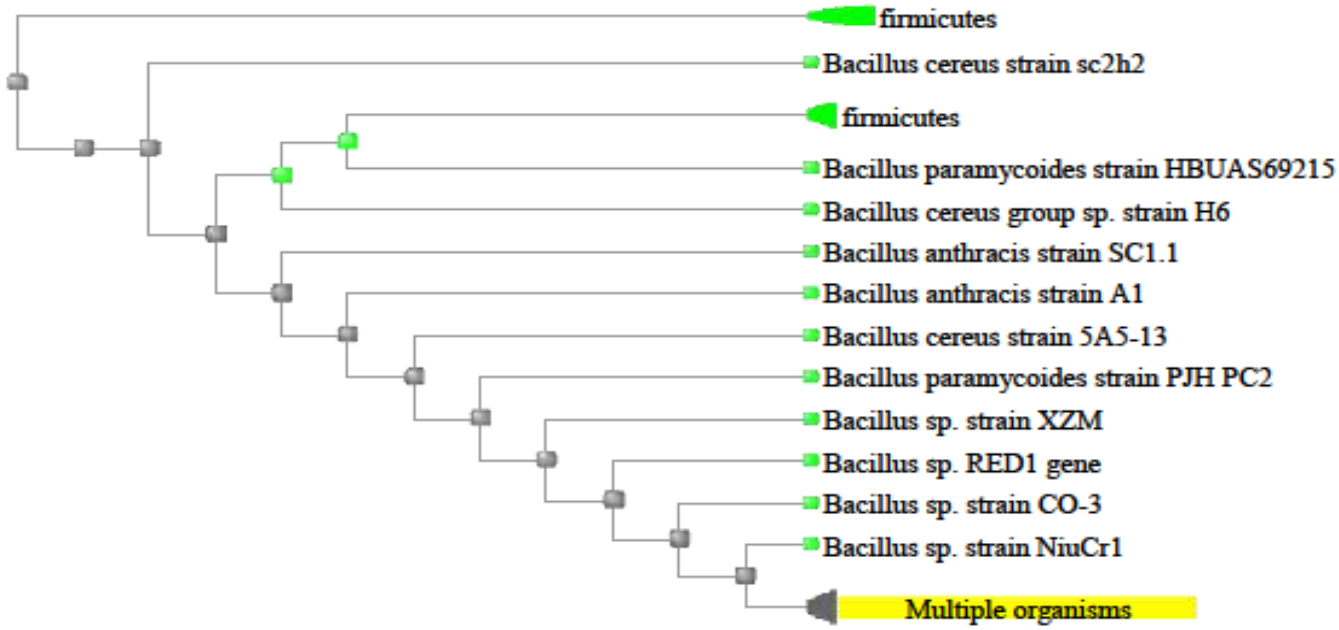

Figure 5b Cladogram of the organism identified in non-malignant tissues

In mucinous adenocarcinoma, the organisms identified were remarkably different. The non-cancerous tissues were predominated by *Bacillus sp, Geobacillus stearothermophilus* and *Lipophrys pholis*. On the other hand, cancerous tissues had nine different species of bacteria. Synchronous adenocarcinoma had a lot of similarity in all the organisms identified in both cancerous and non-cancerous tissue except *Enterococcus mundtii* which was only present in non-cancerous tissues Invasive adenocarcinoma only shared four species out

of the twenty-nine identified in both cancerous and non-cancerous tissues. The four species include: *Bacillus sp* (in *firmicute*), *Planococcaceae bacterium*, *Bacillus subtilis* and *Firmecutes bacterium*. Female patients of age 48-57 and older than 58 years with mucinous and invasive adenocarcinoma had 8 and 6 species of the organisms identified respectively. Out of the total 14 species, 3 of them were common in both cases. These were identified as *Bacillus sp, Firmicutes sp* and *Eubacteria sp*. The females aged 48-57 years had 5 distinct species that were unique to this group. This included *Oscillospiraceae bacterium, Clostidiales bacterium, Texoconibacillus sp, Xylanivirga bacterium* and *Valitelen sp*. Females who had more than 58 years had 3 unique species which included *Planococcaceae sp, Vulcanibacillus sp* and *Staphillococcus sp* (Figure 5c-5d). The predominant organism in males aged more than 58 years was *Enterococcus sp*. Other organisms which were present included *Vagococcus sp, Facklamia sp, Aeroshaera sp* and *Trichococcus sp*. These organisms were common for both the malignant tumors and the distal adjacent tissue. One unique organism that was found in malignant tumor but not in the adjacent tissue was *camobacterium sp*

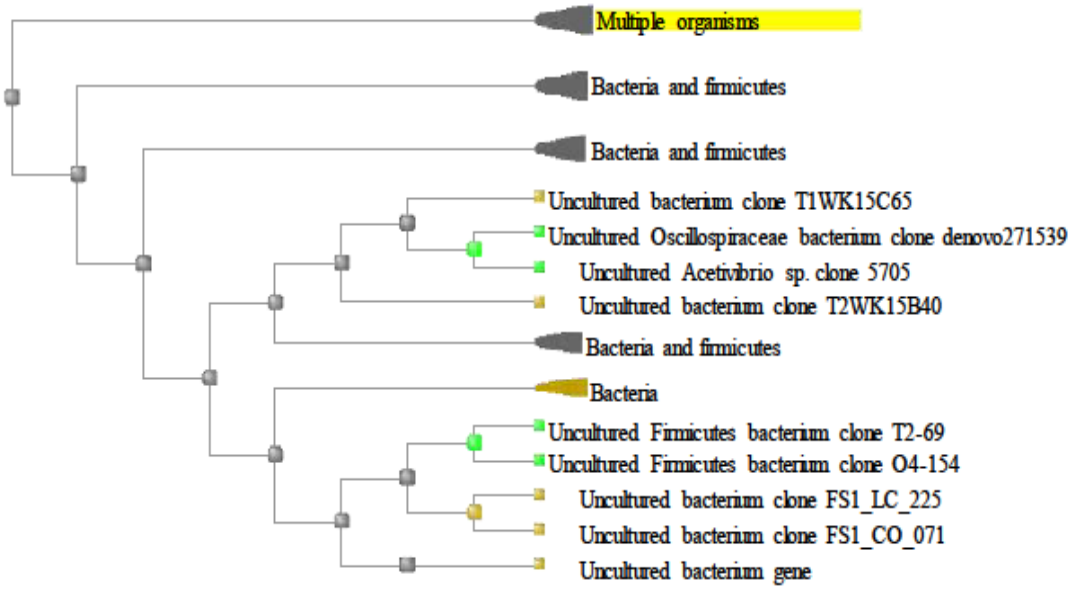

Figure 5c Cladogram of the organism identified in malignant tumors from female patients aged ≥58 years old

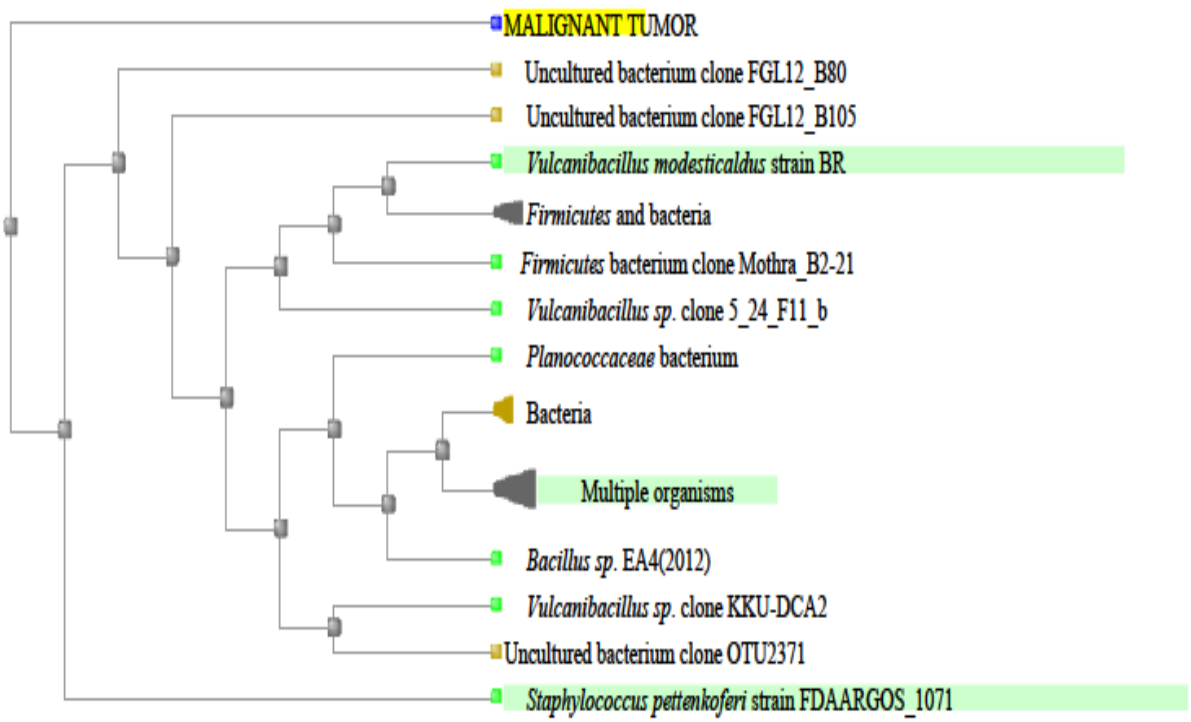

Figure 5d Cladogram for the organisms identified in malignant tumors from females patients aged 48-57 years old

DISCUSSION

This study focused on the mucosa-associated gut microbiota in colorectal cancer (CRC) tissues, and adjacent non-cancerous tissues of subjects in Nairobi, Kenya. The results add to the growing evidence that gut microbiota composition is linked with the pathogenesis of CRC. In addition it supports the variations due to; demographical, geographical, and histopathological factors. Thereby highlighting the interaction between gut microbiota diversity and variable such as age and gender in patients with colorectal cancer.

Microbial diversity in mucinous and Synchronous Adenocarcinoma

Bacillus sp., Geobacillus stearothermophilus, and *Lipophrys pholis* dominated non-cancerous tissues in mucinous adenocarcinoma, whereas a wider range of nine species were found in cancerous tissues. This supports findings that tumor tissues typically have a more varied microbiota than nearby mucosa, indicating microbial dysbiosis linked to carcinogenesis[9]. With the exception of *Enterococcus mundtii,* which was only found in non-cancerous samples, the microbial communities in malignant and non-malignant tissues in synchronous adenocarcinoma were essentially the same. These findings are in line with the stepwise microbial changes described in previous studies on the role of microbiota in the development of colorectal cance[4]. This overlap might indicate early microbial shifts prior to significant dysbiosis.

Microbial shifts in Invasive adenocarcinoma

With only four species shared by cancerous and non-cancerous tissues, invasive adenocarcinoma displayed the most noticeable differences. The idea that greater disruption of microbial homeostasis is linked to deeper tumor invasion is supported by the decreased overlap[5]. The association between microbial imbalance and disease severity is further supported by prior research that similarly reports commensal depletion and opportunistic pathogen enrichment as adenocarcinoma advances[6].

Influence of Age and Sex

Age and sex also had an impact on the microbial composition. Unique species like *Oscillospiraceae* and *Clostridiales* were found in females aged 48–57 years, whereas *Planococcaceae* and *Staphylococcus* were found in females aged 58 years and older. Along with *Vagococcus* and *Facklamia, Enterococcus sp*. predominated in males over the age of 58 years. These results are consistent with earlier findings that gut microbial diversity is influenced by host age, sex, and geographic background[11,12]. Variability in colorectal cancer risk and outcomes across populations may be partially explained by these host-related variations.

Pathogenic Species linked to CRC

It is important that *Enterococcus* and *Streptococcus species* have been found in malignant tissues. Due to their pro-inflammatory and genotoxic properties, *Streptococcus gallolyticus* and *Enterococcus faecalis* have been linked to colorectal carcinogenesis[8]. Although this study did not find *Fusobacterium nucleatum*, a known driver of colorectal cancer progression[7], the presence of other potentially carcinogenic taxa emphasizes the diverse microbial drivers among patient populations.

Comparison with Global data

Fusobacterium nucleatum, Bacteroides fragilis, and other pro-carcinogenic bacteria have been linked to colorectal cancer on a global scale[10,19,20]. On the other hand, *Bacillus, Enterococcus*, and other *Firmicutes* were more prevalent in Kenyan patients in our study. These variations could be due to host genetics environmental factors, and dietary influences[14]. The necessity of locally relevant microbiome studies to guide diagnostic and treatment approaches is highlighted by this regional variation.

Limitations

The use of formalin-fixed paraffin-embedded (FFPE) tissues could have resulted in biases in the quality of the DNA and the representation of microorganisms. Even though strict contamination control procedures were used, FFPE processing can lead to contamination or DNA degradation, which could affect the results of microbial profiling[20].

To validate the observed microbial trends and enhance the predictive power of microbial markers, larger cohort studies are required.

This study did not control for or evaluate dietary practices, antibiotic use or other environmental or lifestyle factors that have a significant impact on the composition of the

gut microbiota which could have biased the association shown.

The study concentrated on taxonomic identification but did not capture functional microbial dynamics.

CONCLUSION

With notable variations between tumor and surrounding non-cancerous tissues across histological subtypes, this study shows that colorectal cancer in Kenyan patients is linked to specific changes in microbial composition. Important regional variation in tumor-associated microbiota is highlighted by the prevalence of *Bacillus* and *Enterococcus species* over *Fusobacterium* and *Bacteroides*, which are frequently reported in global cohorts. In order to better understand local risk factors, enhance diagnostic biomarkers, and guide targeted therapeutic interventions, these findings highlight the necessity of region-specific microbiome profiling.

Recommendations

Larger, multicenter studies are needed to validate the microbial trends observed in this study and enhance the predictive power of identified microbial markers for diagnosis and prognosis.

There is need for future studies to complement FFPE-based analysis with fresh or frozen tissue sampling to minimize DNA degradation and contamination risks.

Future investigations should control for or assess dietary patterns, antibiotic use, and other lifestyle/environmental exposures, given their significant influence on gut microbiota composition and potential role in cancer development.

The distinctive microbial shifts identified could inform the development of non-invasive diagnostic biomarkers and microbiome-targeted therapeutic interventions tailored to the Kenyan and regional context.

Authors' contribution

All the authors were involved in the study from the conception to the initiation, development and actualization of the study.

Conflict of Interest

The authors have no conflicts of interest

REFERENCE

1. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, et al. Global burden of colorectal cancer in 2020 and 2040: Incidence and mortality estimates from GLOBOCAN. Gut. 2023;72(2):338–44.

2. Wambalaba FW, Son B, Wambalaba AE, Nyong'o D, Nyong'o A. Prevalence and Capacity of Cancer Diagnostics and Treatment: A Demand and Supply Survey of Health-Care Facilities in Kenya. Cancer Control. 2019;26(1):1–12.

3. Awedew AF, Asefa Z, Belay WB. Burden and trend of colorectal cancer in 54 countries of Africa 2010–2019: a systematic examination for Global Burden of Disease. BMC Gastroenterol [Internet]. 2022;22(1):1–12. Available from: https://doi.org/10.1186/s12876-022-02275-0

4. Dai Z, Zhang J, Wu Q, Chen J, Liu J, Wang L, et al. The role of microbiota in the development of colorectal cancer. Int J Cancer. 2019 Oct 15; 145(8):2032-41. doi:10.1002/ijc.31908.

5. Cheng Y, Ling Z, Li L. The Intestinal Microbiota and Colorectal Cancer. Front Immunol. 2020;11(November):1–13.

6. Fan X, Jin Y, Chen G, Ma X, Zhang L. Gut Microbiota Dysbiosis Drives the Development of Colorectal Cancer. Digestion. 2021;102(4):508–15.

7. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/β-Catenin Signaling via its FadA Adhesin. Cell Host Microbe. 2013 Aug;14(2):195–206.
8. Boleij A, Tjalsma H. The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant disease. Lancet Infect Dis. 2013 Aug;13(8):719–24.
9. Sheng QS, He KX, Li JJ, Zhong ZF, Wang FX, Pan LL, et al. Comparison of gut microbiome in human colorectal cancer in paired tumor and adjacent normal tissues. Onco Targets Ther. 2020;13:635–46.
10. Guo Q, Qin H, Liu X, Zhang X, Chen Z, Qin T, et al. The Emerging Roles of Human Gut Microbiota in Gastrointestinal Cancer. Front Immunol. 2022;13:915047.
11. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012 May;486(7402):222–7.
12. Porras AM, Shi Q, Zhou H, Callahan R, Montenegro-Bethancourt G, Solomons N, et al. Geographic differences in gut microbiota composition impact susceptibility to enteric infection. Cell Rep [Internet]. 2021;36(4):109457. Available from: https://doi.org/10.1016/j.celrep.2021.109457
13. Sánchez-Alcoholado L, Ramos-Molina B, Otero A, Laborda-Illanes A, Ordóñez R, Medina JA, et al. The role of the gut microbiome in colorectal cancer development and therapy response. Cancers (Basel). 2020;12(6):1–29.
14. Zhao L, Cho WC, Nicolls MR. Colorectal Cancer-Associated Microbiome Patterns and Signatures. Front Genet. 2021;12(December):1–15.
15. Fleiss JL, Levin B, Cho Paik M. Statistical Methods for Rates and Proportions, Third Edition. Stat Methods Rates Proportions, Third Ed. 2004;1–760.
16. Qiagen. AllPrep DNA/RNA FFPE handbook. 2017;(December):1–47.
17. Thermo Fisher Scientific. Thermo Scientific NanoDrop Products NanoDrop Lite User Guide. 2012;(January):1–34. Available from: www.thermoscientific.com/nanodrop Accessed in October 2023
18. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: Architecture and applications. BMC Bioinformatics. 2009;10:1–9.
19. Park J, Kim NE, Yoon H, Shin CM, Kim N, Lee DH, et al. Fecal Microbiota and Gut Microbe-Derived Extracellular Vesicles in Colorectal Cancer. Front Oncol. 2021;11(September):1–14.
20. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012 Aug;488(7410):178–84.
21. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, et al. The long-term stability of the human gut microbiota. Science. 2013 Jul;341(6141):1237439.
22. Lam SY, Ioannou A, Konstanti P, Visseren T, Doukas M, Peppelenbosch MP, et al. Technical challenges regarding the use of formalin-fixed paraffin embedded (FFPE) tissue specimens for the detection of bacterial alterations in colorectal cancer. BMC Microbiol [Internet]. 2021;21(1):1–11. Available from: https://doi.org/10.1186/s12866-021-02359-z