Monday-450

Inactivation of Vegetative Bacteria During Production of SER-109, a Microbiome-Based Therapeutic for Recurrent *Clostridium difficile* Infection

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Introduction

Open-label studies suggest that fecal microbiota transplant (FMT) may be effective in preventing *Clostridium difficile* infection (CDI) in patients with multiple recurrences by restoring the ecology of a healthy microbiome [1,2,3].

However, stool is minimally processed so potential transmission of undetected pathogens or emerging infections is still a concern [4]. This is a particular problem for microbes acquired through diet, like *Listeria*, which can lead to transient, asymptomatic colonization [5] . An alternative treatment for recurrent CDI with an improved risk profile is urgently needed.

SER-109 is an investigational, Ecobiotic® drug, which contains a purified ecology of approximately 50 unique bacterial spore-forming anaerobic Firmicutes fractionated from rigorously screened stool donors [6]. This microbiome-based drug product represents <0.1% of whole stool, facilitating convenient delivery as 4 oral capsules. A randomized, double-blind, placebo-controlled Phase 2 trial evaluating the efficacy and safety of SER-109 for patients with multiple recurrences of CDI has completed enrollment.

The SER-109 manufacturing process includes multiple steps of purification and ethanol inactivation of vegetative bacteria (e.g. *Listeria, Salmonella, Staphylococcus,* or *Enterococcus)*, which can serve as pathogens if not detected during screening. Ethanol is widely known to inactivate vegetative bacteria [7].

Aim

To evaluate inactivation of vegetative bacteria in the SER-109 manufacturing process

Methods

Inactivation kinetics

- Cell banks of *Listeria innocua* SLCC 3379, *Salmonella enterica* LT2, *Staphylococcus aureus* Wichita, and *Enterococcus faecalis* NCTC 775 were added to three separate lots of SER-109 intermediates immediately prior to ethanol inactivation.
- Ethanol was added up to 50% v/v. Inactivations were stopped by diluting 1:10 to reduce the concentration of ethanol to 5% v/v (demonstrated to not be bacteriocidal, data not shown).
- Titers of these samples were taken within 60 minutes of dilution on media selecting for the appropriate spiked organism. Titers in CFU/mL were plotted over time.

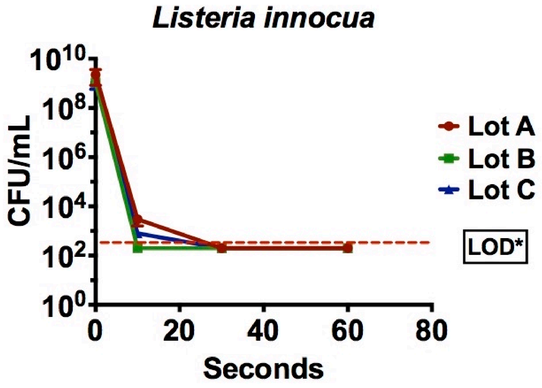
Identification of cultivatable anaerobes in SER-109

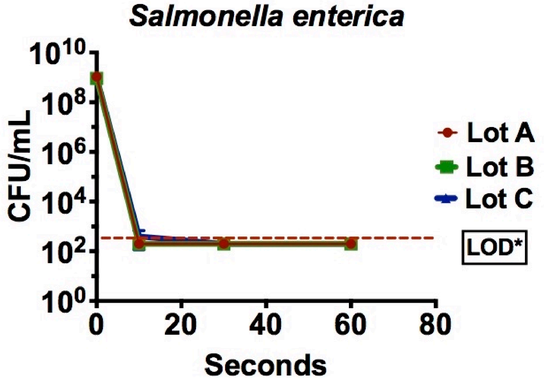
- Samples of SER-109 lots were cultured using standard anaerobic culturing methods. Single colonies were randomly sampled and characterized via sequencing of the 16S rRNA gene.

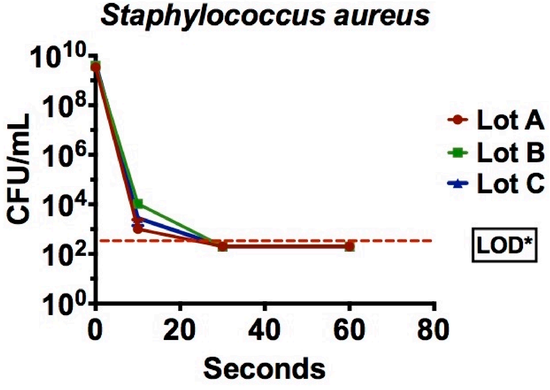
Results

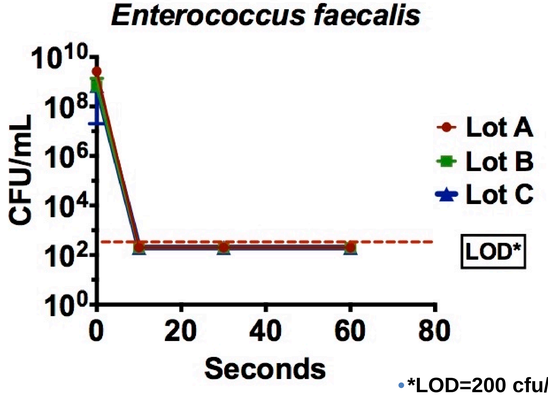
Inactivation kinetics demonstrate that ethanol processing leads to inactivation below the limit of detection in seconds

- Measuring vegetative bacteria in a complex mixture is complicated by the presence of bacterial spores. Three different lots of SER-109 were processed up to the ethanol inactivation step.
- These lots were spiked with cells banks of the indicated organisms. Ethanol was added to 50% v/v, and titers were determined post-ethanol inactivation.
- All samples show inactivation below the limit of detection within seconds









- *LOD=200 cfu/mL

SER-109 consists of spore-forming organisms of the phylum Firmicutes

- Samples of SER-109 lots were cultured using standard anaerobic culturing methods.
- Single colonies were randomly sampled and characterized via sequencing of the 16S rRNA gene.
- A total of 16,810 colonies from a number of SER-109 lots were sampled.
- All colonies were classified as spore-forming organisms belonging to the phylum Firmicutes.
- Taxonomic families detected are listed.

Family
Clostridiaceae
Erysipelotrichaceae
Eubacteriaceae
Lachnospiraceae
Oscillospiraceae
Peptostreptococcaceae
Ruminococcaceae
Unclassified Clostridiales
Total colonies sampled: 16,810

Conclusions

- These spike recovery studies demonstrate >6 Log of inactivation of vegetative cells by ethanol within seconds. When considering the full length of ethanol exposure that occurs within the inactivation process, greater than 10-Log of vegetative bacteria would be expected to be inactivated.
- Determination of the identity of 16,810 recovered viable anaerobes from SER-109 lots demonstrates that the ethanol inactivation step is effective at removing vegetative bacterial cells that would otherwise be present.
- The manufacturing process of SER-109 reduces the risk of pathogen transmission to a level that cannot be achieved by donor screening alone.

References

1. Van Nood Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile infection NEJM 2013
2. Cammarota G Randomized Clinical Trial of FMT for Recurrent CDI Alim Pharm Ther 2015
3. Orenstein R. Safety and Durability of RBX2660 for Recurrent Clostridium difficile infection CID 2016
4. Hecht, G What is the value of an investigational New Drug Application for FMT to treat recurrent CDI Clin Gastro Hep 2014
5. Grif K. Incidence of Fecal Carriage of Listeria monocytogenes in 3 healthy volunteers: A one year prospective stool survey Eur J Clin Micro Infect Dis 2003
6. Khanna, S., et al. (2016). A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent Clostridium difficile Infection. *J Infect Dis 2016*
7. Block S 2001. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins.