P1009
Paper Poster Session
Microbiota

The quantitative analysis of intestinal microbiota by fluorescent real-time PCR as an alternative to bacteriological assays

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Background: It is well known that the majority of the intestinal species are non-cultivable. The purpose of this study was to approve the rapid, reliable non-cultural method for the estimation of gut microbiota composition. The fluorescent real-time PCR was chosen for this aim. The method must meet the following requirements: to include the optimal combination of intestinal microorganisms enough to determine the normal flora, to reveal the excessive growth of potentially harmful opportunistic microorganisms and appearance of pathogenic species. To further develop this method it ought to analyze a big size of samples and elaborate the practical recommendations for introducing it into routine clinical practice.

Material/methods: DNA was extracted from stool samples by using a DNA-Express kit (LITEX) with a modified protocol. Oligonucleotides and oligonucleotide's probes for PCR were designed on the basis of gene sequences available from the GenBank database. Group- and species-specific primer sets were used for identification and counting of Bacteroides fragilis group, Bifidobacterium group, Lactobacillus group, Escherichia coli and enteropathogenic Escherichia coli, Enterococcus group, Faecalibacterium prausnitzii, Clostridium perfringens, Clostridium difficile, Staphyllococcus aureus, Proteus spp., Klebsiella pneumonia, Klebsiella oxytoca, Candida spp, Shigella spp., Salmonella spp. An MiniOpticon apparatus (Bio-Rad, USA) was used for the real-time PCR. To confirm the specificity of amplification, the sequences of PCR products were performed.

Results: A total of 350 samples were analyzed. Real-time PCR showed the high level of comparability with the cultivation method for bacterial groups belonging to normal intestinal flora and for the pathogenic microorganisms such as Klebsiella, Staphyllococcus, Shigella and Salmonella. The main advantages of real-time PCR are the capability to reveal specific changes for the certain types of pathology. One of it is decreasing in Fecalibacterium prauznitzii and significantly (more than 100) increased Bacteroides fragilis group/ Fecalibacterium prauznitzii ratio, associated with the inflammatory processes in the gut. The other benefit due to the species-specific primers is Fusobacterium nucleatum detection pointed to the risk of colorectal neoplasia development. The normal count of Bifidobacterium and Lactobacillus species and lack of excessive number of potentially harmful enterobacteria marked the appropriate response to probiotic therapy.

Conclusions: The using of real-time PCR instead of classical cultivation techniques has been shown to provide a good tool for the monitoring of gut bacterial community. Real-time PCR allows to selecting the unique composition of group- and species-specific primers for detection the main types of disturbances in the intestinal microbiota.