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Study of the bacterial community of the gastric mucosa in patients with gastro-duodenal region diseases using metatranscriptomic analysis

Kupriyanova E.*, Abdulkhakov S., Markelova M., Grigoryeva T.
Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia
** fewrandomletters@mail.ru*

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Motivation and Aim: It has now been proven that *H. pylori* infection can lead to chronic inflammatory changes in the gastric mucosa, and subsequently to the development of precancerous changes in the mucous membrane [1]. However, other types of bacteria, in addition to *H. pylori*, can provoke the development of chronic inflammatory changes in the gastric mucosa, and even contribute to the development of gastric cancer [2]. But the mutual influence of *H. pylori* and other representatives of the gastric microbiota remains poorly understood. Taking into account the morphological and functional characteristics of different parts of the stomach, including those caused by the production of hydrochloric acid, it is obvious that the microbial composition in different parts of the stomach may differ.

In the vast majority of studies, the gastric microbiota has been studied at the bacterial DNA level using sequencing of 16S rRNA gene libraries, which include DNA from dead or destroyed cells. In this regard, the purpose of this study was to analyze the resident, metabolically active representatives of the microbiota in the two main parts of the stomach – the body and the antrum – using sequencing of RNA-derived libraries.

Methods and Algorithms: We obtained 120 paired gastric mucosal biopsies (one from the antrum and one from the corpus, respectively) from 60 patients who underwent esophagogastoduodenoscopy (EGD) for suspected gastroduodenal disease. Total RNA from each sample was extracted with TRIzol reagent. The RNA fraction was then used to synthesize cDNA. Amplicon libraries of the V3-V4 variable region of the 16S rRNA gene were sequenced on the Illumina MiSeq platform.

Results: In the majority of *H. pylori*-positive patients, a significant (up to 90 %) dominance of the *Helicobacter* genus was found. Bacteria of the *Streptococcus* genus, in particular the species *Streptococcus vestibularis*, as well as the *Prevotella* and *Alloprevotella* genera, were represented to a lesser extent. Previous studies examining the composition of the gastric microbiota in patients with chronic gastritis showed that there is a significant negative correlation between the presence of *H. pylori* and the proportion of the *Streptococcus* genus: the presence of *H. pylori* inhibits the growth of *Streptococcus*, and in *H. pylori*-negative patients their number increases [3]. Indeed, in the absence of *H. pylori* infection, we found an increase in the representation of *Streptococcus* by more than 2 times, as well as the presence in a larger number of the *Prevotella*, *Alloprevotella Gemella*, *Haemophilus*, *Staphylococcus*, *Sphingomonas* genera, which, according to the literature, can be considered as representatives of the normal gastric microbiota [4].

There is also a difference between the bacterial composition of the antrum and body of the stomach, which is especially important for *H. pylori*-positive patients. For example, the abundance of *Helicobacter* species in individual samples was 25, 35, and 36 % in the gastric antrum and 87, 81, and 72 % in the corpus, respectively, calling into question the rationale for taking biopsies only from the antrum to confirm the diagnosis of *H. pylori* infection. Also, bacteria from the *Lautropia*, *Enhydrobacter*, *Rikenellaceae*, *Acinetobacter*, *Leptotrichia*, and *Capnocytophaga* genera were found in low numbers in many samples, so they likely represent a minor, poorly studied part of the gastric microbiota.

Based on the data obtained, it can be assumed that *H. pylori* tends to predominate in the stomach of *H. pylori*-positive patients, especially when analyzing the body of the stomach compared to the antrum. *H. pylori* exhibits antagonistic activity against bacteria of the *Streptococcus* genus; in *H. pylori*-negative patients, the number of *Streptococcus* increases significantly. The use of gastric corpus biopsy is likely to have greater prognostic value in the diagnosis of *H. pylori* infection. Also, the use of RNA, compared to classical DNA-based methods, allows the identification of a greater diversity of bacterial genera.

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