Panels A and B show plots of the initial rate of citrate synthase activity (Y-axis, in units of μmole/mL/sec) versus OAA/AcCoA substrate concentration (X-axis, in millimolar units), for wildtype citrate synthase and the A313P and A313V mutants. Panel (A) shows enzymatic parameters for wildtype, A313P mutant, and A313V mutant with variable OAA (0-0.625 mM) and fixed AcCoA (0.3 mM). Panel (B) shows enzymatic parameters for wildtype, A313P mutant, and A313V mutant with fixed OAA (0.5 mM) and variable AcCoA (0-2.5 mM). Three replicates were performed for each condition, with error bars showing ± 1 SD.

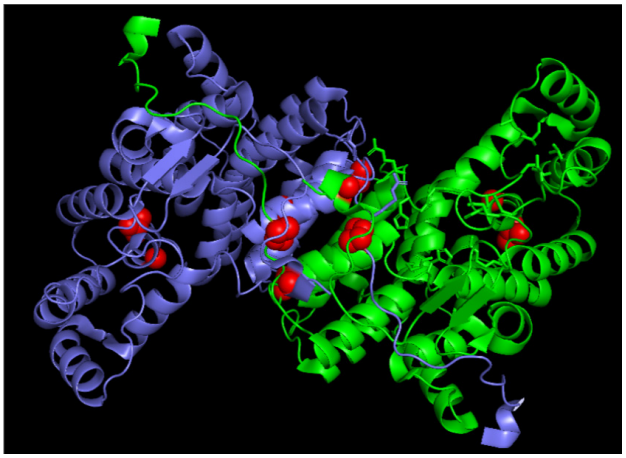

Figure 4. Citrate synthase mutants disrupt functional dimerization and destabilize alpha-helix packing

Identified citrate synthase mutants mapped onto the crystal structure of T. thermophilus citrate synthase (PDB 1IOM) show that the mutations are located either at dimerization interfaces or within the interior of hydrophobic packing interfaces.

Conclusion. Cellular metabolism and virulence regulation are interconnected in *S. aureus*, as alterations in TCA cycle activity lead to increased persister formation and host macrophage inactivation. Our findings that inactivating *citZ* mutations are enriched can provide a potential explanation for the mechanism of persistent bacteremia.

Disclosures. **All Authors**: No reported disclosures

129. Antimicrobial Resistance Genes Were Reduced Following Administration of Investigational Microbiota-Based Live Biotherapeutic RBX2660 to Individuals with Recurrent *Clostridioides difficile* Infection

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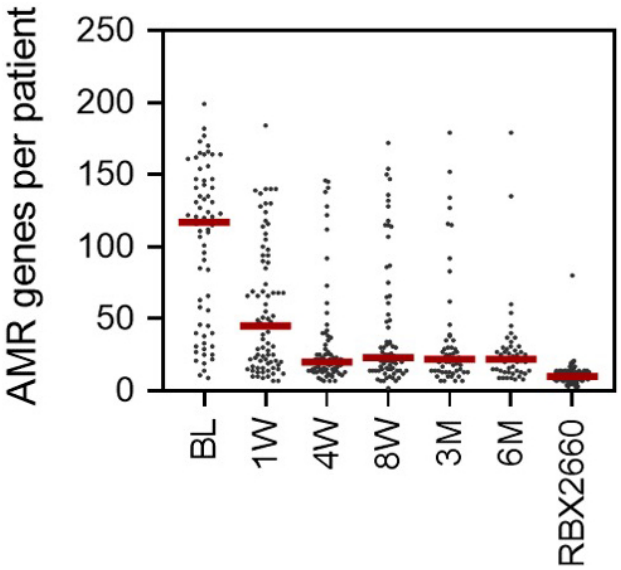
Session: O-26. New Insights into Microbial Pathogenesis

Background. Intestinal colonization by antimicrobial resistant (AMR) pathogens is a known health and infection risk, and is common among individuals with recurrent *Clostridioides difficile* infections (rCDI). Accordingly, therapeutic approaches that decolonize the gut of AMR pathogens could be valuable to patients to reduce risk of associated illnesses. Herein, we assessed gut colonization with AMR bacteria before and after treatment with RBX2660—a microbiota-based investigational live biotherapeutic—in the PUNCH CD3 Phase 3 trial for reducing CDI recurrence.

Methods. rCDI participants enrolled in PUNCH CD3 received a blinded single dose of RBX2660 or placebo within 24 to 72 hours after completing antibiotic treatment for the most recent rCDI episode. Clinical response was the absence of CDI recurrence at eight weeks after treatment, and participants were asked to submit stool samples prior to RBX2660 or placebo treatment (baseline) and 1, 4 and 8 weeks, 3 and 6 months after study treatment. Samples were extracted and sequenced using a shallow shotgun method. The presence and number of AMR genes was determined for 175 participant samples and 116 RBX2660 samples using 90% K-mer sequence coverage based on the MEGARes database. AMR gene count data were collapsed into count columns to adjust for sparseness in the matrices and were analyzed using a Generalized Linear Mixed Model.

Results. Clinically, RBX2660 demonstrated superior efficacy versus placebo (70.4% and 58.1%, respectively), and the total AMR genes per participant decreased significantly from before to after treatment in RBX2660-treated responders ($p < .05$, Figure 1) and remained low to at least 6 months. Among genes that decreased in RBX2660 responders were clinically important extended-spectrum beta-lactamase (bla_{TEM}, bla_{SHV}, bla_{CTX-M}), vancomycin resistance (*vanA*, *vanB*), and fluoroquinolone resistance genes (*gyrA*, *parC*).



Total AMR genes per PUNCH CD3 participant among RBX2660-treated responders at the indicated time points and in the RBX2660 investigational product. The red lines indicate timepoint group medians.

Conclusion. In the PUNCH CD3 Phase 3 trial of RBX2660 for rCDI, AMR gene content decreased after RBX2660 treatment and remained low to at least 6 months, consistent with prior RBX2660 trials. This underscores the potential of microbiota-based biotherapeutics for decolonizing AMR bacteria from gut microbiota and thereby reducing AMR infection risks.

Disclosures. **Heidi Hau, PhD**, **Rebiotix Inc.** (Employee) **Dana M. Walsh, PhD**, **Rebiotix** (Employee) **Ken Blount, PhD**, **Rebiotix Inc., a Ferring Company** (Employee)

130. Design and Preclinical Characterization of SER-155, an Investigational Cultivated Microbiome Therapeutic to Restore Colonization Resistance and Prevent Infection in Patients Undergoing Hematopoietic Stem Cell Transplantation

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Session: O-27. Novel Antimicrobial Agents

Background. During allogeneic hematopoietic stem cell transplant (HSCT), the diversity and stability of the GI microbiome is disrupted, increasing the risk of domination by pathogens associated with bacteremia, aGvHD, and mortality. SER-155 is an investigational, oral microbiome therapeutic composed of cultivated spores and vegetative bacterial strains rationally designed to reduce the risk of bacteremia and aGvHD in HSCT recipients by decolonizing potential pathogens and restoring GI colonization resistance. SER-155 was evaluated *in vitro* for key pharmacological properties associated with colonization resistance, and *in vivo* to assess its ability to restore colonization resistance by reducing *Enterococcus* and *Enterobacteriaceae* carriage.

Methods. The design of SER-155 leveraged genomic data from interventional and observational human datasets to include taxa associated with reduced risk of infection and aGvHD in HSCT. Strains of interest were phenotyped, and over 50 candidate consortia containing different combinations of over 150 species were designed and tested *in vitro* and *in vivo*. *In vivo*, candidate compositions were evaluated in mouse models of vancomycin-resistant *Enterococcus faecium* (VRE) and carbapenem-resistant *Klebsiella pneumoniae* (CRE) colonization.

Results. Oral administration of SER-155 led to a 2-3 Log_{10} reduction in VRE and CRE titers compared to untreated mice (Figure 1). *In vitro*, the carbon source utilization profile of VRE, CRE, and SER-155 strains were assessed using a panel of 85 carbon sources. All 56 carbon sources used by CRE or VRE for anaerobic growth were also utilized by SER-155 strains, supporting a model in which nutrient competition may contribute to reducing CRE and VRE carriage and restoring colonization resistance.