**Session:** O-6. Antimicrobial insights

***Background.*** Cefazolin (Cz) is commonly used to treat methicillin-sensitive *Staphylococcus aureus* (MSSA) bacteremia. Yet, some MSSA isolates producing the staphylococcal β-lactamase (BlaZ) exhibit the Cz inoculum effect (CzIE), defined as an increase in the minimum inhibitory concentration (MIC) to ≥ 16 µg/mL at high inoculum ($10^7$ CFU/mL, HI-MIC). Retrospective clinical data linked the CzIE to increased 30-day mortality and Cz treatment failure in patients with MSSA bacteremia, yet the mechanistic bases of this phenomenon are unknown. We aimed to explore the contribution of *blaZ* regulation, via BlaR (antibiotic sensor) and BlaI (transcriptional repressor) (**Fig 1**) to the CzIE by **i)** *in trans* expression assays and **ii)** analysis of their sequences in a set of isolates

Figure 1. Structure of the Staphylococcal bla Operon

***Methods.*** The *blaZ* genes (with putative promoters) of strains exhibiting and lacking the CzIE (TX0117 and ATCC29213, respectively) were expressed *in trans* in RN4220 (*blaZ* neg) using the promotor-less vector pWM401 (**Figure 2**). We subsequently cloned the *blaR* and *blaI* genes of each TX0117 and ATCC29213 upstream of each *blaZ* allele (**Figure 3**). The presence of the CzIE was assessed in transformants using broth microdilution at standard ($10^5$ CFU/mL, SI-MIC) and high inoculum. We also performed whole-genome sequencing (WGS) in 104 MSSA isolates exhibiting and lacking the CzIE to compare the sequences of BlaZ, BlaR, and BlaI and classified them by allotypes (unique amino acid sequences) using ATCC29213 as reference.


Figure 2. In trans expression of blaZ genes from a CzIE+ strain (TX0117) and a CzIE- strain (ATCC29213) in RN4220

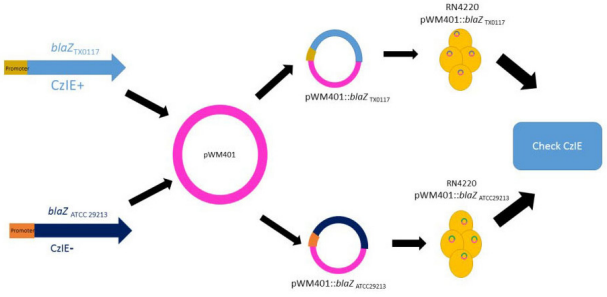



Figure 3. In trans expression of the bla Operons from a CzIE+ strain (TX0117) and a CzIE- strain (ATCC29213) in RN4220

***Results.*** Expression of $blaZ_{TX0117}$ and $blaZ_{ATCC29213}$ with their native promoters in RN4220 resulted in the CzIE with Cz HI-MICs ≥ 64 µg/mL regardless of the origin of the allele (**Table 1**). Inclusion of the regulatory elements *blaR* and *blaI* from TX0117 (CzIE+) did not change the phenotype. In contrast, addition of *blaR* and *blaI* from ATCC29213 (CzIE-) led to a marked decrease in the Cz HI-MIC (**Table 1**). Sequence analyses of 104 MSSA isolates revealed 10, 17 and 6 BlaZ, BlaR and BlaI allotypes, respectively (**Table 2**). BlaZ-2 and BlaR-4 were linked to the CzIE in 90% of isolates.

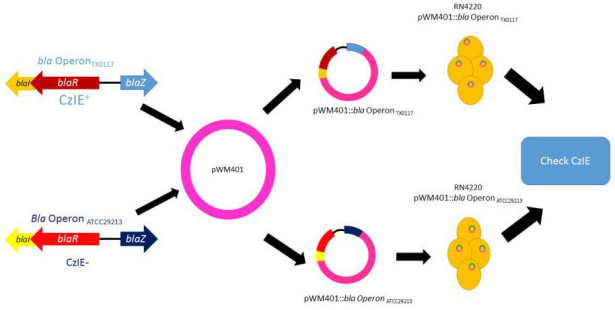



Table 1. MIC values of transformans after In trans expression of blaZ genes and bla Operons from a S. aureus CzIE+ strain(TX0117) and a CzIE- strain (ATCC29213) in RN4220

| Strain | Cz SI-MIC (µg/mL) | Cz HI-MIC (µg/mL) |
|---|---|---|
| ATCC29213 | 0.5 | 2 |
| TX0117 | 1 | 64 |
| RN4220 | 0.25 | 0.25 |
| RN4220 pWM401 | 0.25 | 0.25 |
| RN4220 pWM401::$blaZ_{ATCC29213}$ | 0.5 | 128 |
| RN4220 pWM401::$blaZ_{TX0117}$ | 0.5 | 64 |
| RN4220 pWM401::*bla* $Operon_{ATCC29213}$ | 0.5 | 16 |
| RN4220 pWM401::*bla* $Operon_{TX0117}$ | 0.5 | 64 |

Table 2. BlaZ allotypes of 104 Staphylococcus aureus isolates and their association with the CzIE

***Conclusion.*** Our results suggest that overexpression of *blaZ* can lead to the CzIE in any MSSA strain. Thus, the regulation of *blaZ* expression via BlaR and BlaI seem to play a major role in the CzIE. Identification of specific BlaR and BlaI allotypes could predict the presence of the CzIE.

| BlaZ allotype | Nº Isolates | % of Isolates | % CzIE + |
|---|---|---|---|
| BlaZ 2 | 10 | 9,6 | 90 |
| BlaZ 6 | 11 | 10,5 | 73 |
| BlaZ 1 | 37 | 35,6 | 19 |
| BlaZ 4 | 14 | 13,4 | 0 |
| BlaZ 5 | 6 | 5,7 | 0 |
| BlaZ 20 | 3 | 2,8 | 0 |
| BlaZ 11 | 3 | 2,8 | 0 |
| BlaZ 3 | 2 | 1,9 | 0 |
| BlaZ 8 | 1 | 0,96 | 0 |
| BlaZ 9 | 1 | 0,96 | 0 |
| BlaZ neg | 16 | 15,3 | 0 |

***Disclosures.*** **Cesar A. Arias, M.D., MSc, Ph.D., FIDSA**, **Entasis Therapeutics** (Scientific Research Study Investigator)**MeMed** (Scientific Research Study Investigator)**Merck** (Grant/Research Support)

**29. Rapid Restoration of Bile Acid Compositions After Treatment with Investigational Microbiota-based Therapeutic RBX2660 for Recurrent clostrioides Difficile Infection**

Nicky Ferdyan, BS[1]; Romeo Papazyan, PhD[1]; Dana Walsh, PhD[2]; Sarah Klein, BA[2]; Steve Qi, PhD[1]; Ken Blount, PhD[2]; Karthik Srinivasan, PhD[3]; Bryan Fuchs, PhD[1]; [1]Ferring Research Institute, San Diego, California; [2]Rebiotix, Inc., Roseville, Minnesota; [3]Ferring Pharmaceuticals, San Diego, California

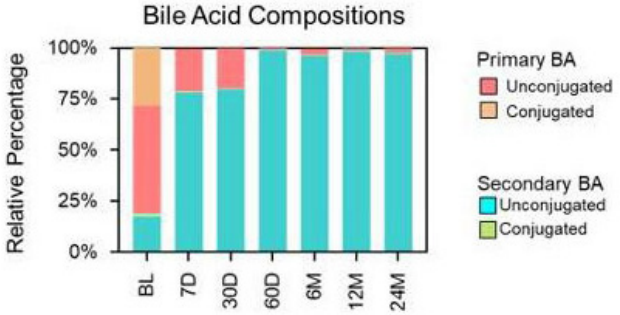
**Session:** O-6. Antimicrobial insights

***Background.*** Recurrent *Clostrioides difficile* infection (rCDI) is a public health threat associated with intestinal microbiome disruption (dysbiosis), which is postulated to increase CDI recurrence risk via disruption of bile acid(BA)-mediated resistance to *C. difficile* colonization. RBX2660 is an investigational microbiota-based therapeutic in clinical development for reducing rCDI recurrence. Herein, we assessed BA composition among participants in a Phase 2 trial of RBX2660 for rCDI.

***Methods.*** In a double-blinded trial (PUNCH CD2), rCDI participants were randomized to receive RBX2660 or placebo. Primary efficacy was defined as absence of CDI recurrence at 8 weeks after the last study treatment. Participants were asked to provide stool samples before (baseline) and up to 24 months after treatment. A liquid chromatography tandem mass spectrometry method was developed to extract and quantify 36 BAs from a total of 167 participant stool samples from 47 participants. Participant-matched samples at baseline and 1, 4, and 8 weeks were compared with a linear mixed effects model.

***Results.*** Primary BAs predominated at baseline but were significantly reduced ($p< .02$) as early as 1 week after treatment and remained so to 24 months. Concurrently, secondary BAs, most notably deoxycholic acid (DCA) and lithocholic acid (LCA), were significantly increased ($p< .01$) after treatment and remained so throughout. Moreover, increases in DCA and LCA were associated with treatment response ($p=.05$ and $p< .01$, respectively), recognizing the limited sample size of treatment failures. Observed BA changes coincided with changes in taxonomic compositions—a shift from Gammaproteobacteria and Bacilli predominance before treatment to Clostridia and Bacteroidia predominance after treatment.

Figure 1: BA restoration of successfully-treated participants



***Conclusion.*** In a trial of RBX2660 for rCDI, participant BA compositions significantly changed from before to after treatment, remained so for at least two years, and correlated with treatment outcome. The resulting predominance of secondary BAs coincided with microbiome compositional changes. Because secondary BA are thought to repress *C. difficile* colonization, these changes may partly explain how RBX2660 reduced CDI recurrence. Continued evaluation of RBX2660 for rCDI is underway.

***Disclosures.*** **Romeo Papazyan, PhD**, **Ferring Pharmaceuticals** (Employee) **Dana Walsh, PhD**, **Rebiotix Inc.** (Employee) **Steve Qi, PhD**, **Ferring Pharmaceuticals** (Employee) **Ken Blount, PhD**, **Rebiotix Inc.** (Employee) **Karthik Srinivasan, PhD**, **Ferring Pharmaceuticals** (Employee) **Bryan Fuchs, PhD**, **Ferring Pharmaceuticals** (Employee)

**30. Antimicrobial Resistance Genes Are Reduced Following Administration of Investigational Microbiota-based Therapeutic RBX7455 to Individuals with Recurrent clostrioides Difficile Infection**

Dana Walsh, PhD[1]; Carlos Gonzalez, MS[2]; Bill Shannon, PhD MBA[2]; Ken Blount, PhD[1]; [1]Rebiotix, Inc., Roseville, Minnesota; [2]BioRankings, LLC, St. Louis, Missouri

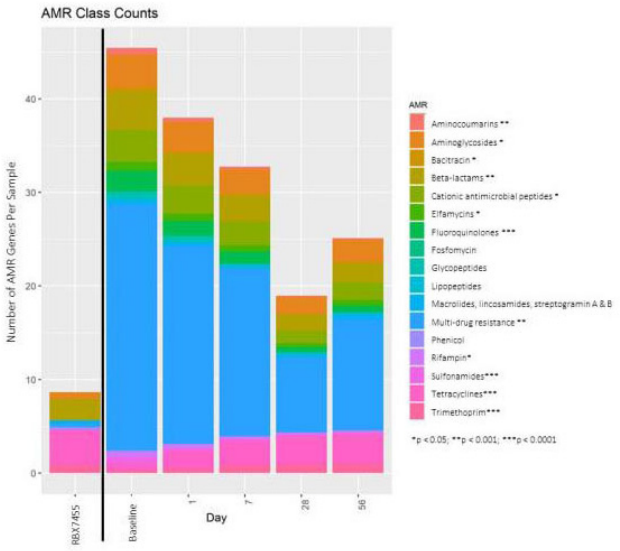
**Session:** O-6. Antimicrobial insights

***Background.*** Antimicrobial resistance (AMR) is a challenge in individuals at risk for recurrent *Clostrioides difficile* infection (rCDI). Recognizing that AMR bacteria colonize the intestinal microbiota, therapeutic approaches that decolonize the gut of AMR bacteria would be valuable. Herein, we assessed the microbial resistome before and after treatment with RBX7455—a room temperature-stable, orally-administered investigational microbiota-based therapeutic—in a Phase 1 trial for reducing CDI recurrence.

***Methods.*** This investigator-sponsored trial enrolled 30 rCDI patients in 3 open-label treatment groups (n=10 per group): 1) Four RBX7455 capsules BID for 4 days, 2) Four RBX7455 capsules BID for 2 days, 3) Two RBX7455 capsules BID for 2 days. RBX7455 administration began 48 hours after finishing CDI antibiotics. Participants were asked to submit stool samples at baseline, 1, 7, 28 and 56 days after treatment. These were extracted and sequenced using a shallow shotgun method. Relative taxonomic abundances at the class level and the presence of AMR genes were determined for 148 participant samples and 11 product samples using 90% K-mer sequence coverage based on the MEGARes database.

***Results.*** Ninety percent of participants met the primary endpoint of no CDI recurrence through 8 weeks after treatment, and participant microbiome compositions became more similar to RBX7455 after treatment. The total AMR counts per participant decreased from before to after treatment ($p < .05$, mixed effects model), with the pattern of AMRs identified (resistome) becoming more like the RBX7455 resistome (Figure 1). Most notably, AMRs associated with multi-drug, fluoroquinolone, and beta-lactam resistance decreased from before to after treatment. There was no significant difference among the groups with respect to clinical response or changes in microbiome composition and AMR content.

Figure 1 Average total and per-class AMR gene counts in participant samples before and after RBX7455 treatment.




***Conclusion.*** In a Phase 1 trial of RBX7455 for rCDI, AMR gene content decreased after treatment. This underscores the potential of microbiota-based therapies for decolonizing AMR bacteria from the gut microbiota. Continued clinical evaluation of RBX7455 is underway.

***Disclosures.*** **Dana Walsh, PhD**, **Rebiotix Inc.** (Employee) **Carlos Gonzalez, MS**, **BioRankings, LLC** (Employee) **Bill Shannon, PhD MBA**, **BioRankings, LLC** (Employee) **Ken Blount, PhD**, **Rebiotix Inc.** (Employee)

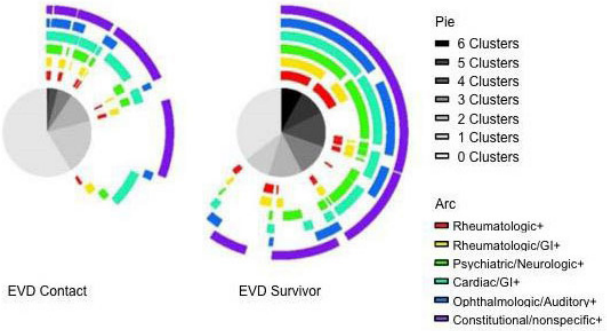
**31. Post-ebola Syndrome Presents with Multiple Overlapping Symptom Clusters: Evidence from an Ongoing Cohort Study in Eastern Sierra Leone**

Sarah Talia Himmelfarb, MD[1]; Nell Bond, PhD[2]; Adaora Okoli, MD, MPH[3]; John Schieffelin, MD, MSPH[1]; Jeffrey Shaffer, PhD[1]; Robert J. Samuels, M.B.Ch.B[4]; Emily J. Engel, MPH&TM[1]; [1]Tulane University, New Orleans, Louisiana; [2]Tulane University School of Medicine, New Orleans, Louisiana; [3]Tulane University school of medicine, New Orleans, Louisiana; [4]Vanderbilt School of Medicine, Visiting Scholar, Nashville, Tennessee

**Session:** O-7. Around the World - Understanding Infectious Disease and Health Interventions

***Background.*** Since the outbreak of Ebola Virus Disease (EVD) in West Africa from 2013–2016, a large cohort of survivors with persistent health complaints has emerged. This constellation of issues is termed post-Ebola syndrome. Here we characterize the symptoms and physical exam findings of this syndrome in a cohort of survivors from Sierra Leone 2.6 years after resolution of disease.

Ebola survivors present with clusters of symptoms that represent sub phenotypes of post-Ebola syndrome




***Methods.*** Potential survivor participants in Eastern Sierra Leone were identified and recruited through the Sierra Leone Association of Ebola Survivors. Household contacts of survivors were identified by enrolled survivors. Both groups were administered a questionnaire assessing self-reported symptoms. A physical exam was performed by a limited number of trained providers. Symptoms were then compared using hierarchical clustering. Statistical analysis of the correlations between clusters was conducted using conditional logistic regression. Both SPICE and principal component (PCA) analyses were performed to explore the relationships between symptom clusters.

***Results.*** Between March 2016 and January 2019, 375 Ebola survivors and 1040 contacts were enrolled. At enrollment, Ebola survivors of all age groups reported significantly more symptoms than their contacts in all categories. Six symptom clusters were identified representing distinct organ systems. SPICE revealed 2 general phenotypes: with or without rheumatologic symptoms. Clusters including rheumatologic symptoms were correlated with one another ($r = 0.63$) but not with other clusters ($r < 0.35$). Ophthalmologic/auditory symptoms were moderately correlated with the non-rheumatologic clusters ($r > 0.5$). Interestingly, psychologic/neurologic, cardiac/GI and constitutional clusters correlated with one another ($r > 0.6$) $p < 0.0001$ in all cases. The symptom clusters were then mapped onto a PCA. Each symptom cluster separated from the remainder along PC1, particularly the phenotypes with rheumatologic symptoms.

***Conclusion.*** This study presents an in-depth characterization of post-Ebola syndrome in Sierra Leonean survivors. The interrelationship between symptom clusters indicates that post-Ebola syndrome is a heterogeneous disease. The phenotypes identified may have unique mechanisms of pathogenesis, and require distinct therapies.

***Disclosures.*** **John Schieffelin, MD, MSPH**, **Wolters-Kluwer** (Independent Contractor)

**32. Risk Factors for Vertical Transmission of t. Cruzi infection in an Endemic Setting**

Melissa D. Klein, BS[1]; Freddy Tinajeros, PhD[2]; Edith Malaga, BS[3]; Manuela Verástegui, PhD[3]; Beth J. Condori, BS[3]; Federico Urquizu, MD[4]; Robert Gilman, MD[5]; Natalie M. Bowman, MD, MPH[6]; [1]University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [2]Asociación Benéfica PRISMA, Santa Cruz de la Sierra, Santa Cruz, Bolivia; [3]Universidad Peruana Cayetano Heredia, Lima, Lima, Peru; [4]Percy Boland Women's Hospital, Santa Cruz de la Sierra, Santa Cruz, Bolivia; [5]Johns Hopkins University, Baltimore, Maryland; [6]University of North Carolina, Chapel Hill, North Carolina

**Session:** O-7. Around the World - Understanding Infectious Disease and Health Interventions

***Members of the Chagas Disease Working Group in Peru and Bolivia include:*** Edith Hinojosa, Clariza Chavez, Jean Karla Velarde, Carla Chavarria, Victoria Serrudo, Roberto Araya, Alcides Buitron, Rita Mendieta, Holger Mayta, Maritza Calderon, Holger Mayta and Yagahira Castro.

***Background.*** Vertical transmission of *Trypanosoma cruzi* infection accounts for a growing proportion of new cases of Chagas disease. Congenital infection is curable if