SYN-004 (ribaxamase), a Beta-Lactamase, Protects the Gut Microbiome from IV Antibiotics and Reduces Propagation of Antibiotic-Resistance Genes in a Porcine Dysbiosis Model

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ABSTRACT

Background: Disruption of the intestinal microbiome is a major, unintended consequence of antibiotic treatment that can lead to overgrowth of pathogenic organisms such as *Clostridium difficile*. SYN-004 (ribaxamase) is an orally-delivered beta-lactamase for use with intravenous (IV) penicillins and cephalosporins and is designed to degrade residual antibiotics in the GI tract, thereby protecting the microbiome. A phase 2b, proof of concept study demonstrated statistically significant reduction of *C. difficile* disease and new colonization with vancomycin-resistant enterococci (VRE) in patients that received ribaxamase with ceftriaxone (CRO), compared to placebo. Here, a porcine model of antibiotic-mediated gut dysbiosis was established and used to assess the ability of ribaxamase to protect the gut microbiome from IV beta-lactam antibiotics and mitigate propagation of antibiotic resistance.

Materials and Methods: Ribaxamase, a 29 kDa, engineered recombinant protein was manufactured in *E. coli* and formulated for oral delivery into enteric-coated pellets to release the enzyme in the duodenum (at pH >5.5). Normal piglets (~20 kg, n=5 per cohort) were treated with IV CRO (50 mg/kg SID for 7 days) with a separate cohort receiving both oral ribaxamase (75 mg QID) and CRO. CRO serum levels were quantified using a validated HPLC-based assay. Whole genome shotgun sequence analyses of pig fecal DNA were performed to assess the effect on the gut microbiota and quantify antibiotic-resistance genes as a measure of antibiotic-resistant bacteria present in the fecal microbiome.

Results: CRO serum levels were similar in antibiotic alone and antibiotic+ribaxamase cohorts, indicating that ribaxamase did not alter the systemic antibiotic level. CRO exposure caused significant changes to the gut microbiome while CRO+ribaxamase microbiomes were not significantly different from pre-treatment microbiomes. Exposure to CRO resulted in greater abundance of resistance genes compared to treatment with CRO+ribaxamase. As expected, many of the resistance genes encoded extended spectrum beta-lactamases, conveying resistance to third generation cephalosporins, including CRO. However, other genes, such as those encoding components of multidrug efflux transporter systems that convey resistance to a broad range of antibiotics, increased in the presence of CRO but were attenuated with CRO+ribaxamase. Finally, genes confering resistance to non-beta-lactam antibiotics, such as aminoglycosides and tetracycline, increased in the presence of CRO.

Conclusions: Ribaxamase protected the porcine microbiome from dysbiosis caused by CRO exposure and attenuated propagation of a broad range of antibiotic-resistance genes. Antibiotic inactivation represents a new treatment paradigm for preservation of a patient's native, pre-disease microbiome and prevention of opportunistic infections, such as *C. difficile* disease, caused by antibiotic-mediated dysbiosis. These data support the potential for ribaxamase to mitigate emergence and spread of antibiotic resistance following exposure to broad spectrum antibiotics.

BACKGROUND

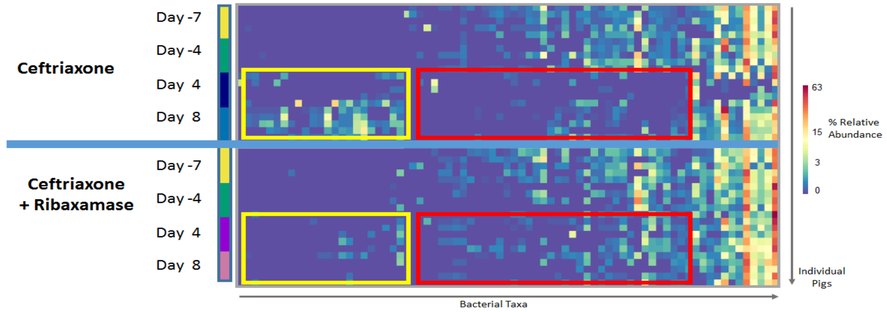
IV beta-lactam antibiotics, including cephalosporins, are excreted via the bile into the intestine where they can disrupt the intestinal microbiota and potentially lead to outgrowth of pathogens like *Clostridium difficile*. SYN-004 (ribaxamase) is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics and is designed to preserve gut microbiota by degrading residual antibiotics in the intestine. The intended indication is prevention of opportunistic infections, including *C. difficile*.

Ribaxamase was manufactured in *E. coli* and formulated to release enzyme in the duodenum (at pH >5.5). A piglet model of antibiotic induced dysbiosis was established. Normal piglets (~20 kg, n=5 per cohort) were treated for 7 days with ceftriaxone (50 mg/kg, IV, SID) -/+ ribaxamase (75 mg, PO, QID), started the day before antibiotic for 9 days. Serum was collected on day 2 of antibiotic treatment, and feces were collected on days -7, -4, 4, and 8. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

RESULTS

Ribaxamase Protects the Microbiome from IV Ceftriaxone

Heat maps of the fecal microbial community, based on relative abundance, were generated. Each square represents a bacterial species in individual animal microbiomes. Species are indicated horizontally and fecal collection day and animal are displayed vertically. Yellow and red boxes display changes in species diversity.

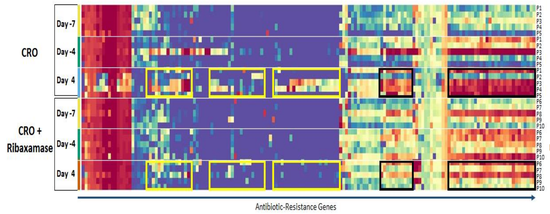


Comparison of fecal microbiota in ceftriaxone (CRO)-treated pigs reveals depletion of some species (red boxes) and overgrowth of others (yellow boxes). Ribaxamase reduced the CRO-mediated microbiota changes.

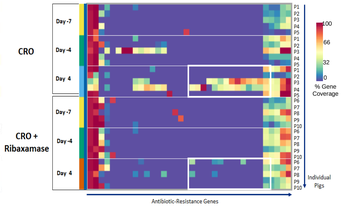
Ribaxamase Reduced Propagation of Antibiotic-Resistance Genes

Heat maps of the fecal resistome, based on % gene frequency as a measure of relative abundance, were generated. Each square represents an antibiotic-resistance (AR) gene in individual animal microbiomes. The genes are displayed horizontally and fecal collection day and animal are displayed vertically. The yellow, black, and white boxes display changes in AR gene abundance caused by antibiotic treatment.

Antibiotic-Resistance Gene Heatmap



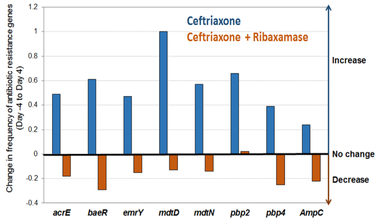
Beta-Lactamase Gene Heatmap



A broad spectrum of antibiotic-resistance genes were propagated in response to ceftriaxone (CRO), not just those conferring resistance to beta-lactams. Ribaxamase reduced the emergence and the propagation of beta-lactamase genes and additional antibiotic-resistance genes.

Ribaxamase Reduced Propagation of Antibiotic-Resistance Genes

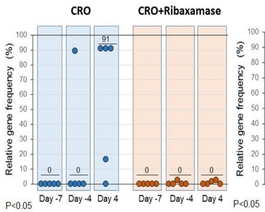
Change in Frequency of AR Genes



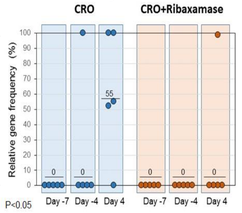
The change in relative frequency (mean) of AR genes from day -4 to day 4 is shown for ceftriaxone (blue) and ceftriaxone+ribaxamase (orange). A negative value indicates reduction, zero indicates no change, and a positive value, an increase. Genes: *acrE, baeR, emrY, mdtD,* and *mdtN* encode components of drug efflux pumps. *Pbp2* and *pbp4* encode penicillin binding proteins, and *AmpC* is a class C beta-lactamase gene. The relative frequency of each gene increased with CRO and decreased with ribaxamase.

Frequency of Aminoglycoside_*strA*, a phosphotransferase, and Tetracycline_*tet39*, an efflux pump, is displayed for each time point. Blue = ceftriaxone (CRO) and orange= ceftriaxone + ribaxamase. Significant reduction in the frequency of *strA* and *tet39* was observed with ribaxamase, compared to ceftriaxone alone (p<0.05).

Aminoglycoside_*strA*



Tetracycline_*tet39*



CONCLUSIONS

- Ribaxamase is intended as an orally-delivered beta-lactamase to protect the gut microbiome from IV penicillins and cephalosporins to prevent *C. difficile* infection (CDI)
- A phase 2b proof-of-concept study demonstrated statistically significant reduction in CDI and new VRE colonization in ribaxamase+ceftriaxone patients compared to placebo
- Ribaxamase protected the gut microbiome from ceftriaxone-mediated dysbiosis in pigs
- Ribaxamase reduced emergence and propagation of antibiotic-resistance genes in pigs

Ribaxamase has the potential to become the first prophylactic therapy designed to prevent antibiotic-mediated microbiome damage, reduce antibiotic resistance, and prevent *C. difficile* infection

DISCLOSURES

SC, JAB, SH, CFF, JS, and MK are employees of Synthetic Biologics, Inc. PS, NAH, and RRC are employees of CosmosID, Inc., a fee-for-service provider engaged by Synthetic Biologics, Inc.