SYN-006, a Novel Carbapenemase, Intended to Protect the Gut Microbiome from Antibiotic-Mediated Damage, May Also Reduce Propagation of Carbapenem-Resistant Pathogens

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ABSTRACT

Background: Beta-lactam antibiotics that are excreted in bile can damage the gut microbiota leading to serious adventitious infections and propagation of antibiotic resistance. SYN-004 (ribaxamase) is a beta-lactamase intended for oral use with certain IV beta-lactam antibiotics to degrade the antibiotic in the GI tract to protect the microbiome. Ribaxamase was evaluated in a phase 2b clinical study that met its primary endpoint of significantly reducing *C. difficile* infection in patients treated with ceftriaxone. Ribaxamase degrades penicillins and cephalosporins, but not carbapenems. SYN-006, a carbapenemase, was identified to expand this prophylactic approach to all classes of beta-lactam antibiotics.

Methods: SYN-006, a metallo-beta-lactamase derived from *B. cereus,* was produced in *E. coli*. Antibiotic degradation was assessed using a bacterial growth assay. SYN-006 (1 mg/kg, PO) was delivered to fistulated dogs that received the carbapenem, meropenem (30 mg/kg, IV). Blood and intestinal antibiotic levels were assessed. A pig model of carbapenem-mediated microbiome disruption was established with microbiome and resistome analysis performed using fecal DNA using whole genome shotgun sequence data.

Results: *In vitro*, SYN-006 displayed a broad antibiotic degradation profile that included carbapenems, cephalosporins, and penicillins, and was resistant to beta-lactamase inhibitors. SYN-006 degraded meropenem in the GI tract of dogs without affecting systemic antibiotic levels. In pigs, ertapenem significantly changed the composition of the gut microbiome and mediated emergence and propagation of a wide range of antibiotic resistance genes, including extended spectrum beta-lactamase genes such as the carbapenemase gene, IMP-27.

Conclusion: SYN-006 efficiently degraded all classes of beta-lactam antibiotics including carbapenems *in vitro*, and meropenem within the dog GI tract. The pig model of carbapenem-mediated microbiome disruption is intended to be used to evaluate the ability of SYN-006 to protect the gut microbiome and attenuate antibiotic resistance. SYN-006 has the potential to protect the gut microbiome from all classes of beta-lactam antibiotics and to reduce the emergence of carbapenem resistant pathogens.

BACKGROUND

Many IV beta-lactam antibiotics are excreted via the bile into the intestine where they can disrupt the intestinal microbiota and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. Ribaxamase is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics to preserve the gut microbiome by degrading residual antibiotics in the intestine. A phase 2b study met its primary endpoint of significantly reducing *C. difficile* infection (CDI) in patients treated with ceftriaxone and ribaxamase.

While SYN-004 degrades penicillins and cephalosporins, it does not inactivate carbapenems [1]. To expand this prophylactic approach to all classes of beta-lactams, we are developing SYN-006, a broad spectrum carbapenemase.

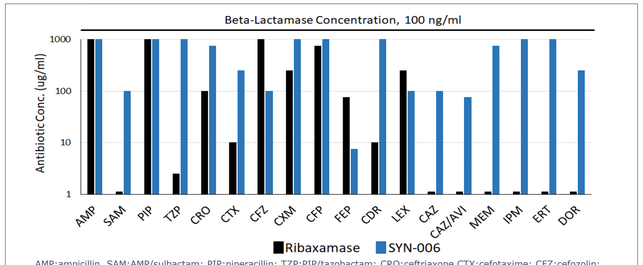
SYN-006 is manufactured in *E. coli* and purified using a single chromatographic step. Resistance to degradation by intestinal proteases is a key attribute for orally-delivered enzymes. SYN-006 displayed stable biological activity for at least 6 hours after incubation in human chyme.

Oral delivery formulations of SYN-006, engineered to protect SYN-006 from low stomach pH and allow enzyme release in the upper small intestine, are currently being developed. The formulated SYN-006 will be evaluated in pigs and/or dogs treated with IV ertapenem to assess the ability of SYN-006 to protect the gut microbiome by degrading excreted antibiotic in the GI tract.

RESULTS

Antibiotic Degradation Profile

Purified beta-lactamase enzymes were assessed for antibiotic hydrolysis potency with a microtiter plate activity assay using *E. coli* growth as the read-out for antibiotic inactivation. A total of 10, 100, or 1000 ug/ml of each antibiotic was mixed with 100 ng/ml of ribaxamase or SYN-006. *E. coli* was added and growth quantified. The graph displays the highest antibiotic concentration at which bacterial growth was observed, indicating antibiotic inactivation.



AMP:ampicillin, SAM:AMP/sulbactam; PIP:piperacillin; TZP:PIP/tazobactam; CRO:ceftriaxone CTX:cefotaxime; CFZ:cefozolin; CXM:cefuroxime; CFP:cefoperazone; FEP:cefepime; CDR:cefdinir; LEX:cephalexin; CAZ:ceftazidime; CAZ/AVI:CAZ/avibactam; MEM:meropenem; IPM:imipenem; ERT:ertapenem; DOR:doripenem

SYN-006 displayed a broader antibiotic degradation profile that included the carbapenems (MEM, IPM, ERT, DOR), compared to ribaxamase. SYN-006 showed good activity against the penicillins (AMP, PIP) and the cephalosporins (CRO, CTX, CFZ, CXM, CFP, CDR, LEX, CAZ) and was active in the presence of beta-lactamase inhibitors.

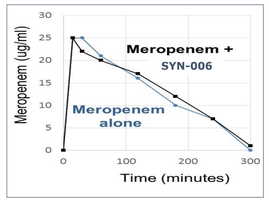
SYN-006 Degrades Meropenem in the Dog GI Tract

Jejunally-fistulated dogs (n=6) received meropenem (30 mg/kg, IV) alone or with a liquid formulation of SYN-006 (1 mg/kg, PO).

Chyme Meropenem and SYN-006

Treatment (n=3)	Dog	SYN-006 (U/g)	Meropenem (ug/g)
Meropenem Alone	1	NA	3.0
	2	NA	3.2
	3	NA	3.0
Meropenem + SYN-006	4	80	0
	5	0.5	0
	6	0.2	2.0

Serum Meropenem



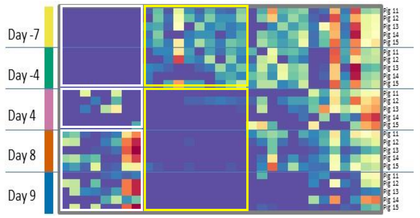
Meropenem levels in the chyme of animals treated with meropenem alone were ~3.0 ug/g. In the presence of SYN-006, meropenem was undectable in two animals, or 2.0 ug/g in one animal. SYN-006 chyme levels were extremely variable, ranging from 80 U/g to 0.2 U/g. This variability is likely due to SYN-006's sensitivity to acid, as the enzyme was delivered orally in a non-enteric-coated liquid formulation. SYN-006 had no effect on systemic meropenem levels. Therefore, when present in the chyme at levels of 0.5 U/g or higher, SYN-006 completely degraded the meropenem in the dog GI tract without affecting meropenem serum levels.

Ertapenem Rapidly Disrupts the Pig Gut Microbiome

To evaluate SYN-006 efficacy in protecting the microbiome from beta-lactam antibiotics, including carbapenems, a pig model of ertapenem-mediated dysbiosis was established. Normal pigs (20 kg, n=5) were treated with IV ertapenem (30 mg/kg, IV, SID) for 7 consecutive days. Feces were collected at two pre-antibiotic time points (Days -7 and -4), during treatment (Day 4), and after antibiotics were stopped (Days 8 and 9). Fecal DNA was subjected to whole genome shotgun sequence analyses.

Heat map analyses of the fecal microbial community was based on species relative abundance. Each square represents a bacterial species present in individual animal microbiomes. The yellow and white boxes display changes in species diversity caused by ertapenem treatment. The Dirichlet-Multinomial model likelihood ratio test was used to compare microbiome populations prior to and after antibiotic treatment.

Heatmap



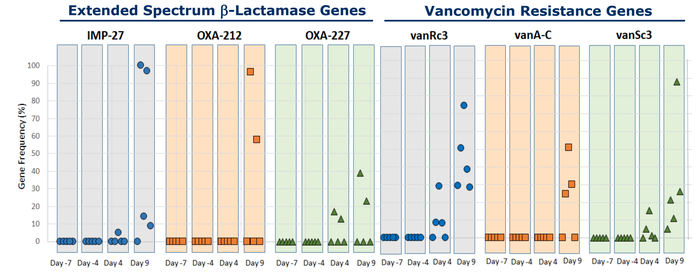
Likelihood Ratio Test

Treatment Group	Chi squared	P value
Day -4	-31.72	1.00
Day 4	387.73	<0.0001
Day 8	720.56	<0.0001
Day 9	655.16	<0.0001

Heatmap analyses compared bacterial species present in microbiomes of pigs prior to and after ertapenem treatment and reveal that the antibiotic caused the depletion of some species (yellow boxes) and the overgrowth of others (white boxes). The likelihood ratio test compared the microbiomes prior to ertapenem treatment (Day -4) to the microbiomes after antibiotic exposure. Microbiomes after antibiotic treatment were significantly different from pre-exposure microbiomes. A pig model of ertapenem-mediated dysbiosis was successfully established.

Emergence of Antibiotic Resistance (AR) Genes after Ertapenem Exposure

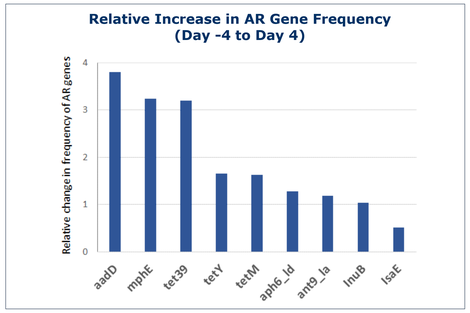
Fecal resistomes were analyzed based on the % gene coverage as a measure of AR gene relative abundance. Relative abundance of selected genes was graphed individually for each animal.



A broad spectrum of AR genes was propagated in response to ertapenem. Most notably, IMP-27, a carbapenemase, reported to be rare in pigs [2], several ESBLs, and vancomycin resistance genes emerged by Day 4 of ertapenem exposure and were not detected prior to antibiotic administration.

Emergence of AR Genes after Ertapenem Exposure

Change in relative frequency (%) is displayed as the ratio of post treatment (Day 4) to pretreatment (Day -4) gene relative abundance.



A broad spectrum of AR genes was propagated following ertapenem exposure including genes conferring resistance to beta-lactam and non-beta-lactam antibiotics.

CONCLUSIONS

- SYN-006 has a broad antibiotic degradation profile including penicillins, cephalosporins, and carbapenems
- In dogs, oral SYN-006 resulted in degradation of intestinal meropenem and did not affect meropenem serum levels
- A pig model of ertapenem-mediated dysbiosis was established
- Ertapenem exposure rapidly resulted in the emergence of antibiotic-resistance genes, including a rare carbapenemase gene, IMP-27
- An enteric-coated formulation of SYN-006 is currently in production and is expected to be tested in the pig dysbiosis model

SYN-006 has the potential protect the gut microbiome from certain IV beta-lactam antibiotics including carbapenems and to reduce emegence of antibiotic resistance

REFERENCES

1. Kaleko, M., *et al.* (2016) Development of SYN-004, an Oral Beta-Lactamase Treatment to Protect the Gut Microbiome from Antibiotic-Mediated Damage and Prevent *Clostridium difficile* infection. *Anaerobe 41:58-67.* doi: 10.1016/j.anaerobe.2016.05.015
2. Mollenkopf, D.F., *et al.* (2017) Carbapenemase-Producing Enterobacteriaceae Recovered from the Environment of a Swine Farrow-to-Finish Operation in the United States. *Antimicrob Agents Chemother* 61. doi: 10.1111/zph.122993