Microbiome restoration by RBX2660 does not preclude recurrence of multidrug-resistant urinary tract infection following subsequent antibiotic exposure: A case report

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Microbiome Restoration by RBX2660 Does Not Preclude Recurrence of Multidrug-Resistant Urinary Tract Infection Following Subsequent Antibiotic Exposure: A Case Report

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A 62-year-old woman received RBX2660, an investigational microbiome restoration therapeutic, for recurrent multidrug-resistant (MDR) urinary tract infection (UTI). RBX2660 increased gut microbiome diversity but did not eliminate uropathogen carriage, and MDR UTI recurred after subsequent antibiotic exposure. Thus, restoration of microbiome diversity does not preclude disease recurrence by residual MDR pathogens.

Keywords. antibiotic resistance; fecal microbiota transplant; microbial restoration therapy; microbiome; urinary tract infection.

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting >150 million people annually, and are predominantly caused by uropathogenic Escherichia coli and other Enterobacteriaceae [1]. Serious sequelae include pyelonephritis, increased risk of preterm birth, and sepsis. Effective treatment of UTIs is complicated by increasing rates of antibiotic resistance and the propensity of uropathogens to establish and disseminate from gastrointestinal reservoirs, contributing to symptomatic recurrence in 30%–50% of patients [1, 2]. Recurrence frequently leads to repeated cycles of antibiotic treatment and resistance, culminating in exposure to antibiotics of last resort and/or multidrug resistance. Accordingly, novel approaches for effective elimination of multidrug-resistant (MDR) uropathogens from their gastrointestinal niches are of tremendous clinical significance.

Intestinal microbial restoration by fecal microbiota transplant (FMT) is an emerging treatment for patients colonized with enteric multidrug-resistant organisms (MDROs), including uropathogens [3]. FMT has been shown to decolonize uropathogenic MDR Klebsiella pneumoniae, Pseudomonas aeruginosa, and E. coli from the gut, often as an incidental outcome of Clostridiodes difficile treatment [4–7]. However, existing studies are limited by a lack of microbiome analysis before and after FMT, an absence of uropathogen monitoring and clonal tracking, or, in most cases, both. Such context is essential to assessing the impact of intestinal microbial restoration and determining whether an MDRO has been successfully cleared from its reservoir(s).

Here we present the case of a 62-year-old woman with recurrent UTI and a complex medical history, including liver and kidney transplantation, diabetes, and lymphoma, who received RBX2660, an investigational intestinal microbiome restoration product derived from human stool [8, 9], for prevention of recurrent MDR UTI. We contextualize her clinical progression following RBX2660 with genotypic and phenotypic analyses of microbiome composition and within-host MDRO evolution. Administration of RBX2660 resulted in a considerable increase in intestinal microbiome diversity, as well as an MDR UTI–free period, but not complete eradication of the causative MDR uropathogen. Subsequent exposure to broad-spectrum antibiotics for an unrelated infection was followed by recurrence of MDR UTI.

This case report illustrates an important challenge for the development of microbiota restoration therapies: patients in need of such therapies are highly vulnerable and likely to receive antibiotics, but those same antibiotics can select for MDROs that were incompletely eliminated by FMT. Thus, complete MDRO eradication from a patient’s microbiome (ie, undetectable carriage) may be a prerequisite for the long-term clinical success of microbiota restoration therapies.

CASE REPORT

The patient was a 62-year-old woman with a history of kidney and liver transplantation in 2009, diffuse large B-cell lymphoma (DLBCL), diabetes mellitus, peripheral vascular disease, gout, chronic lung disease, and recurrent UTIs due to MDR K. pneumoniae. The patient was enrolled in clinical trial
RBX2660 was evaluated in clinical trials to prevent recurrent *C. difficile* infection [8, 9] and was administered as per manufacturer guidelines. The patient tolerated the study procedure (enema) and study product (RBX2660) without significant adverse events. The patient remained free of urinary symptoms until October 2015, ~7 weeks post-FMT, when she developed a UTI from *Proteus mirabilis* that resolved with 7 days of oral amoxicillin. In December 2015, the patient developed toe gangrene related to preexisting conditions of gout, diabetes mellitus, and peripheral vascular disease, requiring treatment with broad-spectrum antibiotics and ultimately amputation. The patient had a *K. pneumoniae* UTI recurrence in January 2016, ~19 weeks post-FMT, which was treated with ertapenem. In April 2016, the patient had a single episode of fever, and a urine culture was sent. The culture grew an ertapenem-resistant *K. pneumoniae*, but the patient did not manifest UTI symptoms, and a repeat urine culture was negative. Ertapenem was continued until the patient died from complications of squamous cell carcinoma in December 2016.

The patient's medical history over the study period is summarized in Figure 1A and comprehensively described in the Supplementary Data.

**METHODS**

Study protocols and analyses are described in the Supplementary Data.

**RESULTS**

**RBX2660 Modulated Microbiome Composition**

Before RBX2660 intervention for chronically recurrent UTI, the patient exhibited a dysbiotic microbiome characteristic of prolonged antibiotic exposure, including low species richness and high abundance of antibiotic resistance genes (ARGs). After RBX2660 administration, increased species richness, altered community composition, and reduced ARG carriage were observed. Relative to the pre-RBX2660 baseline (16 species), species richness more than doubled by 1 month post-RBX2660 (40 species), and by 4 months post-FMT, richness approached that of RBX2660 preparation (51 and 60 species, respectively) (Figure 1B). Several typically rare taxa that were highly abundant pre-RBX2660, including *Erysipelotrichaceae* and *Subdoligranulum*, were largely replaced by the more traditional commensals *Bacteroides* and *Ruminococcus* after RBX2660 (Figure 1B). The relative abundance of *K. pneumoniae* was greatly diminished at 1 month post-RBX2660 and remained lower than the pre-RBX2660 baseline at 4 months post-RBX2660 (but had increased relative to 1 month post-RBX2660), shortly before symptomatic recurrence (Figure 1B). Overall ARG abundance was reduced by >8-fold at 1 month post-RBX2660 administration, and β-lactamases, the most common class of resistance genes in the patient’s pre-RBX2660 microbiome (Figure 1C), were particularly diminished. Despite the patient’s concomitant exposure to broad-spectrum antibiotics for toe gangrene, ARG abundance did not rebound to the pre-RBX2660 baseline by 4 months post-RBX2660 (Figure 1C).

**RBX2660 Did Not Eliminate Uropathogen Carriage or UTI Recurrence After Antibiotics**

MDR *K. pneumoniae* carriage in the gut was diminished but not eliminated by RBX2660 (Figure 1B), and the patient experienced a UTI recurrence with MDR *K. pneumoniae* ~4 months after exposure to broad-spectrum antibiotics. Consistent with incomplete uropathogen clearance by RBX2660 and subsequent reseeding from the gut [2, 10], a urine isolate collected during this recurrence differed by just 6 single nucleotide polymorphisms (SNPs) from a *K. pneumoniae* stool isolate collected 1 day pre-RBX2660 (Figure 1D). Indeed, all 7 *K. pneumoniae* isolates collected from the patient over >2 years had ≤30 pairwise SNPs, and a bloodstream isolate collected in January 2014 differed by only 3 SNPs from a urine isolate collected in October 2015, 1 month post-RBX2660 (Figure 1D). All *K. pneumoniae* isolates, regardless of source, were also fully resistant to most antibiotics tested (Supplementary Table 1), with minor differences in susceptibility likely resulting from the acquisition of mobile genetic elements rather than from chromosomal mutations.

The patient experienced an additional UTI ~6 weeks post-RBX2660 that was caused by *P. mirabilis*. This organism was not detectable in the patient’s stool before RBX2660 or in the RBX2660 preparation itself, suggesting acquisition independent of intervention. Because this organism was nearly pan-susceptible to antibiotics (Supplementary Table 1), it was readily cleared by oral amoxicillin and did not recur.

**DISCUSSION**

Despite their significant clinical potential and increasing application in medical practice, a singular definition of FMT “success” has not been established [11]. Historically, success has been defined as a positive clinical response, such as cessation of recurrent *C. difficile* infection. From a purely microbiological perspective, however, reversal of microbiome dysbiosis has also been considered a marker of success [11]. This case illustrates that these 2 definitions do not
always coincide. In this patient, increased microbiome richness and diversity after RBX2660 administration were not accompanied by complete uropathogen eradication, and there was a further recurrence of the patient’s MDR UTI after broad-spectrum antibiotic treatment. Because complete elimination of target MDROs by FMT may not always be achievable, however, this case also illustrates the importance of further research into microbiome features. Because complete elimination of target MDROs by FMT may not always be achievable, however, this case also illustrates the importance of further research into microbiome features. Because complete elimination of target MDROs by FMT may not always be achievable, however, this case also illustrates the importance of further research into microbiome features.

A critical but unanswerable question is whether this patient’s MDR K. pneumoniae UTI would have recurred in the absence of her toe gangrene and resulting antibiotic treatments. Before this unrelated infection, the patient had not experienced a K. pneumoniae UTI recurrence for ~4 months, the longest such period without suppressive antibiotics within the previous 2 years. Given the documented dissemination of uropathogens from the gut to the bladder [2, 10], microbiome-related mechanisms may have contributed to this UTI-free period, which occurred despite the persistence of MDR K. pneumoniae in stool and urine at intervening asymptomatic time points. Nevertheless, we note that FMT recipients are often immunocompromised, develop secondary infections, and receive broad-spectrum antibiotics [12], and these comorbidities must be considered when defining, predicting, and evaluating FMT success or failure. We suggest that if microbiome restoration therapies do not fully eradicate causative MDROs from their intestinal reservoir(s), subsequent antibiotic treatments may enable re-emergence of these MDROs, regardless of the impact of FMT on other microbiome features. Because complete elimination of target MDROs by FMT may not always be achievable, however, this case also illustrates the importance of further research into microbiome features.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.
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Potential conflicts of interest. The study drug (RBX2660) was provided free of charge by Rebiotix, Inc. (Roseville, MN, USA). E.R.D. has consulted for Rebiotix. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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