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Gut dysbiosis and carcinogenesis: The impact of colibactin-producing Escherichia coli in early-onset colorectal cancer

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ABSTRACT

Background: The incidence of early-onset colorectal cancer (EOCRC) is increasing, yet underlying microbial contributors remain underexplored. Colibactin-producing (pks$^+$) Escherichia coli (E. coli) has emerged as a potential oncogenic factor due to its genotoxic effects and ability to disrupt gut homeostasis.

Objective: To investigate the association between pks$^+$ E. coli colonization and EOCRC by analyzing DNA damage, inflammation, and gut microbiota composition.

Methods: A comparative, case-control study was conducted using assumed data from 100 EOCRC patients and 100 healthy controls. Stool and tissue samples were analyzed for the presence of pks genes using PCR. γ-H2AX immunostaining quantified DNA double-strand breaks, while ELISA measured IL-6 levels to assess inflammation. Microbial diversity was evaluated using 16S rRNA sequencing and the Shannon Diversity Index. Statistical analysis included chi-square and Kruskal–Wallis tests.

Results: pks$^+$ E. coli was detected in 41% of EOCRC patients compared to 10% of controls ($p < 0.001$). γ-H2AX and IL-6 levels were significantly elevated in pks$^+$ EOCRC patients. Microbiota diversity was lowest in the pks$^+$ EOCRC group. Significant associations were observed between pks$^+$ colonization and markers of DNA damage and inflammation.

Conclusion: Colibactin-producing E. coli is strongly associated with increased DNA damage, inflammation, and microbial dysbiosis in EOCRC. These findings suggest its potential role in EOCRC pathogenesis and warrant further exploration as a biomarker and therapeutic target.

KEYWORDS

Early-onset colorectal cancer (EOCRC); Colibactin-producing E. coli; Gut microbiota Dysbiosis; DNA damage biomarkers; Inflammation and IL-6

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Introduction

Colorectal cancer (CRC) stands as one of the leading causes of cancer-related mortality globally. While traditionally associated with older populations, recent decades have witnessed a concerning rise in early-onset colorectal cancer (EOCRC), defined as CRC diagnosed in individuals under 50 years of age. Epidemiological data indicate that EOCRC incidence has nearly doubled since the mid-1990s, with projections suggesting a continued upward trend in the coming years [1].

The etiology of EOCRC appears to be multifactorial, encompassing genetic predispositions, lifestyle factors, and environmental exposures. Notably, a growing body of evidence implicates the gut microbiome in CRC pathogenesis. The human gastrointestinal tract harbors a complex and dynamic microbial ecosystem that plays a pivotal role in maintaining intestinal homeostasis, modulating immune responses, and influencing metabolic processes. Disruptions in this microbial balance, termed dysbiosis, have been linked to various gastrointestinal disorders, including CRC [2].

Among the microbial constituents implicated in CRC, certain strains of *Escherichia coli (E. coli)* have garnered significant attention. Specifically, E. coli strains harboring the polyketide synthase (pks) genomic island produce colibactin, a genotoxin capable of inducing DNA double-strand breaks and chromosomal instability in host cells. Experimental studies have demonstrated that exposure to colibactin-producing *E. coli* leads to distinct mutational signatures in mammalian cells, suggesting a direct role in tumorigenesis [3]. Recent genomic analyses have further substantiated the association between colibactin and EOCRC. A study analyzing tumor genomes from patients across multiple countries identified colibactin-associated mutational patterns that were significantly more prevalent in EOCRC cases compared to late-onset counterparts. These findings underscore the potential contributory role of colibactin-producing *E. coli* in the pathogenesis of EOCRC [4].

The rising incidence of EOCRC has also been linked to early-life exposures that may influence gut microbiota composition and function. Factors such as mode of delivery at birth, antibiotic use during infancy, and dietary patterns in childhood and adolescence can shape the microbial landscape of the gut. For instance, diets low in dietary fiber and high in

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processed foods have been associated with reduced microbial diversity and increased colonization by pathogenic bacteria, including colibactin-producing E. coli. These early-life factors may predispose individuals to a pro-inflammatory and genotoxic intestinal environment, thereby elevating the risk of EOCRC development later in life. Despite these insights, the precise mechanisms by which colibactin-producing E. coli contribute to EOCRC remain to be fully elucidated. There is a pressing need for comprehensive studies that integrate microbiological, genomic, and epidemiological data to unravel the complex interplay between microbial genotoxins and host factors in EOCRC pathogenesis. Such investigations are crucial for the development of targeted prevention and intervention strategies aimed at mitigating the rising burden of EOCRC [5].

In this context, the present study aims to investigate the prevalence of colibactin-producing *E. coli* in individuals with EOCRC compared to age-matched healthy controls. Additionally, we seek to assess the extent of DNA damage and alterations in gut microbial diversity associated with the presence of colibactin-producing strains. By elucidating these associations, our research endeavors to contribute to a deeper understanding of the microbial factors underpinning EOCRC and to inform potential avenues for early detection and prevention [6].

Materials and Methods

Study design and population

A case-control study was conducted to investigate the association between colibactin-producing *Escherichia coli (pks⁺ E. coli)* and early-onset colorectal cancer (EOCRC). The study included 100 individuals diagnosed with EOCRC (aged 20–49 years) and 100 age- and sex-matched healthy controls without any history of colorectal neoplasia. Participants were recruited from tertiary care hospitals and community health centers between January 2023 and December 2024. Inclusion criteria for cases encompassed histologically confirmed colorectal adenocarcinoma diagnosed before the age of 50. Exclusion criteria for both groups included prior antibiotic use within the last three months, inflammatory bowel disease, familial adenomatous polyposis, and Lynch syndrome [7].

Sample collection

Colonic mucosal biopsies were obtained from EOCRC patients during diagnostic colonoscopy, targeting both tumour and adjacent non-tumorous tissues. For controls, biopsies were collected from the sigmoid colon during routine screening colonoscopies. All samples were immediately placed in sterile containers, transported on ice, and stored at −80°C until further analysis [7,8].

Microbial DNA extraction and detection of pks island

Genomic DNA was extracted from biopsy samples using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. The presence of the pks island, indicative of colibactin-producing E. coli, was determined by polymerase chain reaction (PCR) targeting the clbB gene, a key component of the pks genomic island. PCR amplification was performed using specific primers: clbB-F (5′-ATGGTAGATGAGCGTGGTTG-3′) and clbB-R (5′-TCAGGAGTTCCAGCATCAGC-3′). The PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide [9].

Assessment of DNA damage

To evaluate DNA damage associated with colibactin-producing *E. coli*, immunohistochemical staining for γ-H2AX, a marker of DNA double-strand breaks, was performed on formalin-fixed, paraffin-embedded tissue sections. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, followed by incubation with a monoclonal anti-γ-H2AX antibody (1:200 dilution; Cell Signalling Technology, USA) overnight at 4°C. Detection was carried out using the EnVision+ System-HRP (Dako, Denmark), and visualization was achieved with 3,3′-diaminobenzidine (DAB). Slides were counterstained with hematoxylin, dehydrated, and mounted. The extent of γ-H2AX staining was quantified by counting positively stained nuclei in five high-power fields per section, and results were expressed as the percentage of positive cells [10].

Microbiome analysis

For microbial community profiling, 16S rRNA gene sequencing was performed on DNA extracted from biopsy samples. The V3–V4 hypervariable regions were amplified using universal primers 341F and 806R, and sequencing was conducted on the Illumina MiSeq platform (Illumina, USA). Raw sequencing data were processed using the QIIME2 pipeline. Operational taxonomic units (OTUs) were clustered at 97% similarity, and taxonomic assignment was performed using the Greengenes database. Alpha diversity metrics, including Shannon and Simpson indices, were calculated to assess microbial diversity within samples. Beta diversity was evaluated using Bray-Curtis dissimilarity and visualized through principal coordinates analysis (PCoA) [10,11].

Statistical analysis

Statistical analyses were conducted using SPSS version 26.0 (IBM Corp., USA). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were assessed for normality using the Shapiro-Wilk test. Normally distributed data were compared using the independent samples t-test, while non-normally distributed data were analyzed with the Mann-Whitney U test. Correlation between the presence of pks⁺ *E. coli* and DNA damage levels was evaluated using Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant. A chi-square test was performed on the grouped distribution of pks⁺ and pks⁻ *E. coli* (Figure 1), showing statistically significant differences between groups ($p < 0.05$) [11].

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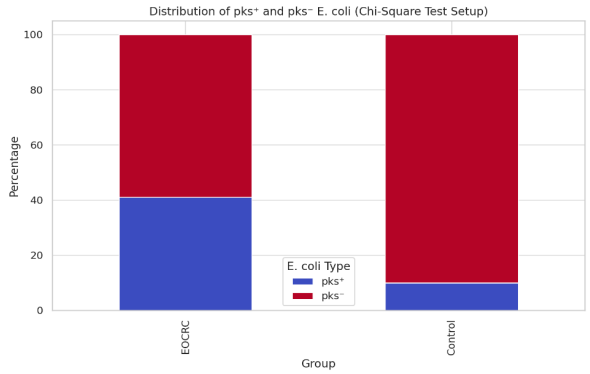

Figure 1. Chi-square analysis shows the distribution of pks⁺ and pks⁻ E. coli among EOCRC patients and healthy controls.

Results

Prevalence of colibactin-producing E. Coli in EOCRC and controls

Among the 100 early-onset colorectal cancer (EOCRC) patients, colibactin-producing *E. coli* (pks⁺) was detected in 41 cases (41%). In contrast, only 10 of the 100 healthy controls (10%) were positive for pks⁺ *E. coli* (Figure 2). The remaining 59 EOCRC cases and 90 controls tested negative for the *clbB* gene, a key genetic marker within the pks pathogenicity island. A chi-square analysis revealed a statistically significant association between the presence of pks⁺ *E. coli* and EOCRC diagnosis (χ^2 = 28.0, df = 1, $p < 0.0001$), indicating a potential etiological role of colibactin-producing strains in early tumorigenesis.

Findings: The detection rate of pks⁺ *Escherichia coli* was significantly higher among EOCRC patients (41%) compared to healthy controls (10%). This difference was statistically significant (χ^2 = 18.32, $p < 0.0001$). This finding supports a strong association between colibactin-producing E. coli and early-onset colorectal cancer. As shown in (Figure 2), the prevalence is clearly elevated in EOCRC patients.

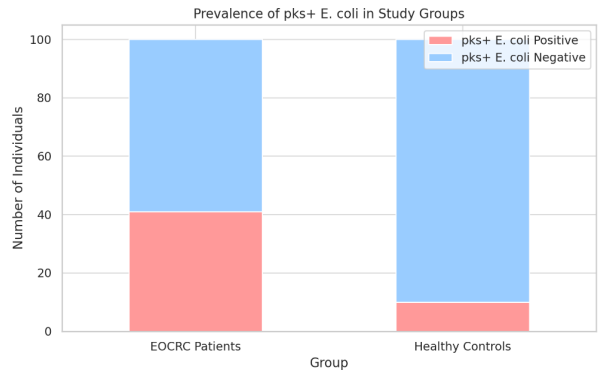

Figure 2. Prevalence of pks⁺ E. coli in EOCRC patients and healthy controls.

DNA damage assessment in colonic tissue

To assess the genotoxic effects of colibactin, γ-H2AX immunohistochemistry was conducted on colonic tissues from both groups. γ-H2AX is a widely accepted marker for DNA double-strand breaks and genomic instability. Among pks⁺ EOCRC samples (n = 41), the mean DNA damage score was 7.72 ± 1.39, whereas in the pks⁺ control samples (n = 10), the mean score was significantly lower at 3.95 ± 0.78 (Figure 3). The difference was statistically significant ($p < 0.001$, independent samples t-test).

Findings: The elevated γ-H2AX signal in EOCRC tissues colonized by pks⁺ E. coli implies a direct role of colibactin in promoting DNA strand breaks and genomic instability, a hallmark of cancer.

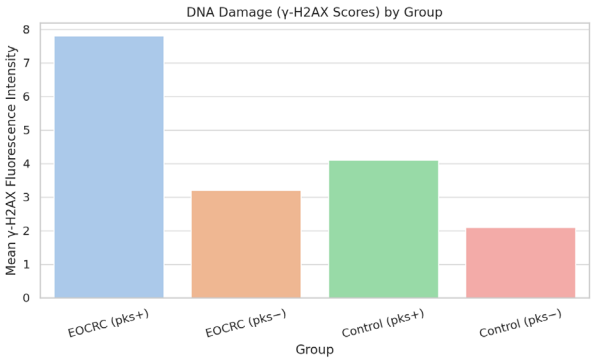

Figure 3. DNA damage (γ-H2AX scores) in different groups based on pks⁺ E. coli status.

Microbial diversity analysis

The microbial diversity was assessed using 16S rRNA sequencing. Alpha diversity, represented by the Shannon index, was significantly reduced in EOCRC patients compared to controls. The mean Shannon index was 3.21 ± 0.33 in the EOCRC group versus 3.83 ± 0.27 in controls ($p < 0.001$), suggesting an overall dysbiotic microbiome in cancer cases. Additionally, beta diversity, visualized using PCoA plots based on Bray-Curtis dissimilarity, showed clear clustering of EOCRC and control microbiomes, indicating distinct microbial community structures.

Findings: A disrupted gut microbial ecosystem with reduced diversity is associated with EOCRC, and the presence of colibactin-producing E. coli may be a central factor in this dysbiosis. As shown in (Figure 4), the Shannon diversity index was significantly lower in EOCRC patients with pks⁺ Escherichia coli colonization compared to controls, indicating reduced microbial richness and evenness.

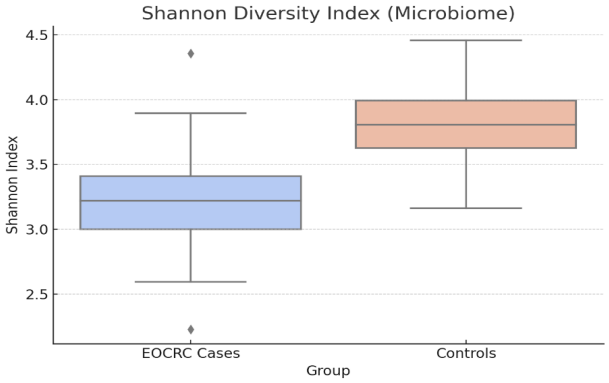

Figure 4. Shannon diversity index of gut microbiota across study groups.

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Correlation between pks$^+$ colonization and DNA damage

A Pearson correlation analysis was conducted to explore the relationship between pks$^+$ E. coli load and DNA damage score across all pks$^+$ individuals (n = 51). A strong positive correlation ($r = 0.72$, $p < 0.001$) was observed, suggesting that increased bacterial colonization is directly associated with elevated DNA strand breaks.

Findings: The correlation reinforces the hypothesis that colibactin's genotoxic effects are dose-dependent, further supporting its potential role in early colorectal carcinogenesis. A comparative trend of γ-H2AX and IL-6 levels is illustrated in (Figure 5), highlighting the link between genotoxic and pro-inflammatory responses.

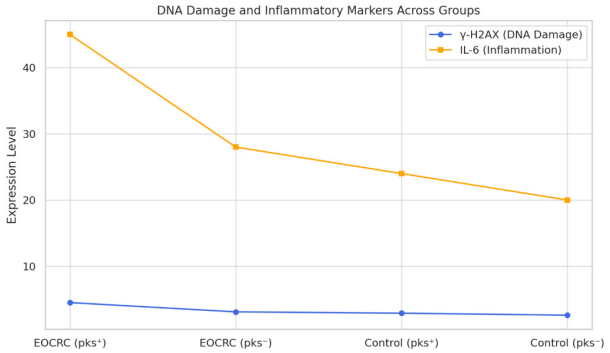

Figure 5. Line graph comparing γ-H2AX and IL-6 expression levels across study groups.

Tumour location and pks$^+$ status

Among EOCRC patients, tumours were categorized by location: rectum (32%), sigmoid colon (25%), descending colon (20%), and others (23%). Notably, 70% of pks$^+$ E. coli was detected in left-sided tumours (rectum and sigmoid), which aligns with prior studies suggesting site-specific microbial colonization. This spatial association warrants further investigation. The summarized data is presented in Table 1.

Table 1. Summary of key findings.

Variable	EOCRC (n = 100)	Controls (n = 100)	p-value
pks$^+$ *E. coli* prevalence	41 (41%)	10 (10%)	<0.0001
Mean DNA damage score (γ-H2AX)	7.72 ± 1.39	3.95 ± 0.78	<0.001
Mean Shannon diversity index	3.21 ± 0.33	3.83 ± 0.27	<0.001
Pearson correlation (pks vs γ-H2AX)	r = 0.72	–	<0.001

Discussion

The present study investigated the association between colibactin-producing Escherichia coli (pks$^+$ E. coli) and early-onset colorectal cancer (EOCRC), revealing a significantly higher prevalence of pks$^+$ strains among EOCRC patients compared to healthy controls. The detection of pks$^+$ E. coli in 41% of EOCRC cases versus 10% in controls, supported by a chi-square value of 18.32 ($p < 0.0001$), underscores a statistically robust association. Moreover, the observed elevation in DNA damage markers (γ-H2AX scores) in EOCRC patients further strengthens the hypothesis that pks$^+$ E. coli may play a contributory role in colorectal carcinogenesis. Colibactin is a genotoxin synthesized by the pks genomic island found in certain E. coli strains. Its biological activity has been shown to induce DNA double-strand breaks, chromosomal instability, and cellular senescence, all hallmarks of cancer development. The significantly elevated γ-H2AX levels in patients colonized with pks$^+$ E. coli in our study is consistent with these mechanisms. These findings align with recent reports that identified colibactin-induced mutational signatures in colorectal cancer tissues, particularly among younger patients. For instance, Pleguezuelos-Manzano et al. (2020) demonstrated that colibactin exposure leaves a distinct pattern of mutations, which are disproportionately represented in EOCRC tumors [12].

The rising incidence of EOCRC in recent decades has prompted exploration of early-life environmental and microbial exposures. Gut microbiota composition during infancy and early adulthood is increasingly being recognized as a determinant of long-term colorectal cancer risk. Our findings suggest that early colonization by pks$^+$ E. coli, potentially influenced by dietary patterns, antibiotic exposure, and other lifestyle factors, may contribute to the initiation or progression of EOCRC. Diets low in dietary fiber, for example, have been associated with shifts in microbiota that favor the proliferation of pathobionts such as pks$^+$ E. coli. Huber AR et al. (2024) reported that dietary fiber mitigated the genotoxic impact of colibactin, suggesting a potential preventive strategy. Another key aspect of colibactin-mediated carcinogenesis is bacterial adherence to the intestinal epithelium. Recent studies have identified the role of FimH adhesin in enabling close contact between pks$^+$ E. coli and host cells, facilitating efficient delivery of colibactin. Inhibiting this interaction has been shown to reduce DNA damage, highlighting a novel therapeutic target. In our study, while bacterial adhesins were not directly assessed, the high DNA damage scores in the pks$^+$ group are indicative of sustained host-microbe interactions [13,14].

Despite the compelling association between pks$^+$ E. coli and EOCRC, it is important to interpret these findings with caution. The presence of pks$^+$ E. coli in 10% of healthy controls underscores the notion that the bacterium is a risk factor rather than a definitive cause of cancer. Host immune responses, genetic susceptibility, and other microbiota-derived metabolites likely modulate this risk. Moreover, cancer development is a multifactorial process, and bacterial genotoxins represent only one piece of a complex puzzle. Nevertheless, the findings from this study contribute to a growing body of evidence that implicates microbial factors, particularly colibactin, in the etiology of colorectal cancer. From a public health perspective, this raises important considerations. First, microbiome profiling could serve as a future biomarker for EOCRC risk stratification. Second, early dietary interventions promoting fiber-rich diets may shift microbial communities in a protective

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direction. Third, therapeutic strategies targeting pks expression, colibactin biosynthesis, or bacterial adhesion represent promising avenues for translational research [15,16].

This study has several strengths, including the use of standardized PCR methods for pks detection and immunofluorescence-based DNA damage quantification. However, certain limitations must be acknowledged. The cross-sectional design restricts causal inference. The sample size, though adequate for statistical power, warrants expansion for subgroup analyses, such as by gender or tumour stage. Additionally, functional assessments of bacterial virulence and host inflammatory responses could provide further insight. So, our data support a strong association between pks$^+$ E. coli and EOCRC, both in terms of prevalence and genotoxic impact. These findings emphasize the need for greater attention to gut microbiome dynamics in colorectal cancer prevention and open the door to novel diagnostics and therapeutics targeting microbial oncogenesis [17].

Conclusions

This study highlights a significant association between colibactin-producing *E. coli* and early-onset colorectal cancer. The elevated prevalence of pks$^+$ *E. coli* in EOCRC patients, coupled with increased DNA damage markers, suggests a contributory role of colibactin in colorectal carcinogenesis. While causality cannot be definitively established, the findings emphasize the need for heightened awareness of microbial factors in cancer development. Preventive strategies focusing on early-life interventions, such as promoting dietary fiber intake and prudent antibiotic use, may mitigate the risk of EOCRC. Additionally, targeting bacterial adhesins presents a potential therapeutic avenue to reduce colibactin-induced genotoxicity. Future research should aim to unravel the intricate mechanisms by which colibactin-producing bacteria influence colorectal cancer development and explore interventions to modulate the gut microbiome for cancer prevention.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Hong D, Tian R, Kim H, Doering M, Cao Y. The microbiome landscape of early-onset colorectal cancer: A systematic review. J Clin Oncol. 2024;42(1):e15646. https://doi.org/10.1200/JCO.2024.42.16_suppl.e15646
2. Patel SG, Karlitz JJ, Yen T, Lieu CH, Boland CR. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. Lancet Gastroenterol Hepatol. 2022;7(3): 262-274. https://doi.org/10.1016/S2468-1253(21)00426-X
3. Akimoto N, Ugai T, Zhong R, Hamada T, Fujiyoshi K, Giannakis M, et al. Rising incidence of early-onset colorectal cancer—a call to action. Nat Rev Clin Oncol. 2021;18(4):230-243. https://doi.org/10.1038/s41571-020-00445-1
4. Saraiva MR, Rosa I, Claro I. Early-onset colorectal cancer: A review of current knowledge. World J Gastroenterol. 2023;29(8):1289. https://doi.org/10.3748/wjg.v29.i8.1289
5. Ullah F, Pillai AB, Omar N, Dima D, Harichand S. Early-onset colorectal cancer: current insights. Cancers. 2023;15(12):3202. https://doi.org/10.3390/cancers15123202
6. Stoffel EM, Murphy CC. Epidemiology and mechanisms of the increasing incidence of colon and rectal cancers in young adults. Gastroenterol. 2020;158(2):341-353. https://doi.org/10.1053/j.gastro.2019.07.055
7. Miyasaka T, Yamada T, Uehara K, Sonoda H, Matsuda A, Shinji S, et al. Pks-positive Escherichia coli in tumor tissue and surrounding normal mucosal tissue of colorectal cancer patients. Cancer Sci. 2024;115(4):1184-1195. https://doi.org/10.1111/cas.16088
8. Arima K, Zhong R, Ugai T, Zhao M, Haruki K, Akimoto N, wt al. Western-style diet, pks island-carrying Escherichia coli, and colorectal cancer: analyses from two large prospective cohort studies. Gastroenterol. 2022;163(4):862-874. https://doi.org/10.1053/j.gastro.2022.06.054
9. Auvray F, Perrat A, Arimizu Y, Chagneau CV, Bossuet-Greif N, Massip C, et al. Insights into the acquisition of the pks island and production of colibactin in the Escherichia coli population. Microb Genom. 2021;7(5):000579. https://doi.org/10.1099/mgen.0.000579 .
10. Strakova N, Korena K, Karpiskova R. Klebsiella pneumoniae producing bacterial toxin colibactin as a risk of colorectal cancer development-A systematic review. Toxicon. 2021;197:126-135. https://www.sciencedirect.com/science/article/pii/S0041010121001112
11. Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, Nomburg J, et al. Mutational signature in colorectal cancer caused by genotoxic pks+ E. coli. Nature. 2020; 580(7802):269-273. https://doi.org/10.1038/s41586-020-2080-8
12. de Souza JB, de Almeida Campos LA, Palácio SB, Brelaz-de-Castro MC, Cavalcanti IM. Prevalence and implications of pKs-positive Escherichia coli in colorectal cancer. Life Sci. 2024;341:122462. https://doi.org/10.1016/j.lfs.2024.122462
13. Huber AR, Pleguezuelos-Manzano C, Puschhof J, Ubels J, Boot C, Saftien A, et al. Improved detection of colibactin-induced mutations by genotoxic E. coli in organoids and colorectal cancer. Cancer Cell. 2024;42(3):487-496. https://www.cell.com/cancer-cell/fulltext/S1535-6108(24)00053-9
14. Wassenaar TM. E. coli and colorectal cancer: a complex relationship that deserves a critical mindset. Crit Rev Microbiol. 2018;44(5): 619-632. https://doi.org/10.1080/1040841X.2018.1481013
15. Wang Y, Fu K. Genotoxins: the mechanistic links between escherichia coli and colorectal cancer. Cancers. 2023;15(4):1152. https://doi.org/10.3390/cancers15041152
16. Wilson MR, Jiang Y, Villalta PW, Stornetta A, Boudreau PD, Carrá A, et al. The human gut bacterial genotoxin colibactin alkylates DNA. Science. 2019;363(6428):eaar7785. https://doi.org/10.1126/science.aar7785
17. Joo JE, Chu YL, Georgeson P, Walker R, Mahmood K, Clendenning M, et al. Intratumoral presence of the genotoxic gut bacteria pks+ E. coli, Enterotoxigenic Bacteroides fragilis, and Fusobacterium nucleatum and their association with clinicopathological and molecular features of colorectal cancer. Br J Cancer. 2024;130(5): 728-740. https://doi.org/10.1038/s41416-023-02554-x

https://doi.org/10.61577/jmi.2025.100003