Microbial Alterations in Colorectal Cancer: Insights from Tunisia

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Introduction

Colorectal cancer (CRC) is a common cancer linked to intestinal microbiota, a key part of the tumor microenvironment. Many studies have showen that CRC is associated with intestinal dysbiosis of bacteria, fungi, viruses, and Archaea, which may play a causative role. This study assessed disruptions in intestinal microbial composition, focusing on bacteria, and their correlation with CRC **(1)**.

Purpose

This study aimed to evaluate the disturbance in the composition of bacterial intestinal flora and to determine its relationship with colorectal cancer.

Materials and methods

• **Case-control study**: 27 fecal samples (14 from colorectal cancer patients and 13 from controls) collected between March and August 2021 at Charles Nicolle Hospital, Tunis. Samples were frozen at −80°C and transported to the Institute of Mediterranean Infection (IHU).

• **Statistical analysis**: Pearson's chi-square and Fisher's exact tests for categorical variables; Mann-Whitney U test for continuous variables. An OR of 0.375 (p = 0.1) was calculated for the association between BMI (>25 vs. ≤25) and colorectal cancer.

Microbiota profiling: DNA was extracted using multiple protocols to optimize recovery, including mechanical lysis and the EZ1 Advanced XL extraction kit. Targeted Real-time quantitative PCR qPCR identified biomarkers such as Streptococcus gallolyticus and Enterococcus faecalis.

Bacterial cultures under various conditions, combined with high-throughput MALDI-TOF mass spectrometry, were used to optimize the isolation of oxygen-intolerant and clinically relevant bacteria in colorectal cancer (CRC) **(Figure 1).**

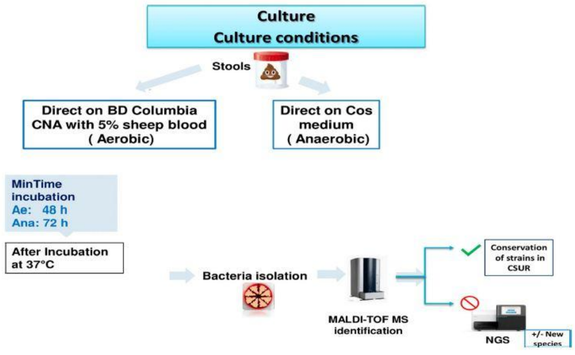

Figure 1. Methodology of bacterial identification (2)

Results

• CRC was more common in men (10) than women (4), male gender, tobacco, alcohol, diabetes, and family history were associated with increased CRC risk (OR > 1), only diabetes was statistically significant (OR = 12, p = 0.03)**(Table 1).**

Table 1. Demographic data recorded for each study participant for each group colorectal cancer and controls (2)

Characteristics	Colorectal cancer group (N=14)	Control group (N=13)	OR	P
Median Age (years-IQR)	58.0 (51—67.2)	59.0 (50.5 -66.0)	–	0.9
Gender (Male/female)				0.69
Male	10	8	1.56	
Female	4	5		
Median weight (Kg-IQR)	66.0 (58.7–66.0)	68.0 (64.5–72.5)	–	0.4
Tobacco consumption				0.16
Yes	8	4	3.0	
No	6	9		
Alcohol consumption				1.0
Yes	3	2	1.5	
No	11	11		
Diabetes				0.03
Yes	7	1	12	
No	7	12		
Family history of colorectal cancer				0.18
Yes	5	2	3.05	
No	9	11		
Median BMI (kg/m^2-IQR)	22.61 (24.35–20.38)	23.03 (25.95–22.20)	0.375	0.1

• **Bacterial biomarkers:** Two candidate biomarkers, Streptococcus gallolyticus and Enterococcus faecalis, were selected for qPCR quantification. S. gallolyticus was more prevalent in the CRC group than in controls, and similarly, E. faecalis showed higher prevalence in the CRC group (Figure 2).

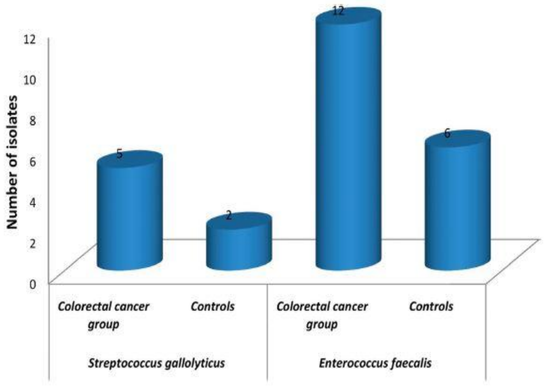

Figure 2. Distribution of candidate bacteria based on the number of isolates in the two groups: colorectal cancer group and controls (2).

• **Bacterial isolates:** A total of 117 bacterial strains were isolated from 27 stool samples—63 (53.85%) from CRC patients and 54 (46.15%) from controls. Overall, 38 species were identified, with 24 species among CRC cases and 26 species in controls. Of these, 14 species (53.8%) were specific to the control group, while 12 species (50%) were specific to CRC patients (p = 0.7). Ten species (26.31%) were strict anaerobes present in both groups (*Flavonifractor plautii, Eggerthella lenta, Clostridium bifermentans, Lactobacillus crispatus*, etc.). The median number of bacterial species per stool sample was not significantly different between CRC cases (5.5; IQR = 4.75) and controls (7; IQR = 3.5, p = 0.69). Unique species in the control group included *Pediococcus acidilactici, Weissella confusa, Lactococcus garvieae, Enterococcus mundtii*, among others **(Figure 3).**

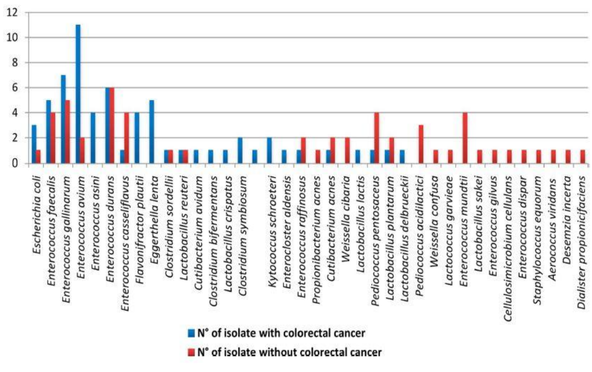

Figure 3. Representation of number of isolates for each bacteria identified between the two groups: colorectal cancer group and control group (2)

Conclusion

This study underscores the potential of gut microbiota biomarkers as a promising, non-invasive tool for the early detection and differentiation of colorectal cancer, offering new avenues for precision diagnostics

References

(1) Zhang J, He Y, Xia L, Yi J, Wang Z, Zhao Y, et al. Expansion of colorectal cancer biomarkers based on gut Bacteria and viruses. Cancers.2022;14(19):4662.
(2) Zrelli, M., Ferjani, A., Nouira, M. et al. Diversity in gut microbiota among colorectal cancer patients: findings from a case–control study conducted at a Tunisian University Hospital. Discov Onc 15, 402 (2024).