Exploring the role of the microbiota: in defence against Clostridioides difficile and multidrug resistant Gram-negatives

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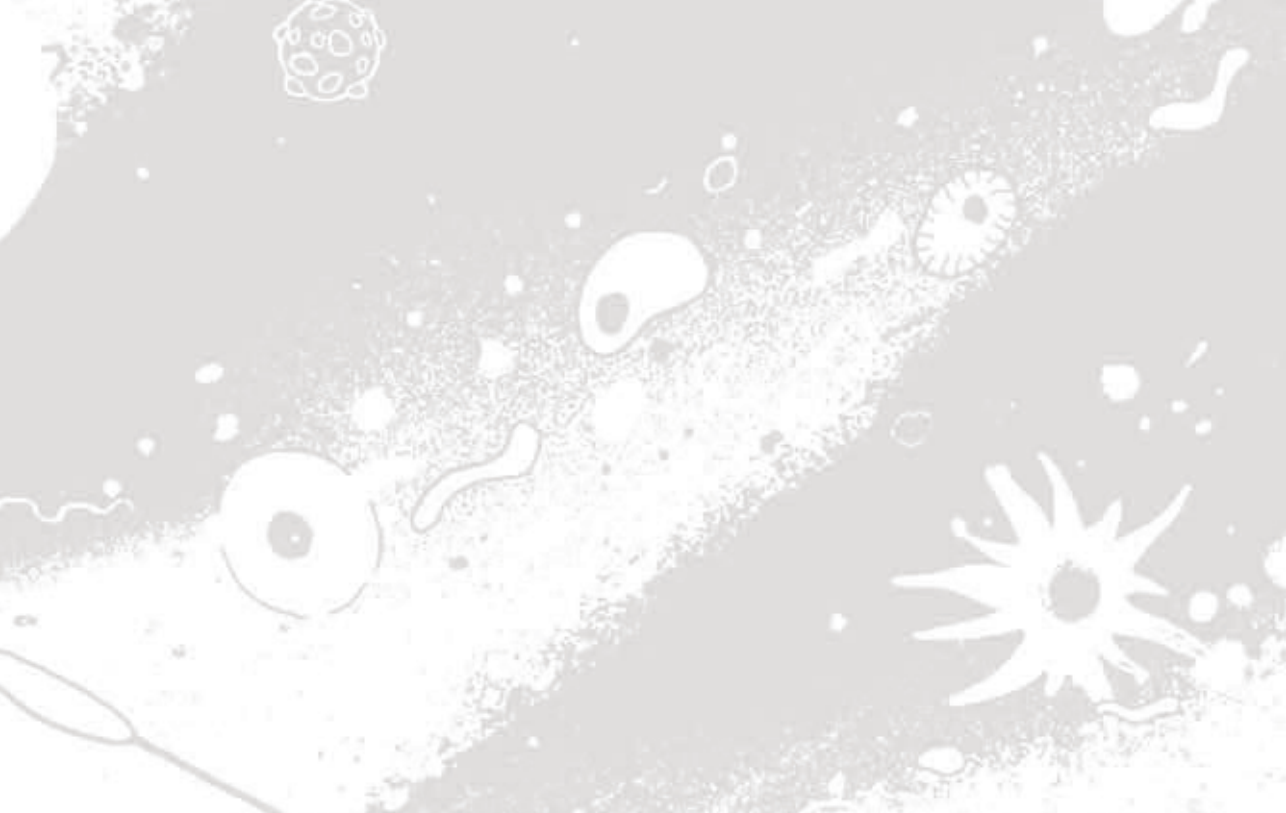The handle https://hdl.handle.net/1887/3188577 holds various files of this Leiden University dissertation.

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Fecal microbiota transfer for multidrug resistant Gram-negatives; a clinical success combined with microbiological failure

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Chapter 8. Fecal microbiota transfer for multidrug resistant Gram-negatives; a clinical success combined with microbiological failure

Abstract

Combined fecal microbiota transfer (FMT) and antibiotic treatment pre-vented recurrences of urinary tract infections with multidrug resistant (MDR) *P. aeruginosa*, but failed to eradicate intestinal colonization with MDR *E. coli*.

Based on microbiota analysis, failure was not associated with distinct diminished microbiota diversity.

Introduction

Multidrug resistance (MDR) of *Enterobacteriaceae* is an increasing worldwide problem that challenges the treatment of common bacterial infections. MDR has been declared one of the greatest challenges to global public health today, and innovative strategies for decolonization of MDR bacteria are urgently needed to reduce the use of reserve antibiotics and prevent transmission [1]. A few reports mention success with fecal microbiota transfer (FMT) to eliminate extended-spectrum β-lactamase (ESBL) pro-ducing *Enterobacteriaceae*. Failures have not been reported. We present a 34-year old patient on peritoneal dialysis, treated with FMT to eradicate a Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *Pseudomonas aeruginosa* causing recurrent urinary tract infections, which hampered planned kidney-pancreas transplantation. Microbiome analysis was performed prior to and after infusion of fecal microbiota.

Case description

A 34-year old male with type 1 diabetes mellitus was referred to our tertiary hospital because of diabetic nephropathy. Screening for combined kidney pancreas trans-plant started. Two months after starting hemodialysis, he was admitted because of bacteremia and catheter related thrombophlebitis of the brachiocephalic vein by

Staphylococcus aureus, which was treated with flucloxacillin for 6 weeks. Because the extensive thrombosis prohibited shunt or catheter placement, he was converted to peritoneal dialysis (PD). During admission, a transurethral catheter was placed because of neurogenic bladder dysfunction. Shortly after discharge he returned to our hospital with a febrile catheter-related urinary tract infection (UTI) and was treated empirically with ceftazidim. Urinary cultures were positive with a bla_{VIM} carbapenemase producing *P. aeruginosa*, resistant to carbapenems, cephalosporins, quinolones, aminoglycosides and fosfomycin, only susceptible to colistin with a MIC of 4mg/L. The same *P. aeruginosa* was isolated from a rectal swab and the PD-catheter exit site. The patient received colistin intravenously (IV) for 2 weeks and the urinary and PD catheter were replaced. In the following months, the patient suffered from recurrent febrile UTIs due to the MDR *P. aeruginosa* (details on antibiotic use shown in *Figure 1*). Because of the high likelihood of recurrence of UTI caused by this MDR organism for which the only antibiotic was nephrotoxic, kidney transplantation was considered contraindicated and the patient was removed from the waiting list. During colistin treatment of the third episode, a plan for decolonization was developed. The transurethral catheter was removed and intermittent catheterization with twice weekly prophylactic intravesical high dose gentamicin instilments was started. Repeated negative cultures of urine, PD catheter-skin interface, skin, ears and throat excluded chronic prostatitis or colonization at other sites than the gut. No oral selective digestive decontamination was given. After consultation with our ethics committee, informed consent was obtained from the patient for treatment with fecal microbiota. Six weeks after the last IV course of colistin, the infusion of FMT was performed.

Material and methods

Donor feces infusion was performed using the support of the National Donor Feces Bank (http://www.ndfb.nl/) according to the FECAL trial protocol with minor modifications [2]. In summary, donor feces was obtained from an unrelated healthy volunteer. Donor serum and feces were extensively screened for fecal and blood transmitted diseases including MDR bacteria. 75 gram of feces was homogenized with saline, and sieved (300μm mesh) to remove undigested food fragments. Within 8 hours after defecation of the donor, 300ml fecal suspension was infused in the duodenum of the patient through a nasoduodenal tube, after full colon lavage. Stool samples were

collected prior to infusion, after 1 week, 2 weeks, 1 month, 2 months and 3 months and screened for MDR presence using selective enrichment media, as described previously [3]. A portion of the feces was stored within 4 hours after delivery at -80°C for microbiome research. To assess the relatedness of bacterial strains, Amplified Fragment Length Polymorphism (AFLP) technique was performed as described previously [4].

Microbiota analysis

Bacterial DNA was isolated from the fecal samples using the ZR Fecal DNA MiniPrep kit (Zymo Research). Library preparation and amplification of the V4 hyper variable region 16S rRNA gene was performed using NEXTflex 16S V4 Ampliconseq kit v2.0. High-throughput sequencing was executed at ServiceXS (Leiden, the Netherlands) on the Illumina Hiseq 2500 platform (Illumina, San Diego, USA) in rapid run mode paired-end 250 base pairs read length. Raw sequences were processed and analysed using the open-source bioinformatics pipeline QIIME 1.9.1 (http://qiime.org/), the Operational Taxonomic Unit were picked using the open-reference protocol. Subsequently, micro-biota profiles were reported at phylum level and visualized using the visualization tool Krona [5].

Results

No adverse event occurred during or after the infusion of microbiota, other than loose stools for 3 days. The stool culture taken prior to FMT was negative for the MDR *P. aeruginosa*, but did contain an ESBL producing *Escherichia coli*, susceptible to carbapenems, gentamicin, piperacillin/tazobactam and colistin. Subsequently 5 stool cultures up to 3 months of follow up remained negative for *P. aeruginosa*, however persisted in containing the ESBL producing *E. coli*. The *E. coli* post-FMT was identical to the *E. coli* found prior FMT, using AFLP. No infectious complications caused by *P. aeruginosa* were noted during 18 months of follow up. However, the patient was treated once with trimethoprim-sulfamethoxazole for cystitis caused by an ESBL positive *E. coli* 8 months after FMT. Unfortunately, this strain was not available for AFLP analysis.

16S analysis of the patient's stool 19 and 1 days prior FMT revealed a diverse microbiota composition, i.e., high Shannon diversity index of 7.8 and 8.1 respectively.

No significant changes in microbiota diversity of the recipient were observed following the FMT *(Figure 1)*. At phyla level a high similarity of donor and recipient microbiota was observed with respect to the *Firmicutes* and *Bacteroidetes* as the expected main phyla of the microbiota *(Figure 1)*.
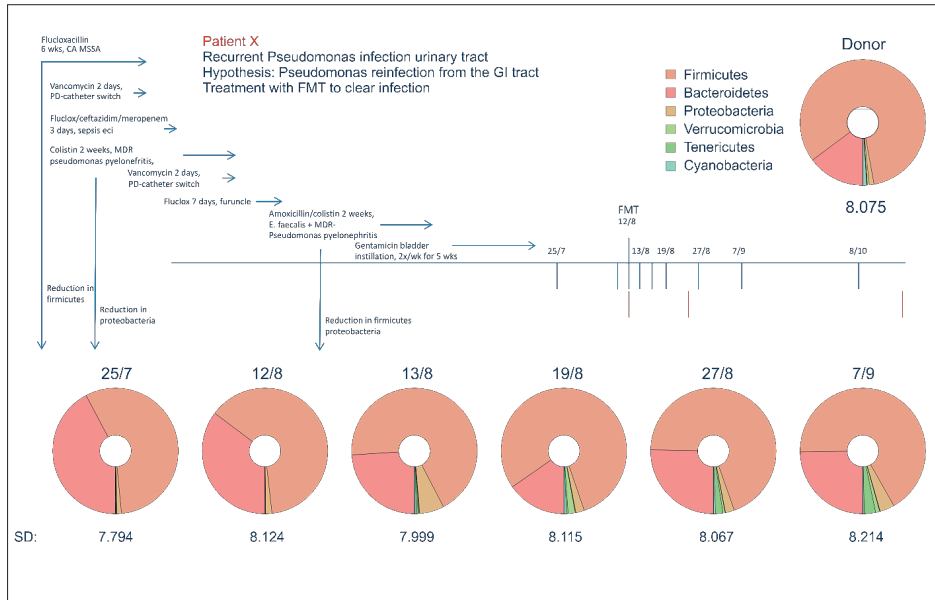
Figure 1. Timeline of recurrent infections, antibiotic use, and microbiota diversity prior to and after fecal microbiota transfer.

CA MSSA: catheter related bacteremia with methicillin sensitive *Staphylococcus Areus*.
MDR: multidrug resistant. PD: peritoneal dialysis. FMT: fecal microbiotica transfer.

Discussion

A 34-year old patient on peritoneal dialysis and recurrent urinary tract infections with a VIM-positive *P. aeruginosa* was treated with infusion of fecal microbiota to eradicate *P. aeruginosa* from the intestinal tract. A clinical success was observed, since at a follow-up period of 18 months no recurrent infections by *P. aeruginosa* were diagnosed. FMT may have contributed to clinical success but it cannot be excluded that MDR *P. aeruginosa* was already eradicated from the gut before FMT, as the *P. aeruginosa* could not be cultured the day before FMT.

A remarkable observation is the persistence of an ESBL positive *E. coli* after FMT. The *E. coli* was presumably acquired after eradication treatment for *P. aeruginosa,* since it had not been detected in earlier cultures. It is possible that the incomplete eradication of the MDR *E. coli* is the result of coexistence of donor and patient *E. coli* strains after FMT. A recent study showed this coexistence of donor and recipient strains, which persisted for at least 3 months after FMT for treatment of patients with metabolic syndrome [6]. This suggests that novel strains, acquired via FMT, can colonize the gut without replacing the indigenous strain population of the recipient.

In contrast to the diminished microbiota of recurrent CDI patients, our patient had an intact microbiota diversity and composition at phylum level prior to FMT. Previous antibiotic treatment *(Figure 1)* had not resulted in a distinct disturbance of the intestinal flora. Only minor changes of the microbiota composition were observed after FMT with a slight increase of cyanobacteria and tenericutes. We suggest that diminished diversity appears not to play a role in MDR carriership as opposed to recurrent CDI [7]. Therefore, one might question the efficacy of fecal transplantation in patients with a normal microbiota diversity. The disturbed microbiota and its recovery after FMT might explain the positive results of MDR eradication in patients with recurrent CDI [8,9]. Interestingly, a recent paper showed that infusion of fecal microbiota in patients with recurrent CDI decreased the number and diversity of anti-microbial resistance genes, particularly by restoring dysbiosis and reducing the number of *Proteobacteria* [10]. Furthermore, beneficial effect of microbiota transfer has been shown in mice colonized with vancomycin resistant *Enterococcus* (VRE) [11]. Clearly, more research on FMT for eradication of colonization of different MDR bacterial species is required.

A total of only eight case reports have been published, showing FMT resulted in intestinal decolonization of ESBL- and carbapenemase-producing Enterobacteriaceae, VRE, or methicillin-resistant *Staphylococcus aureus* [12-15]. Unfortunately, no informa-tion has been provided on microbiota composition before and after transplantation. Five trials are currently underway regarding the use of FMT for MDR bacterial decolonization which should provide more insight on the role of the microbiota on colonisation with specific microorganisms [12].

A limitation of our analysis is that the microbiota was determined by 16S analysis. Although very useful in bacterial taxonomic classification, it lacks the required resolution to track transmission of bacterial strains in the microbiota using single-nucleotide variants in metagenomes [6]. Therefore, it was not possible to compare the composition of the microbiota at strain level, allowing a comparison between the donor and patient *P. aeruginosa* strains. However, no VIM-gene was detected by PCR on DNA from three feces samples after FMT.

In conclusion, combined FMT and antibiotic treatment prevented recurrence of UTI with MDR *P. aeruginosa*. Intestinal colonization with ESBL producing *E. coli* persisted in the presence of a microbiota with intact diversity, suggesting that eradication of *E. coli* requires perhaps other specific strain(s) of microbes. More detailed analysis such as metagenomics, could identify specific strains that add to decolonization and should be applied in current studies on FMT for intestinal eradication of different MDR bacterial species.

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