Antimicrobial Resistance Genes are Reduced Following Administration of Investigational Microbiota-Based Therapeutic RBX2660 to Individuals with Recurrent *Clostridioides difficile* Infection

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Restoration of gut microbiota diversity has been shown to reduce rCDI and decrease colonization with antibiotic-resistant organisms

Clostridioides difficile causes life-threatening diarrhea and is classified as an **urgent public health threat** by CDC.

Antibiotics are the standard of care treatment. Patients with *Clostridioides difficile* infection (CDI) are at risk of recurrence. Risk of recurrence increases with each subsequent episode.

Patients with recurrent CDI (rCDI) undergo repeated rounds of antibiotics, increasing their propensity for microorganisms that harbor antimicrobial resistant genes (AMR)

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Design of PUNCH™ CD3 Clinical Trial for rCDI

Investigational formula RBX2660

Screening; consent; enrollment; randomization

Antibiotic washout (24-72h)

Single dose RBX2660 or placebo

Treatment success defined as recurrence free @ 8 weeks

rCDI (active episode)

ANTIBIOTIC

8 WEEKS

SAFETY MONITORING

Safety and durability of treatment assessed

Laboratory diagnosis by physician SoC

Baseline **7 D** **30 D** **8 W** **Additional timepoints**

Shallow shotgun sequencing to evaluate microbiome changes

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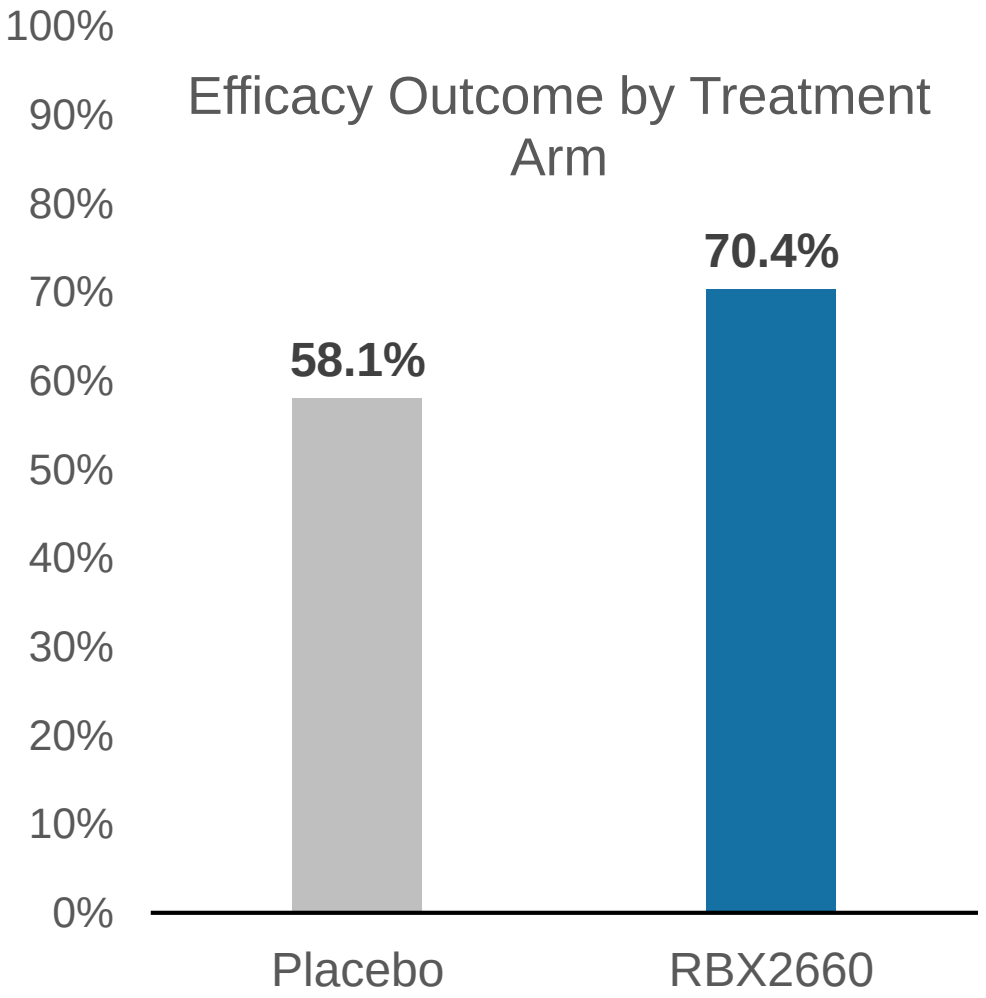
PUNCH™ CD3: Investigational RBX2660 was superior to placebo

- Treatment success: absence of CDI recurrence at 8 weeks after a single dose of blinded investigational treatment
- Novel *a priori* Bayesian analysis leveraged outcome data from the prior Phase 2B trial; success defined as meeting a minimum threshold of 0.975 posterior probability of superiority (RBX2660 versus placebo)
- **RBX2660 met the pre-specified threshold of success over placebo, with a 0.986 posterior probability of superiority**



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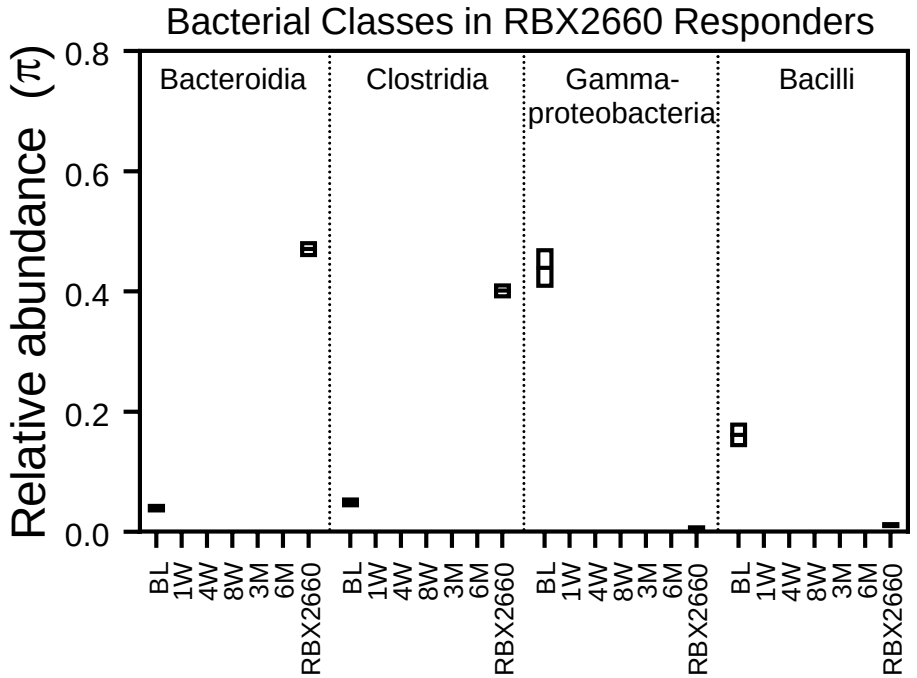
Microbiome composition recovers after treatment with RBX2660

- At baseline (BL), responder's microbiomes differ from RBX2660
- As early as 1 week (1W) after receipt of investigational product RBX2660, treatment responders demonstrated significant alterations, more closely reflecting the profile of RBX2660
- Microbiome recovery is consistent with previous clinical studies of RBX2660



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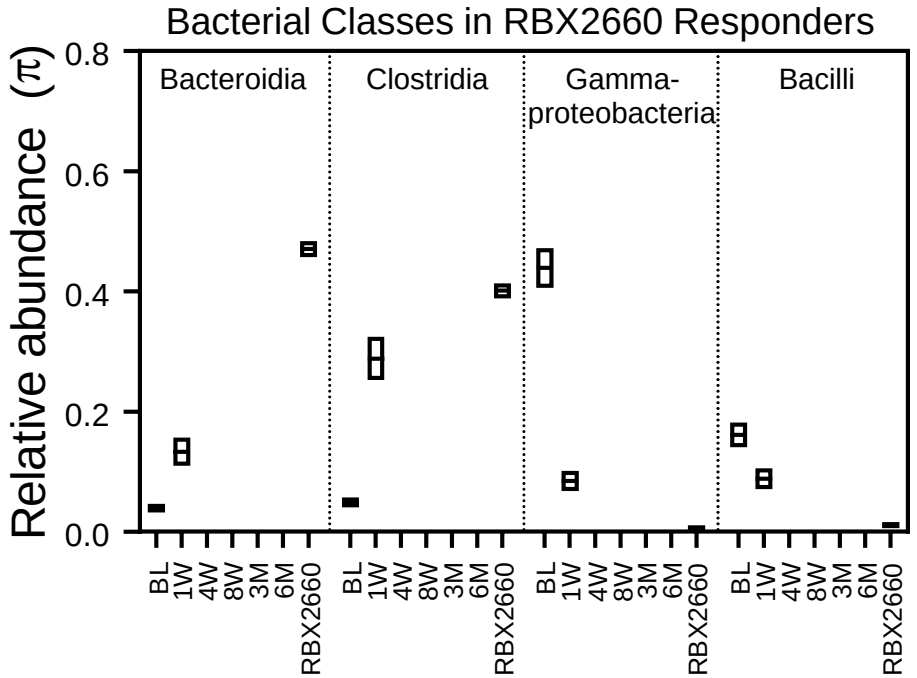

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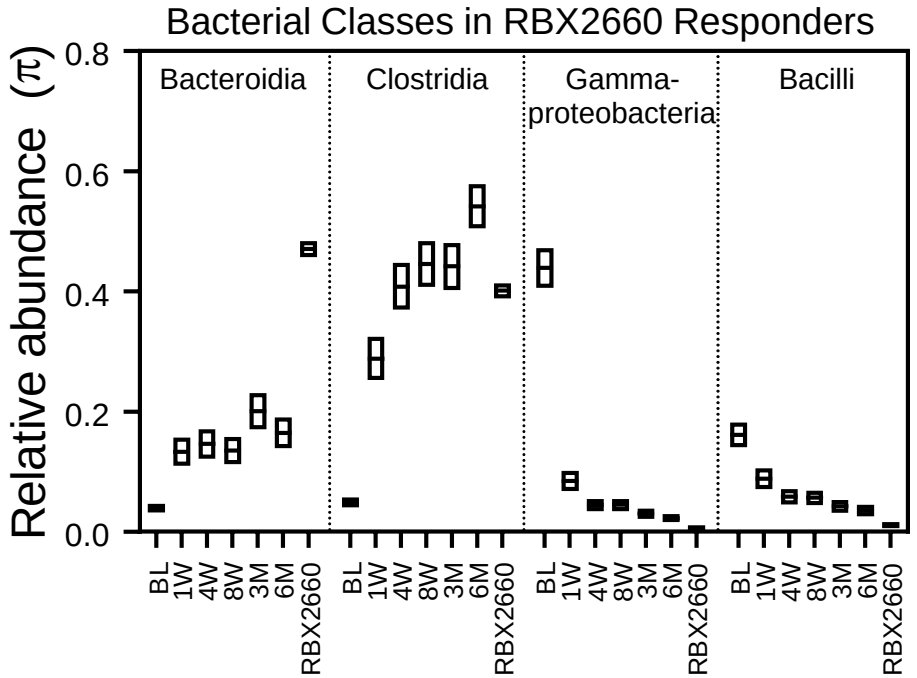
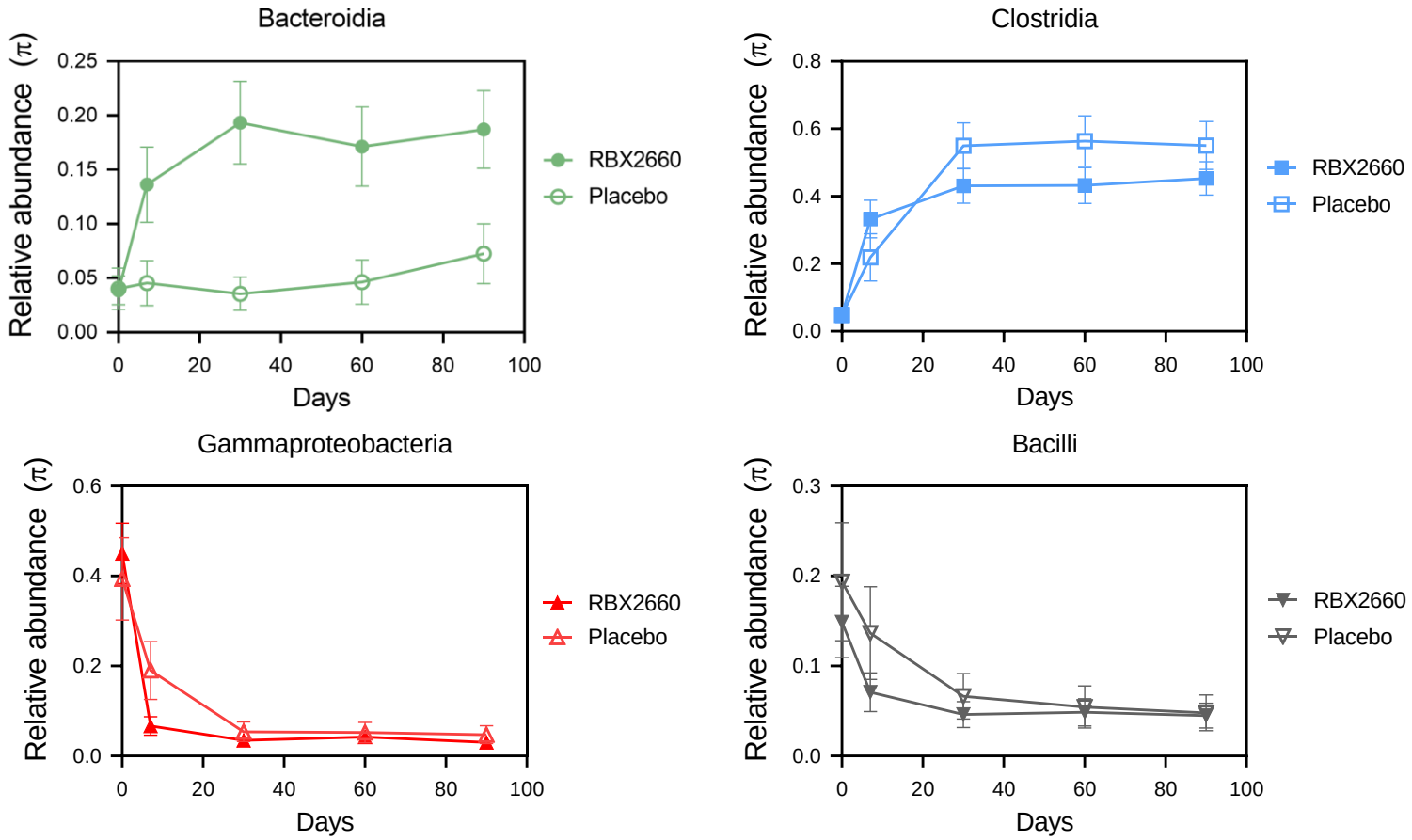


PUNCH™ CD3: Key taxa restored within days after RBX2660 receipt

- Restoration was more rapid and extensive in RBX2660 responders compared to placebo responders as soon as 7 Days

Bacterial Classes after treatment with RBX2660 or placebo



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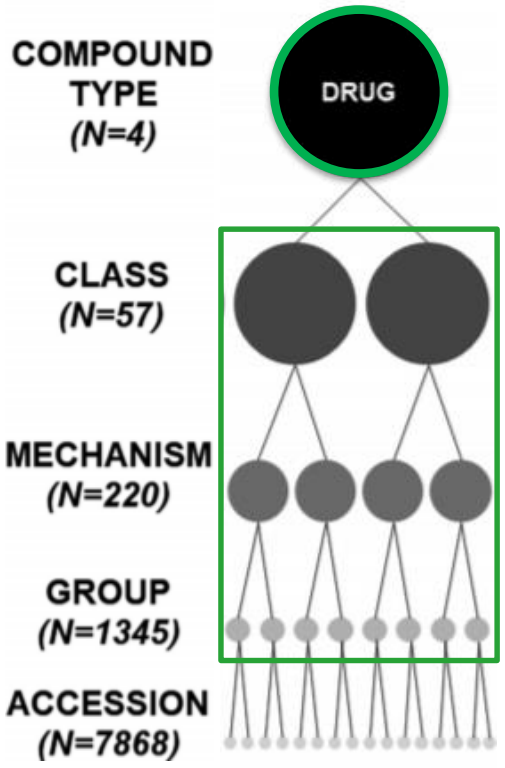
AMR profiles were evaluated for each participant/timepoint using MEGARes 2.0[1]

- Shallow shotgun raw DNA sequences were processed through MEGARes 2.0[1] to generate AMR alignments using Least Common Ancestor
- Required 98% DNA homology, ~90% AMR gene coverage (≥50 seq reads)



Antimicrobial chemical class: β-lactams, aminoglycosides, etc

Biological mechanism of resistance: e.g. Class A β-lactamase

Gene: SHV Class A β-lactamase

[1]**Doster *et al.*** (2019). MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Res. doi:10.1093/nar/gkz1010.

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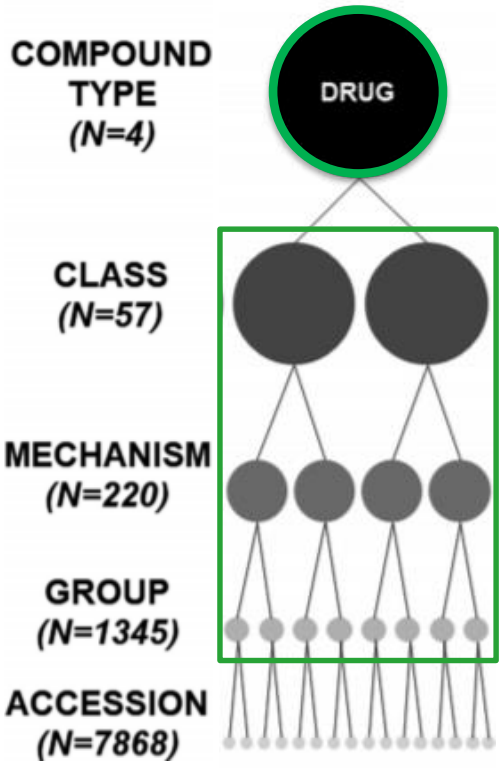

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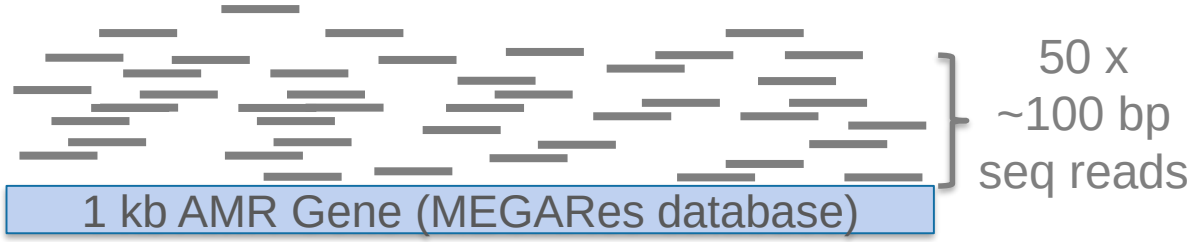

AMR genes not meeting the 50 seq read threshold were considered absent

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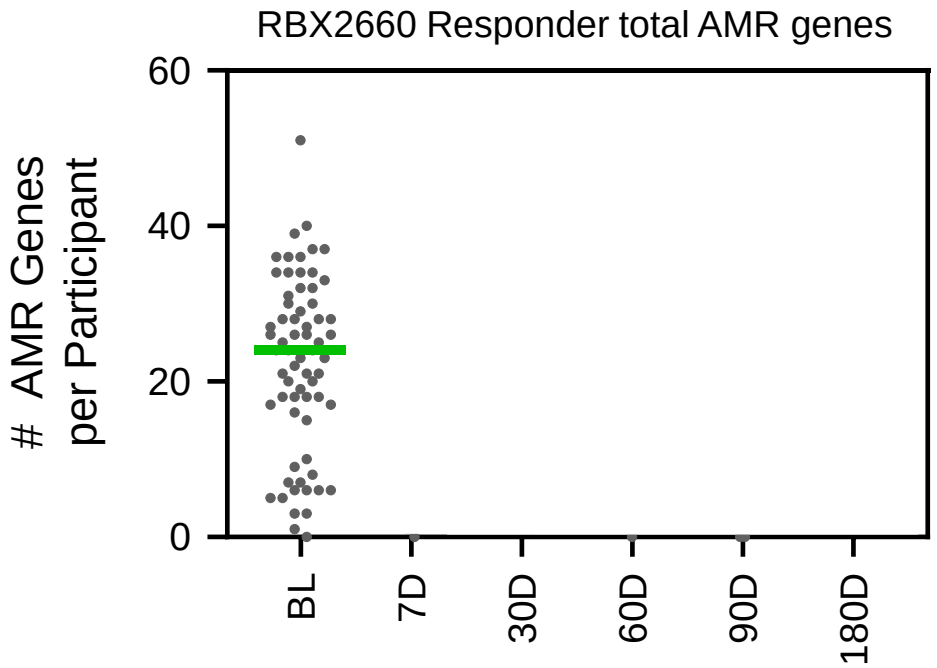
PUNCH™ CD3: AMR gene abundance was decreased after RBX2660

- Prior to RBX2660 treatment (BL), participants had >20 identified AMR genes
- Within 7D, AMR genes per participant decreased among investigational product RBX2660 responders and was sustained to at least 180D (6 months)
- Statistically significant at all time points; $p < 0.05$; Generalized Linear Mixed Effects Model (GLMM)



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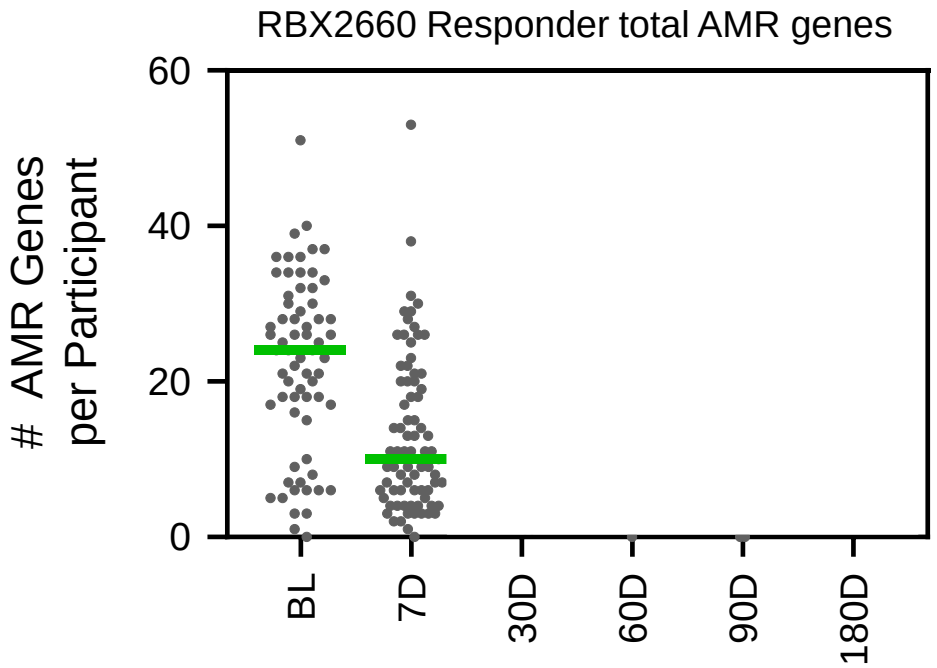

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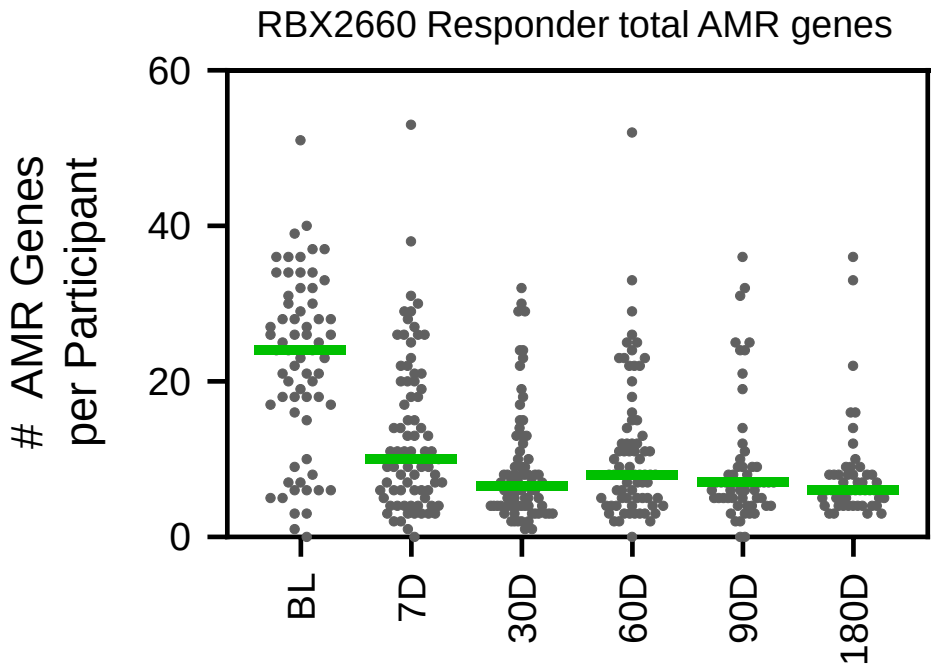
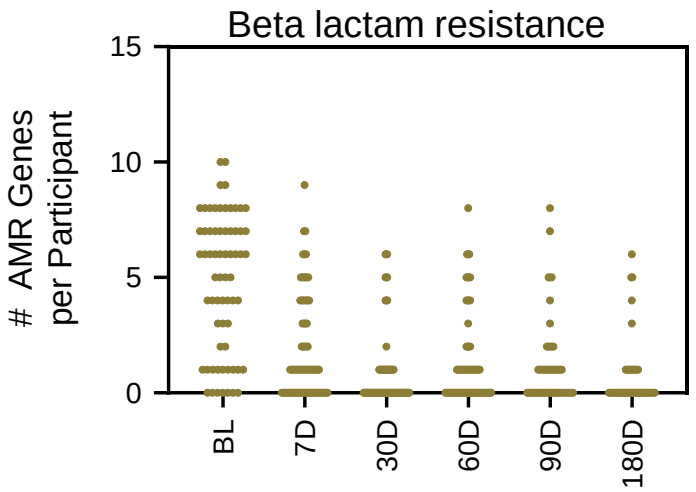
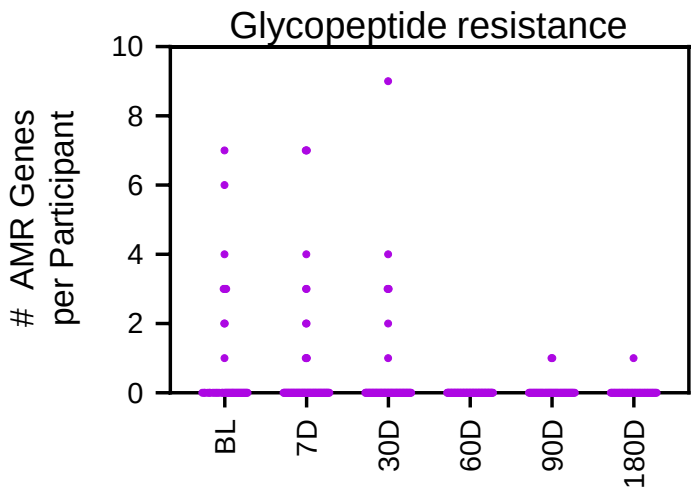


Clinically relevant AMR gene classes were reduced in RBX2660 responders

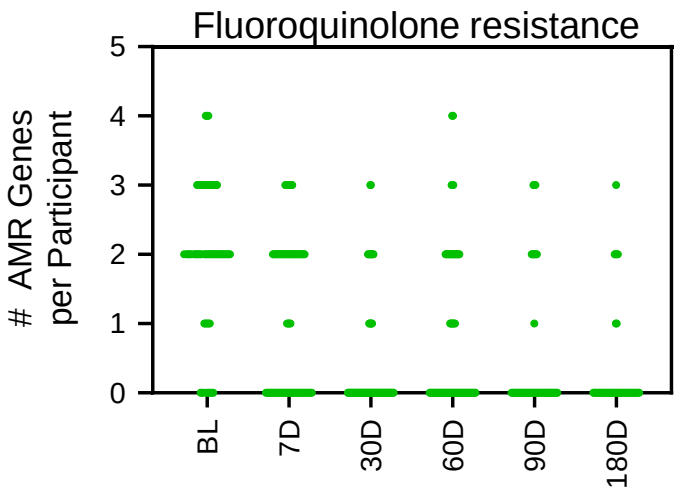
- A statistically significant decrease in genes ($p < 0.05$; GLMM) conferring resistant to beta lactams, glycopeptides and fluoroquinolones were noted in RBX2660 treatment responders across all timepoints when compared to BL



Genes associated with ESBL resistance bla_{TEM}, bla_{SHV} and bla_{CTX} were reduced



Vancomycin resistance gene *vanB* was reduced



Resistance genes *gyrA* and *parC* were reduced

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Summary of key points

- The pivotal Phase 3 PUNCH™ CD3 study met the pre-specified threshold of success (0.986 posterior probability of superiority) of our investigational microbiome-based live biotherapeutic, RBX2660, vs placebo

- Microbiome composition of RBX2660 responders demonstrates restoration as early as 1 week post-treatment and is sustained to 6 months

- Total AMR genes were observed to decrease significantly in RBX2660-treated responders supporting the potential for AMR decolonization through a microbiome-based live biotherapeutic.

- Specific classes of clinically important AMR genes decrease significantly in RBX2660 responders as early as 1 week after treatment

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