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 *Clostridioides 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 investigative 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

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#NCT02312986 ("FMT for Multidrug Resistant Organism Reversal") and received RBX2660 in September 2015 for intestinal uropathogen decolonization. Before intervention, the patient's most recent UTI episodes occurred in November 2013 (treated with meropenem, then ertapenem, then fosfomycin), January 2014 (onset 5 days after fosfomycin was stopped for a previous UTI), and June 2014 (onset 7 weeks after ertapenem was stopped for a previous UTI). Due to the frequency and severity of her UTI recurrences, she remained on suppressive ertapenem from her last UTI until 2 days before receiving RBX2660 in September 2015.

RBX2660 has been 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 post-RBX2660 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

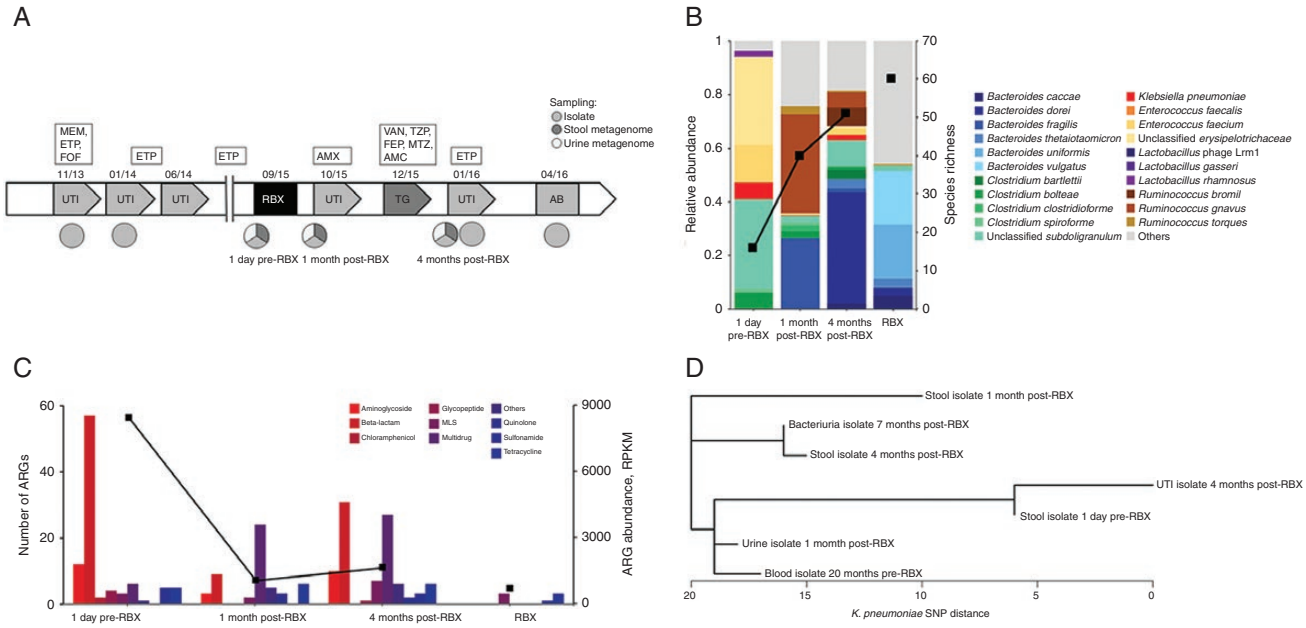

Figure 1. A, The patient experienced 3 episodes of urinary tract infection before RBX2660 (November 2013, January 2014, and June 2014, all from multidrug-resistant *Klebsiella pneumoniae*) and 2 episodes following RBX2660 (October 2015 from *Proteus mirabilis* and January 2016 from multidrug-resistant *K. pneumoniae*). The patient also experienced toe gangrene (TG; December 2015) and asymptomatic bacteriuria (AB; April 2016). These infections necessitated treatment with meropenem (MEM), ertapenem (ETP), fosfomycin (FOF), amoxicillin (AMX), vancomycin (VAN), piperacillin + tazobactam (TZP), cefepime (FEP), metronidazole (MTZ), and amoxicillin + clavulanate (AMC). Stool and urine samples were collected for isolate and/or metagenomic sequencing at the designated time points. B, Stool microbiomes collected before and after RBX2660 administration, as well as the RBX2660 preparation itself, were profiled for relative abundance (left y-axis) and overall richness (right y-axis) of microbial species and for (C) total antibiotic resistance gene (ARG) count by class (left y-axis) and overall ARG abundance (right y-axis) (Supplementary Data). D, Phylogenetic relationships of multidrug-resistant *K. pneumoniae* isolates from stools, urines, and blood (sepsis) before and after RBX2660 administration were established by calculating single nucleotide polymorphism (SNP) distances (Supplementary Data) between isolates. The maximum SNP distance between these isolates is 30 (overall tree length plus branch length).

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 exposure. Although RBX2660 was well tolerated and mediated substantial microbiome diversification, the patient developed an acute UTI from *P. mirabilis* <2 months post-RBX2660. This infection was successfully treated with a narrow-spectrum oral antibiotic and did not recur, nor did it lead to a recurrence of MDR *K. pneumoniae* UTI. However, her chronic MDR *K. pneumoniae* UTI reemerged after an unrelated infection necessitated treatment with 7 different antibiotics, which may have selected for MDROs and contributed to UTI recurrence.

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 protectants [13] and microbiome–pathobiont–host interactions, which could minimize the likelihood of MDRO recurrence even in the absence of eradication. Larger studies should examine how comorbidities and antibiotics impact the clinical success of intestinal microbiota restoration therapies, as well as the broader relationship between microbiome recovery, MDRO clearance, and clinical progression following such therapies.

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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