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Letter to the editor

Microbiota diversity and bacterial load after successful treatment of *Clostridioides difficile* infection with honey lavage in 4 patients

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In this letter, an experimental therapy in four patients with therapy-resistant *Clostridioides difficile* infection is described. These four patients were treated with Manuka honey via colon lavage. First, the patients received a three-day fidaxomicin treatment. The colon lavage was performed on the third day. During a subsequent ileocolonoscopy, 300 mL 15% Manuka honey was applied via a spray catheter. Patients remained in bed for two hours after the procedure and did not defecate. The patient's microbiomes were tested before treatment, after the fidaxomicin treatment, and after honey lavage. A decrease in *C. difficile* load was found in their microbiomes. Additionally, restoration of microbiota diversity after the honey lavage was also noted. The four patients experienced complete cessation of watery stools and remain symptom free. These results indicate the need for more clinical research into this matter.

Key words: Manuka honey, microbiota, honey lavage, *Clostridioides difficile*

To the editor,

Patients infected with *Clostridioides difficile* might experience a recurrence within two months after being treated with conventional antibiotic therapy. In some cases, a therapy-resistant *C. difficile* infection (CDI) occurs, which means that the CDI is no longer susceptible to conventional antibiotic therapy. These patients have a few other experimental options. One of the alternatives for persistent CDI might be honey lavage of the colon. Our research team, as well as other clinical researchers, has performed several *in vitro* studies demonstrating that unprocessed honeys have antibacterial activity against a range of pathogens, including CDI [1]. Although the therapeutic mechanism of honey has not yet been fully elucidated, the antibacterial effect has been attributed to osmolarity, hydrogen peroxide generation, and unidentified additional phytochemical components. Furthermore, besides antibacterial activity, honey has been shown to have an anti-inflammatory function as well. Manuka (*Leptospermum scoparium*) honey has been graded using methylglyoxal (MGO). Methylglyoxal, the antimicrobial compound in Manuka honey, shows antibacterial activity. MGO also demonstrates this effect against bacteria with resistance to antibiotics [2–4].

To date, 4 patients with therapy-resistant CDI who failed treatment with metronidazole, vancomycin, or fidaxomicin have been treated with an experimental honey lavage. The patients received 3 days of fidaxomicin, and a colon lavage was performed on day 3 (4 L Klean-Prep). This was followed by an ileocolonoscopy during which the bowel was sprayed with a 300 mL solution of 15% honey (Manuka MGO 550). The honey was administered from the most distal segment of the small bowel to the sigmoid. After the colonoscopy, we asked the patients to stay in bed for 2 hours and not defecate. *C. difficile* was isolated from feces after the honey lavage. Overall, all 4 patients treated with honey lavage experienced complete cessation of watery stools and have remained symptom free (years later).

Since honey has antibacterial activity against a range of bacteria, we were interested in whether the honey lavage alters the composition of gastrointestinal microbiota besides *C. difficile*. We asked the patients to collect faeces samples before treatment (baseline), after 2 and 3 days of fidaxomicin therapy, and after the honey lavage. The faecal samples were gathered in sterile containers and stored at −20°C within 2 hours of collection. To study the human intestinal microbiota, we used a PCR-based profiling technique (IS-pro) for high-throughput analysis. The IS-pro technique allows the detection of bacterial DNA and its taxonomic classification down to the species level on the basis of 16S-23S ribosomal interspace (IS) fragment length. In contrast to 16S metagenomics, the 16S-23S rDNA IS region is more variable in size and sequence. This makes it more applicable for analysing

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complex communities such as the microbial community of the intestinal tract [5, 6].

To determine the change in microbial diversity, we calculated the sum of the microbial loads and the Shannon diversity of the gut microbiota before and after therapy.

In all 4 faeces samples, we identified *C. difficile* DNA. The IS-pro analyses showed a successively decreasing load of *C. difficile* DNA at baseline, on day two after fidaxomicin therapy, on day three after fidaxomicin therapy, and after the honey lavage. The load of *C. difficile* as reflected by fluorescence intensity decreased respectively from 2879595 RFU at baseline to 135714 RFU and 49828 RFU on days two and three and then to 25 RFU after therapy (Fig. 1). In accordance with the *C. difficile* load, the microbiota diversity decreased successively, with the Shannon indices being 3.36 at baseline, 3.29 after two days of fidaxomicin, and 3.23 after three days of fidaxomicin. After the honey lavage, the microbiota diversity stabilized near the baseline (3.33 Shannon).

Overall, the bacterial load and diversity per sample decreased after fidaxomicin therapy and were restored after the honey lavage. This case report demonstrates the possible therapeutic value of honey lavage as a treatment for therapy-resistant CDI, indicating the need for more clinical research into this matter.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the manuscript. The honey lavage was performed by R.J.F Laheij, MSc, MD, PhD.

CONFLICTS OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

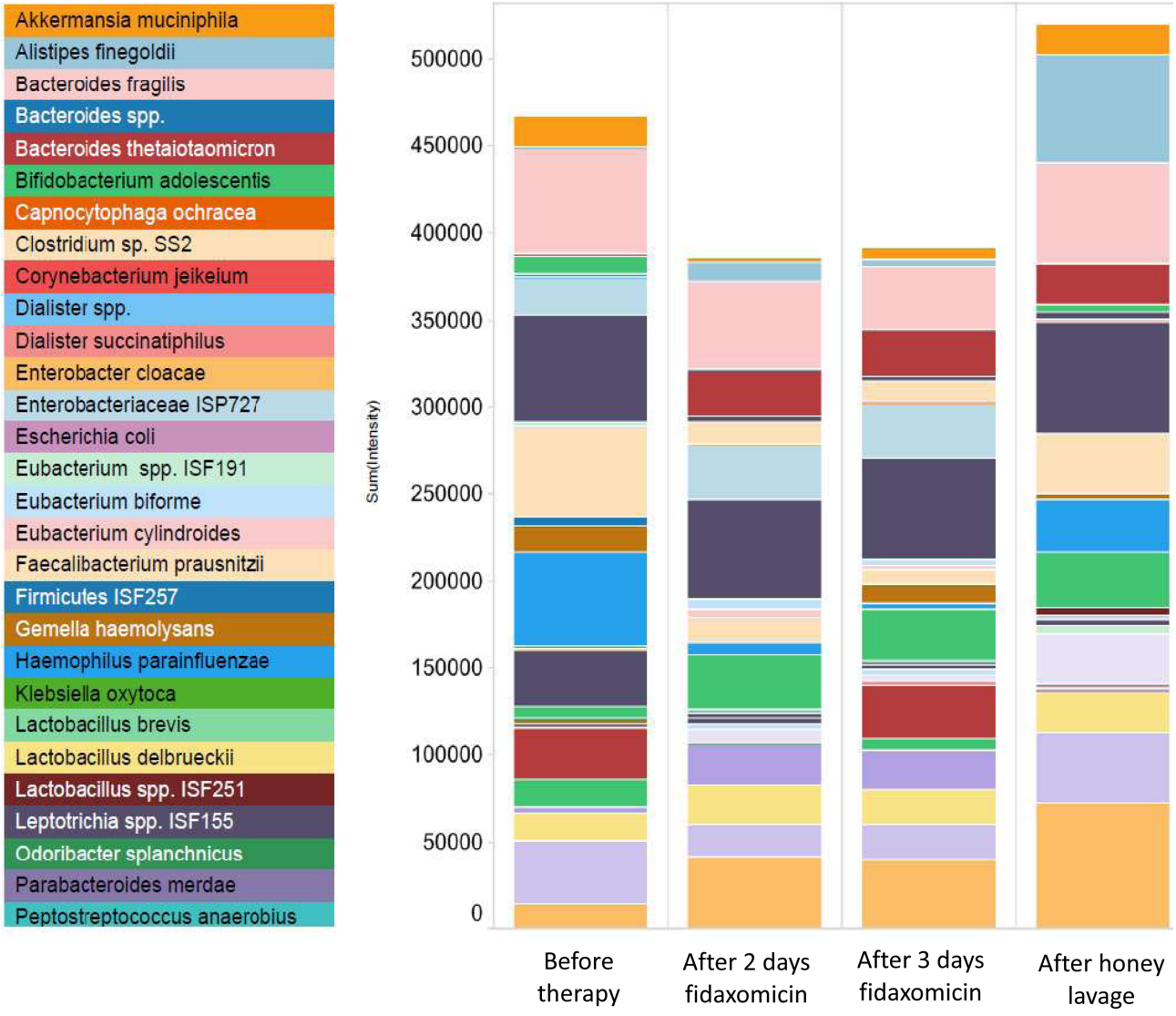

Fig. 1. Microbiota load and diversity before and after therapy.

The figure shows the changes in microbiota load and diversity among the four time points: before therapy, after two and three days of fidaxomicin, and after honey lavage. A clear decrease in *Clostridium* sp. SS2 is shown in this figure, which is noted in the legend next to the stacked bar chart.

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