MICROBIAL DRUG RESISTANCE
Volume 29, Number 1, 2023

DOI: 10.1089/mdr.2022.0031

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A Case of Successful Treatment of Recurrent Urinary Tract Infection by Extended-Spectrum β-Lactamase Producing *Klebsiella pneumoniae* Using Oral Lyophilized Fecal Microbiota Transplant

Naomi Bier,[1,2] Blake Hanson,[1,2] Zhi-Dong Jiang,[1] Herbert L. DuPont,[1] Cesar A. Arias,[3–5] and William R. Miller[3,4]

Recurrent urinary tract infections (UTIs) are a challenging clinical entity that can be frustrating for patient and physician alike. Repeated rounds of antibiotics can select for multidrug-resistant organisms, further complicating care. We describe the successful use of fecal microbiota transplantation (FMT) for the treatment of recurrent extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* UTIs in a patient with an ileal conduit and urostomy. In the 18 months after FMT, the patient had not experienced new infections with ESBL-producing organisms. The urine and stool microbiomes of the patient were tracked before and post-FMT using 16s RNA sequencing with measurement of α-diversity. Sequencing of the recipient microbiota did not mirror the donor stool taxa at either site, but an increase in the relative proportion of the genus *Bacteroides* as compared with *Prevotella* was noted in the stool post-transplant. FMTs may be a promising treatment option for recurrent multidrug-resistant infections.

Keywords: fecal microbiota transplant, FMT, urinary tract infection, UTI, antibiotic resistance, decolonization

Introduction

Urinary tract infections (UTIs) account for >8 million outpatient medical encounters a year in the United States.[1] UTIs typically develop through seeding of the urogenital tract with gut microbes, and pathogens causing recurrent UTIs have been identified in the gut microbiome of patients.[2] Antimicrobial resistance in uropathogens, such as *Escherichia coli* and *Klebsiella pneumoniae*, present a challenge for patients and clinicians as these multidrug-resistant organisms (MDROs) often display resistance to available antibiotics for oral treatment.[3,4]

The gastrointestinal (GI) tract serves as a major reservoir for common uropathogenic MDROs. Typically, an intact gut microbiota can prevent the establishment or clear colonization by opportunistic pathogens through direct interaction between the commensals and pathogens, and/or by mediating an enhancement of the host defenses and response.[5] In the setting of recurrent UTIs, repeated rounds of antibiotics disrupt the normal GI microbiota, and can lead to a loss of this colonization resistance.[5]

Restoration of a healthy gut microbiome has been proposed as an alternative to reduce the burden of MDRO carriage in the GI tract and potentially decrease the incidence of subsequent urinary infection.[6] This strategy of supplementing the intestinal microbiota with that of a healthy donor through fecal microbiota transplantation (FMT) has been used successfully to treat recalcitrant *Clostridioides difficile* infections (CDIs). Recently, a number of case series have reported decreased rates of subsequent infection or GI colonization with an MDRO after FMT.[7,8] In this report, we describe the successful treatment of recurrent UTIs due to an extended-spectrum

[1]Center for Infectious Diseases, University of Texas Health Science Center, School of Public Health, Houston, Texas, USA.
[2]Center for Antimicrobial Resistance and Microbial Genomics, McGovern Medical School, Houston, Texas, USA.
[3]Division of Infectious Diseases, Department of Internal Medicine, Houston Methodist Hospital, Houston, Texas, USA.
[4]Center for Infectious Diseases Research, Houston Methodist Research Institute, Houston, Texas, USA.
[5]Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogota, Colombia.
Part of this study was presented as an abstract at IDWeek 2020, held as a virtual conference.

β-lactamase (ESBL)-producing *K. pneumoniae* using an oral lyophilized FMT in a patient with a urinary ileal conduit.

Materials and Methods

Administration of oral lyophilized FMT

PRIM-DJ2727 (60 g total fecal matter lyophilized to 1 g/dose) was given weekly, for 3 weeks, for a total of three doses, under an expanded access investigational new drug application protocol approved by the U.S. Food and Drug Administration (IND 18545) and the local institutional review board (HSC-MS-18-0878). Urine and rectal swab stool samples were collected directly from the patient before treatment, 1 week after the final FMT dose, at transplant day +70, and transplant day +180. Specimens were stored at −80°C until DNA extraction.

DNA purification and processing

Samples underwent nucleic acid extraction using the Qiagen DNeasy PowerSoil Kit following the manufacturer protocol. A negative control (reagent control) was used for DNA extractions to assess for possible contamination during the extraction protocol.

16S ribosomal RNA microbiome sequencing and data processing

The V1–V3 region of 16S ribosomal RNA (rRNA) was amplified as previously described.[9] To ensure the absence of nucleic acid contamination, and verify amplification capability, a negative (pure molecular grade H_2O) and positive (pure *Pseudomonas aeruginosa* gDNA) PCR control were included in the analysis. 16S PCR products were purified using Agencourt AMPure XP Beads, quantified using Qubit dsDNA HS Kit, pooled at equimolar concentration, and sequenced using an Illumina Miseq with the 2×300 v3 sequencing kit (Cat. No. MS-102-3003; Illumina).

Sequencing data were processed as previously described.[9] In brief, Trimmomatic was used to remove the 16S primers and paired sequences were assembled using PEAR,[10] bbplit was used to remove PhiX, and bmtagger was used to remove human DNA sequencing data.[11,12] Remaining amplicons were clustered into operational taxonomic units using USEARCH,[13] which were classified with the RDP Classifier to identify taxonomy.[14]

Data analysis

All analyses of the 16S rRNA microbiome data were completed with R version 3.6.1. The Phyloseq package was used to assess α-diversity for each sample using the observed diversity.[15] For α-diversity assessment, the data were rarified using the Phyloseq package and an rngseed of 1234 for reproducibility. The extraction controls had minimal reads as expected (360–3,221 reads) compared with 2–3× 10^5 reads in the sample. The PCR controls behaved accordingly, as well with 12 reads in the PCR negative and 395,417 reads in the PCR positive control of purified *P. aeruginosa* genomic DNA.

Results and Discussion

A 50-year-old woman with von Willebrand disease and multiple antibiotic allergies, including sulfa drugs, penicillins, and cephalosporins, presented with recurrent UTIs after complications from a hysterectomy performed ∼15 years prior. During this procedure, she had bladder perforation and subsequent formation of a vesiculovaginal fistula. Owing to recurrent UTIs, she underwent cystectomy and creation of a neobladder, then conversion to a catheterizable pouch due to a second fistula. She was ultimately converted to an ileal conduit with urostomy, and for the prior 2 years had recurrent episodes of pyelonephritis.

In the preceding 6 months she had five symptomatic UTIs (Fig. 1), four of which required treatment with intravenous (IV) ertapenem due to isolation of an ESBL-producing *K. pneumoniae* isolate susceptible to only carbapenems and amikacin (Table 1). Cultures from the other UTI yielded fluoroquinolone-susceptible *P. aeruginosa* and *Enterobacter* spp., and ciprofloxacin therapy precipitated an episode of *C. difficile* colitis treated with oral vancomycin. She had failed prior suppression with nitrofurantoin and fosfomycin due to resistance to these agents.

The patient had stage G3a chronic kidney disease, and the repeated episodes of infection resulted in progression of ureteral scarring with intermittent ureteral obstruction and hydronephrosis requiring the short-term placement of percutaneous nephrostomy tubes. The need for IV administration of antibiotics had a significant negative impact on the patient's quality of life, and there was ongoing concern for recurrence of the *C. difficile* colitis with further antibiotic use.

The patient wanted to avoid further daily IV antibiotic administration, however, given the episode of obstruction

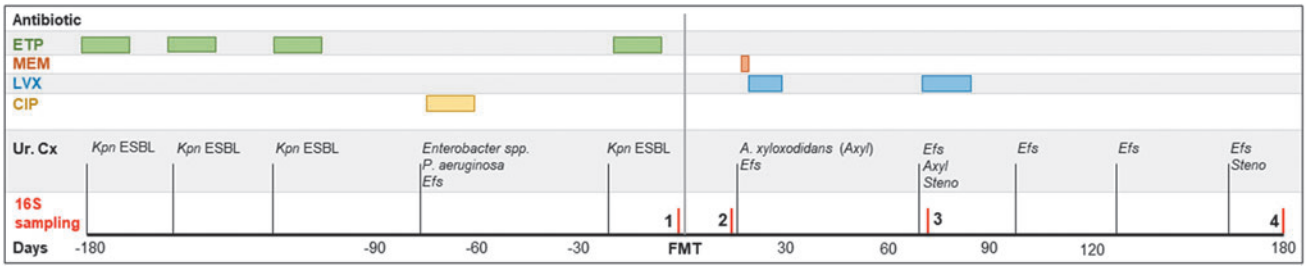

FIG. 1. Timeline of clinical course. Numbers indicate approximate timing of urine and stool collection. *Axyl, Achromobacter xylosoxidans*; CIP, ciprofloxacin; *Efs, Enterococcus faecalis*; ETP, ertapenem; FMT, fecal microbiota transplantation; LVX, levofloxacin; MEM, meropenem; *Steno*, *Stenotrophomonas maltophilia*; Ur. Cx, urine culture.

Table 1. Antimicrobial Susceptibilities of the *Klebsiella pneumoniae* Urinary Isolate

Antibiotic	*MIC*
Amikacin	≤16
Ampicillin	>16
Ampicillin/Sulbactam	>16/8
Cefazolin	>16
Cefepime	>16
Ceftriaxone	>32
Ciprofloxacin	>2
Fosfomycin	>1,024
Gentamicin	>8
Levofloxacin	>4
Meropenem	≤1
Nitrofurantoin	>64
Piperacillin/Tazobactam	R
Tetracycline	>8
Tobramycin	>8
Trimethoprim/Sulfamethoxazole	>2/38

MIC, minimum inhibitory concentration (values in μg/mL); R, resistant.

with underlying chronic kidney disease, aminoglycosides were a less attractive treatment option. Although bacteriophage therapy was considered and has been used as an adjunctive treatment for MDRO infections, including UTIs,[16] access to this treatment option was not readily available. Thus, it was decided to pursue FMT using PRIM-DJ2727, an oral encapsulated lyophilized stool product under investigation for treatment of CDIs.[17]

FMT was administered 1 week after completion of ertapenem for the preceding UTI. In the 6 months subsequent to the FMT, the patient developed two symptomatic UTIs, with cultures positive for *Achromobacter xylosoxidans* during the first episode, and both *A. xylosoxidans* and *Stenotrophomonas maltophilia* during the second episode (Fig. 1). *Enterococcus* spp. was recovered from multiple cultures and was considered a colonizer. *A. xylosoxidans* and *S. maltophilia* were not identified in the FMT product (Fig. 2A).

However, *A. xylosoxidans* was detected in the post-FMT urine samples (Urine_2 and Urine_3) and became undetectable by day +180 (Urine_4). We followed the urine microbiome pre- and post-FMT to determine whether the FMT would have an effect on the microbial composition of the conduit. The observed α-diversity in the urine increased after FMT, however, the identified taxa did not resemble the transplanted microbiota (Fig. 2B). Stool α-diversity (observed) increased after the transplant and stabilized by 6 months post-FMT despite oral fluoroquinolone therapy for a subsequent infection (Fig. 2B).

The stool composition resembled that of the FMT over time with *Bacteroides* increasing in proportion and *Prevotella* decreasing 180 days post-FMT. Interestingly, there was an inverse relationship between stool and urine observed α-diversity. However, when richness and evenness of the microbial population were taken into account using the Shannon α-diversity metric, both stool and urine followed a similar pattern of decreasing initially, increasing, and then finally stabilizing to a similar level as before FMT, although with different microbial components post-FMT.

K. pneumoniae was not detected in the urine before FMT, as expected after a round of ertapenem treatment. *K. pneumoniae* was detected in the stool before FMT with a very low relative abundance of 0.005% (∼20 reads in 351,362), suggesting the preceding antibiotics successfully diminished the population but did not completely eradicate the colonization, and may explain the recurrent infections before FMT. *K. pneumoniae* was not detected post-FMT in the stool throughout the follow-up period.

Eventually, *K. pneumoniae* was detected in the urine (Urine_4) with a relative abundance of 0.02% (∼70 reads in

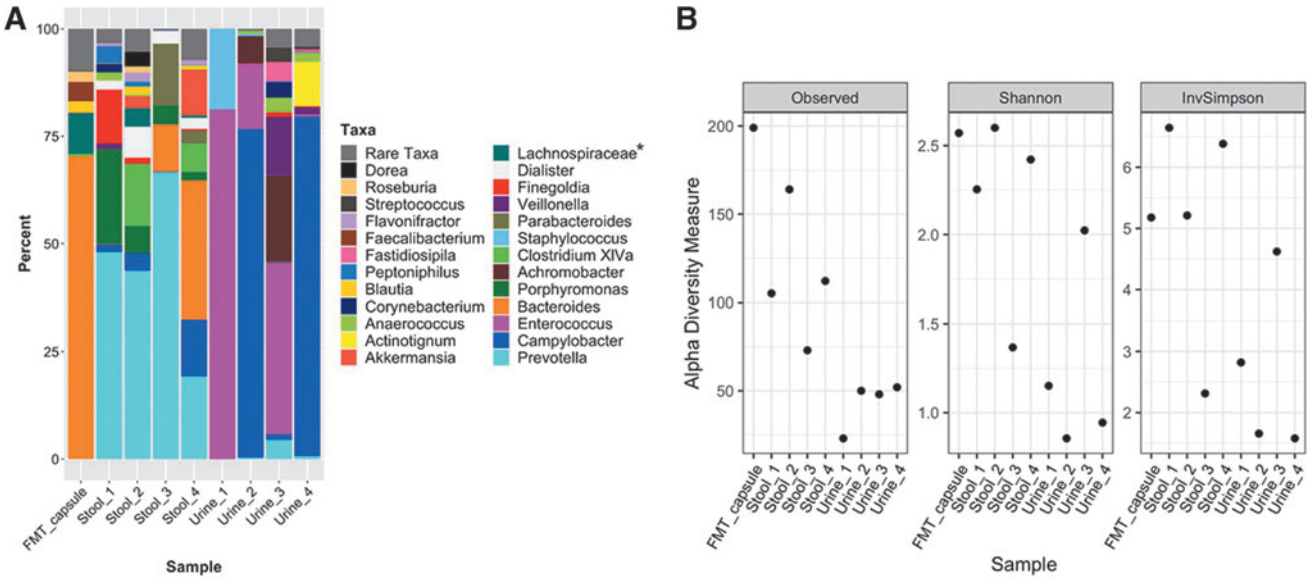

FIG. 2. **(A)** Relative abundance of taxa identified in stool and urine samples. Composition of the FMT capsule is shown in the *first column*. Numbers correspond to the sampling time as indicated in Fig. 1. *Lachnospiraceae—unclassified genus. **(B)** Observed, Shannon, and inverse Simpson α-diversity. Numbers correspond to the sampling time as indicated in Fig. 1.

344,977). It should be noted *K. pneumoniae* was not detected in the extraction control performed during the DNA extraction of the urine and stool samples, and that deep sequencing was performed on each sample to detect rare community members. The detection of *K. pneumoniae* in the final urine sample raises the possibility of recolonization with extremely low abundance relative to the transplanted microbiota or acquisition of a different *K. pneumoniae* strain. However, subsequent urine cultures did not yield growth of *K. pneumoniae* and the patient has not experienced a subsequent *K. pneumoniae* UTI at the time of submission of this publication.

Recurrent UTIs can be challenging problems in patients with indwelling catheters, anatomic abnormalities, or immunosuppressed states such as pregnancy.[18] In the present case, the patient had anatomic abnormalities with recurrent infections requiring IV antibiotics due to drug allergies and bacterial antimicrobial resistance. Despite these challenges, this patient did not have any further ESBL-producing *K. pneumoniae* UTIs after antibiotic therapy followed by FMT. Interestingly, *K. pneumoniae* was found in the urine at low abundance by 16S rRNA sequencing 180 days post-FMT; however, the patient has not experienced any *K. pneumoniae* UTIs since the FMT.

The role of FMT in the successful treatment of this patient's recurring UTIs was likely complex, and may have included decolonization by replacement of a more diverse GI microbiota, alteration in immune function at the mucosal interface, a change in metabolites secreted by the transplanted microbiota, or a combination of these factors.[5] Even directly after the FMT, the stool microbiome composition by 16S rRNA sequencing did not mirror the donor stool taxa, though it was taken before the subsequent antibiotic exposure.

Over time, there was an increase in the relative proportion of the genus *Bacteroides* as compared with *Prevotella*, a pattern that was also observed in the FMT stool capsule. Although the significance of this shift is uncertain, it is conceivable that an overall shift in microbiota, metabolites, and immune modulation may have been protective against recolonization with the ESBL-producing *K. pneumoniae*.[19] Indeed, colonization with *Prevotella* in mice has been associated with alteration of immune function and decreases in abundance and diversity of *Firmicutes*, which play a role in colonization resistance to enteric pathogens such as *Clostridioides difficile*.[20,21]

In addition, the increase in physical distance between the urostomy and the anorectal area in this case, as compared with the unaltered female anatomy where the vaginal mucosa and urethra are in proximity to the anorectal area, may have increased the effectiveness of the FMT in preventing recolonization of the conduit.

Limited data exist regarding the impact of FMT on the microbiome of non-GI sites. Previous reports of FMT to treat recurrent UTIs have not characterized the urine microbiome in their studies.[6,7] The microbiome analysis of the urine in this case is particularly interesting given the presence of a small intestine-derived conduit. The tissue properties of this feature may support a different microbiome than that of the native urogenital tract. As this is a single case, further study specifically addressing the mechanism of FMT impact on recurrent UTIs is needed.

Disclosure Statement

Z.-D.J. and H.L.D. have applied for a patent for the lyophilized FMT product PRIM-DJ2727. C.A.A. has received grants from Merck, MeMed Diagnostics, and Entasis Therapeutics. W.R.M. has received grants and/or honoraria from Merck, Entasis Therapeutics, and Shionogi. N.B. and B.H. report no conflict.

Funding Information

This study was supported by the National Institutes of Health NIH/NIAID Grants R01 AI134637, R01 AI148342, K24 AI121296, and P01 AI152999 to C.A.A. W.R.M is supported by NIH/NIAID K08 AI135093. B.H. is supported by NIH/NIAID K01 AI148593. N.B. is supported by a training fellowship from The University of Texas Health Science Center at Houston Center for Clinical and Translational Sciences TL1 Program, Grant No. TL1 TR003169. The funding agencies had no role in experimental design, data collection, or interpretation of this study.

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Address correspondence to:
William R. Miller, MD
Division of Infectious Diseases
Houston Methodist Hospital
6560 Fannin Street, Scurlock Tower Suite 1408
Houston, TX 77030
USA

E-mail: wrmiller@houstonmethodist.org