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Effects of anti-*Helicobacter pylori* concomitant therapy and probiotic supplementation on the throat and gut microbiota in humans

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ABSTRACT

The microbiota within humans maintains homeostasis and plays important roles in human health. However, some situations such as the use of antibiotics may disrupt the microbiota balance and result in a series of adverse effects. This study aimed to investigate the effects of a commonly used anti-*Helicobacter pylori* concomitant therapy on the composition of the gut and throat microbiota and any antibiotic resistance that may develop. In addition to the standard regimen, two different supplementary probiotic regimens that both used *Saccharomyces boulardii* were included. Microbiological culture-based techniques were used to analyse the microbiota composition and antibiotic resistance. Our results showed marked quantitative and qualitative alterations in both the gut and throat microbiota after treatment with not only the standard concomitant therapy but also with either supplementary probiotic regimen. Nevertheless, most of the changes in the gut microbiota (except for yeast and *Bacteroides* spp. counts) reverted by Day 71, whereas the alterations in the throat microbiota appeared to persist. Patients treated with the eradication therapy in the absence of probiotic supplementation experienced the most pronounced disturbances in the throat microbiota, whereas changes in the throat microbiota appeared to stabilize in the groups that received probiotic supplementation. We also detected higher antibiotic resistance rates for *Enterobacteriaceae*, *Enterococcus* spp. and *Bacteroides* spp. after treatment with the eradication therapy. Co-administration of probiotics is likely to be more effective than post-antibiotic supplementation, and although some beneficial effects were observed, the probiotic combination did not exert significant effects on the unbalanced commensal gut and throat microbiota composition.

1. Introduction

The extensive microbiota harboured in humans is exceedingly vital for bodily function [1]. Normally, the microbiota maintains homeostasis, which plays important roles in human health. And accumulating evidence suggests that an unbalanced commensal microbiota contributes to many diseases, including inflammatory bowel disease, liver disease, and atopic diseases such as eczema and asthma [2,3].

Helicobacter pylori (*H. pylori*) is one of the most common pathogens worldwide; more than half of the world's population is positive for *H. pylori* infection, and in developing countries, the prevalence is as high as 80% among adults [4]. It is well known that *H. pylori* infection is strongly correlated with gastrointestinal disorders such as chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and even gastric cancer [5]. In general, the most widely recommended treatment for the eradication of *H. pylori* is a standard triple therapy, which combines two antibiotics (clarithromycin plus either amoxicillin or metronidazole) with a proton pump inhibitor (PPI) [6]. However, in recent years, the effectiveness of this standard triple therapy has been increasingly compromised by the rapid emergence of antibiotic-resistant strains of *H. pylori* and poor patient compliance [7]. Increasing data suggests that this combination has diminished efficacy and a maximum cure rate is of only 70% [8], which is

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unacceptable. Since no new drugs have been developed for this indication, it was proposed that three antibiotics (amoxicillin, clarithromycin and either metronidazole or tinidazole) should be administered simultaneously with a PPI, a regimen known as concomitant therapy or non-bismuth quadruple therapy [9,10].

However, long-term and high-dose administration of multiple broad-spectrum antibiotics can cause several adverse events. Studies have shown that concomitant therapy can cause diarrhoea, nausea/vomiting, and even *Clostridium difficile* infection (the primary pathogen of antibiotic-induced pseudomembranous colitis) [11,12].

The primary mechanism of these adverse events is thought to be impaired resistance to pathogens due to disruption of the microbiota (especially the gut microbiota) and subsequent changes in the metabolism. Therefore, studying the effects of antibiotics on the composition of the microbiota will help to further elucidate the specific mechanisms. However, to the best of our knowledge, there are no reports regarding the effects of the concomitant therapy on the composition of the indigenous microbiota.

In view of the proposed underlying pathogenesis, the use of probiotics as an adjuvant therapy in *H. pylori* eradication to reduce adverse events and avoid complications has been extensively studied, but its role is still debated [13–17]. Additionally, the optimal supplementation (i.e., the most suitable species, duration and dosage) is the focus of researchers.

In addition to the adverse events, scientists have been studying antibiotic resistance, which is another by-product that can manifest from eradication therapy. Some reports showed that eradication of *H. pylori* using triple therapy can result in increased antibiotic resistance of the gut microbiota [18–21].

In this study, we investigated for the first time the effects of a popular concomitant therapy on the composition of the gut and throat microbiota as well as the antibiotic resistance induced by this therapy. We also explored the effects of two different probiotic supplementary methods in the treatment.

2. Materials and methods

2.1. Ethics statement

Written consent was obtained from all participants involved in the study, and the study was approved by the Human Ethics Committee at Peking University Health Science Center.

2.2. Study population

Thirty patients with non-ulcer dyspepsia (NUD) and *H. pylori* infection were recruited into this study at Peking University Third Hospital between January 2016 and June 2016. The diagnosis was based on endoscopic findings, and patients were considered *H. pylori*-positive if both the rapid urease test (RUT) and ^{13}C-urease breath test (^{13}C-UBT) were positive. Eligible subjects were 18–65 years of age, and the exclusion criteria for the study were as follows: prior documented treatment of *H. pylori*; use of antibiotics, PPI, probiotics, H_2-receptor blockers or bismuth within 12 weeks of enrolment; dietary restrictions or recommendations before inclusion into the study; history of gastrointestinal tract surgery; chronic or severe comorbid diseases; history of allergy to any of the study drugs; currently pregnant or lactating.

2.3. Study design

The study was a randomized, double-blind study. Patients were randomly divided into three groups (ten patients per group). *H. pylori* eradication was achieved with a 14-day regimen (from Day 1 to Day 14) of concomitant therapy: esomeprazole (20 mg, twice daily), amoxicillin (1000 mg, twice daily), clarithromycin (500 mg, twice daily) and tinidazole (500 mg, twice daily). Group A did not receive any probiotic therapy, Group B was provided probiotic sachets (BIOFLOR®, *Saccharomyces boulardii* CNCM I-745®, Biocodex Inc, 500 mg, twice daily) for 2 weeks from Day 15 to Day 28, and Group C received the same probiotic sachets for 2 weeks but ingested them from Day 1 to Day 14 (at least 2 h after administration of antibiotic therapy). All other probiotic products were forbidden during the intervention (2 or 4 weeks) as well as during the follow-up period (6 or 8 weeks).

A throat swab and a faecal sample were collected from each patient one day before the concomitant therapy (Day 0) and one day (Day 15) and eight weeks (Day 71) after completing the concomitant therapy. For the throat swabs, 0.5 ml of 12.5% glycerine solution was added to infiltrate the swab immediately after collection, and the resulting liquid is defined as diluent. All the samples were stored at −80 °C until further analysis. A^{13}C-UBT was used to confirm the presence of *H. pylori* infection 8 weeks after completing the concomitant therapy (Day 71), *H. pylori* was considered eradicated if the test result was negative.

All the patients were informed of the importance of full compliance and were warned of potential adverse events. Compliance and adverse events for the three groups were evaluated by direct questioning with a physician and collection of the medication tablet boxes after completion of the treatment. Compliance was calculated as a percentage using the following formula: (%) = (the expected number of tablets consumed - the number of remaining tablets)/the expected number of tablets consumed × 100%, and values > 90% were considered satisfactory.

2.4. Microbiota analysis

Standard microbiological culture-based techniques were used to analyse the microbiota composition [22]. Faecal samples were thawed in an anaerobic chamber at room temperature, and a 0.5 g sample was accurately weighed, diluted 10-fold in 4.5 ml of normal saline (0.9% NaCl solution) and mixed well by adding 3 sterilized glass beads. The suspension was serially diluted 10-fold with the same saline solution down to a 10^{-8} dilution and inoculated on 9 selective and non-selective agars; these agars are shown in STable 1. The throat swabs were thawed, homogenized, diluted 10-fold to 10^{-5}, and inoculated on 8 different agars (See STable 2). All the aerobic plates were incubated at 37 °C for 24 h except for plates containing Sabourand's agar, which were incubated at 28 °C for 36 h. All the anaerobic plates were incubated under anaerobic conditions (AnaeroPack® - Anaero, Mitsubishi Gas Chemical Co., Inc) at 37 °C for 48 h, and chocolate agar was incubated under 5%–6% CO_2 conditions (AnaeroPack® - CO_2, Mitsubishi Gas Chemical Co., Inc) at 37 °C for 48 h. After implementing the various incubation steps, different colony types were counted and identified to the genus level based on the colony morphology, Gram staining, general morphology and biochemical tests. Colonies on the CCFA agars were determined if they were *Clostridium difficile* using a diagnostic reagent (C. Difficile TEST KIT, Oxoid Ltd.). The concentration was defined as the number of colony forming units (CFU) either per gram (g) of wet faeces or per millilitre (ml) diluent. The detection limit was 10^2 CFU/(g wet faeces) or 10^2 CFU/ml diluent.

2.5. Antibiotic susceptibility tests

Amoxicillin resistance was determined for *Enterobacteriaceae* and *Enterococcus* spp. from the faecal samples and for *Staphylococcus* spp. from the throat samples. Clarithromycin resistance was tested in *Enterococcus* and *Bacteroides* spp. from the faecal samples