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CHARACTERIZATION OF *ESCHERICHIA COLI* ISOLATES FROM PATIENTS WITH COLON CANCER

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Escherichia coli is a one of the most important human pathogens. Besides diarrheal and extraintestinal infections, *E. coli* has been associated with pathologic conditions such as inflammatory bowel diseases and colorectal cancer[1,2].

In this study, we characterized mucosal *E. coli* isolates from 63 patients with colon cancer. Using PCR screening, we classified *E. coli* isolates to four main phylogenetic groups (i.e., A, B1, B2, and D) and analyzed prevalence of encoded virulence factors in *E. coli* genomes.

We foud that mucosal *E. coli* (n = 200) from patients with colorectal neoplasia most frequently belonged to phylogroup B2 (38.0%), followed by phylogroups A (28.5%) and D (26.0%), while phylogroup B1 was not very common (7.5%). Isolates of phylogroup B2 encoded the most of analyzed virulence factors.

Mucosal *E. coli* from cancer patients rarely (<1%) harboured determinants for toxicity (i.e., *lt*, *st*, *stx1*, and *stx2*) and adhesion (i.e., *bfpA* and *ial*), which are typical for diarrheal *E. coli* pathotypes such as EHEC, ETEC, and EPEC. In contrast, these *E. coli* isolates frequently encoded virulence factors typical for extraintestinal pathogenic *E. coli* (ExPEC).

High prevalence was observed for fimbriae (i.e., P-fimbriae (*pap*, 31.5%) and S-fimbriae (*sfa*, 28.0%)), several various iron-acquisition systems (i.e., yersiniabactin (*fyuA*, 68.5%), enterobactin (*fepC*, 63.0%), and salmochelin (*iroN*, 45%)), and toxins such as colibactin (*pks*, 25.5%), UPEC-specific protein (*usp*, 45%); and hemolysin (*α-hly*, 13.0%).

In conclusion, *E. coli* from patiens with colorectal cancer harbored virulence factors, which allow *E. coli* to bind to eukaryotic cells, survive in low-iron conditions, and damage cells and tissues. As a result, specific *E. coli* strains could contribute to the development of neoplastic processes in the large intestine.

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MINION AS A TOOL FOR SEQUENCING OF VARIABLE AND PARALOGOUS GENOMIC LOCI OF *TREPONEMA PALLIDUM*

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Genus *Treponema* includes number of human and animal pathogenic species and subspecies, e.g., *Treponema pallidum* subspecies *pallidum* (TPA) causing syphilis, *Treponema pallidum* subspecies *pertenue* (TPE) causing yaws. Genome similarity among TPA genomes (99.95%)[1] and TPA and TPE subspecies (99.8%)[2] indicates that small differences in the genome sequence play big roles in pathogenicity. Regions that show high sequence variability among strains, repetitive regions, paralogous genes and regions of intragenomic recombination are key for understanding pathogenicity of treponemes and host immune response and many of them code for surface proteins. Those regions are important candidates for vaccine development[3]. Determination of sequences of described genomic regions by Illumina sequencing is often problematic reflecting their paralogous character, thus new approaches need to be developed to determine reliable complete chromosome sequences.

Specific primers were designed to amplify regions of interest (ROI, n=36) with unique 200 nt tags at the start and end of each gene. All PCR products of each clinical sample were equimolarly pooled and barcoded and multiple samples were sequenced together using long-read sequencing technology (Oxford Nanopore, MinION). Unique tags were used for identification of reads origin by mapping to masked reference genome. *De novo* assembly with filtered reads was performed followed by quality assesment of consensus for each ROI. Clinical samples of TPA and TPE with different sequence profiles were selected, sequenced and consensus sequences of ROIs were analyzed.

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