regulators were observed. The highest CAZ-AVI MIC values were noted in an isolate displaying a mutation in an RND transported permease.

Table

| *E. cloacae* isolate | MIC (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Cefepime | | Meropenem | | Ceftazidime-avibactam | |
| | baseline | After 10-day passaging | baseline | After 10-day passaging | baseline | After 10-day passaging |
| 1 | 1 | 16 | 0.03 | 0.5 | 0.5 | 16 |
| 2 | 1 | 32 | 0.03 | 0.5 | 0.5 | 4 |
| 3 | 0.5 | 2 | 0.03 | 0.5 | 0.25 | 0.5 |
| 4 | 1 | 64 | 0.03 | 4 | 0.5 | 4 |
| 5 | 0.25 | 4 | 0.03 | 2 | 0.5 | 1 |
| 6 | 0.25 | 4 | 0.06 | 0.5 | 0.5 | 1 |

***Conclusion.*** FEP displayed more resistant mutants when compared to MER and CAZ-AVI. Despite the low MIC values of the mutant isolates for MER, the fold changes for this agent were higher than in FEP and CAZ-AVI. Resistance mechanisms in the mutants were complex. Only a few isolates had alterations in the genes usually associated with β-lactam resistance in *E. cloacae*. Further studies should be performed to evaluate if the mutations observed *in vitro* are present in clinical isolates resistant to these agents.

***Disclosures.*** **Mariana Castanheira, PhD**, **1928 Diagnostics** (Research Grant or Support)**A. Menarini Industrie Farmaceutiche Riunite S.R.L.** (Research Grant or Support)**Allergan** (Research Grant or Support)**Allergan** (Research Grant or Support)**Amplyx Pharmaceuticals** (Research Grant or Support)**Cidara Therapeutics** (Research Grant or Support)**Cidara Therapeutics** (Research Grant or Support)**Cipla Ltd.** (Research Grant or Support)**Cipla Ltd.** (Research Grant or Support)**Fox Chase Chemical Diversity Center** (Research Grant or Support)**GlaxoSmithKline** (Research Grant or Support)**Melinta Therapeutics, Inc.** (Research Grant or Support)**Melinta Therapeutics, Inc.** (Research Grant or Support)**Melinta Therapeutics, Inc.** (Research Grant or Support)**Merck** (Research Grant or Support)**Merck** (Research Grant or Support)**Merck & Co, Inc.** (Research Grant or Support)**Merck & Co, Inc.** (Research Grant or Support)**Paratek Pharma, LLC** (Research Grant or Support)**Pfizer** (Research Grant or Support)**Qpex Biopharma** (Research Grant or Support) **Jill Lindley**, **Allergan** (Research Grant or Support)**Qpex Biopharma** (Research Grant or Support) **Brieanna Roth, n/a**, **Allergan** (Research Grant or Support) **Timothy B. Doyle**, **Allergan** (Research Grant or Support)**Allergan** (Research Grant or Support)**Cipla Ltd.** (Research Grant or Support)**Melinta Therapeutics, Inc.** (Research Grant or Support)**Pfizer** (Research Grant or Support)**Qpex Biopharma** (Research Grant or Support) **Helio S. Sader, MD, PhD**, **A. Menarini Industrie Farmaceutiche Riunite S.R.L.** (Research Grant or Support)**Allergan** (Research Grant or Support)**Allergan** (Research Grant or Support)**Allergan** (Research Grant or Support)**Cipla Ltd.** (Research Grant or Support)**Cipla Ltd.** (Research Grant or Support)**Melinta** (Research Grant or Support)**Merck** (Research Grant or Support)**Merck** (Research Grant or Support)**Paratek Pharma, LLC** (Research Grant or Support)**Pfizer** (Research Grant or Support)

### 27. The Membrane Antimicrobial Peptide Defense (MadRS) System Orchestrates Resistance Against Antibiotics and Host Innate Immune Peptides in enterococcus Faecalis

William R. Miller, MD[1]; Ayesha Khan, BSC[2]; Samuel G. Erickson, BS[2]; Cedric H. Pettaway, Jr., BS[2]; Rafael Rios, MSc[3]; Melissa R. Cruz, BS, MPH[2]; An Q. Dinh, BS[4]; Lorena Diaz, Ph.D.[5]; Danielle A. Garsin, Ph.D.[2]; Cesar A. Arias, M.D., MSc, Ph.D., FIDSA[6]; [1]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, Texas; [2]McGovern Medical School, Houston, Texas; [3]Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, Bogota, Colombia, Bogota, Distrito Capital de Bogota, Colombia; [4]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, TX,, Houston, Texas; [5]Universidad El Bosque, Bogota, Cundinamarca, Colombia; [6]CARMiG, UTHealth and Center for Infectious Diseases, UTHealth School of Public Health, HOU, TX; Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, BOG, COL, Houston, Texas

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

***Background.*** Enterococci have evolved a network of two component-signaling systems (TCS) to evade killing by cationic antimicrobial peptides (AP) of the host innate immune response. Activation of these pathways can also lead to cross-resistance to AP-like antibiotics such as daptomycin (DAP), complicating treatment of severe enterococcal infections. Our previous work implicated the MadRS (formerly YxdJK) TCS in resistance to DAP. In this work, we show that the MadRS system regulates a network of membrane defense proteins that provide targeted resistance to killing by LL-37.

***Methods.*** We performed a global transcriptional analysis comparing wild type (WT) *E. faecalis* OG1RF and an OG1RF derivative possessing a mutated allele of the *madS* gene that activates the MadRS system (OG1RF$madS_{A202E}$). Selected genes identified in this analysis were knocked out using a CRISPR-*cas9* gene editing system and confirmed by sequencing. Antibiotic minimum inhibitory concentrations (MIC) were determined using gradient diffusion strips. LL-37 killing assays were performed in triplicate in RPMI+5% LB broth. *Caenorhabditis elegans* assays were performed in triplicate, and representative results are shown.

***Results.*** Analysis of the MadRS regulon revealed a significant upregulation of a network of gene targets, including *madLM* (formerly *yxdLM*, involved in bacitracin resistance), *madEFG*, the *dlt* operon, *salA*, and EF2211 (**Fig 1**). The $madS_{A202E}$ allele was associated with increased bacterial survival on exposure to LL-37, while the *madEFG* deletion mutant was more susceptible to killing (**Fig 2A**). Deletion of *madLM* decreased bacitracin MICs, but did not alter survival on exposure to LL-37. In *C. elegans*, inactivation of the MadRS system by deletion of the *madR* response regulator resulted in attenuation as compared to WT OG1RF. The effect was reversed by complementing with wild-type *madR* on a plasmid (**Fig 2B**). The attenuation effect of the mutant was abolished in *pmk-1* knockout worms, which lack the ability to produce innate immune peptides (**Fig 2C**).

Figure 1

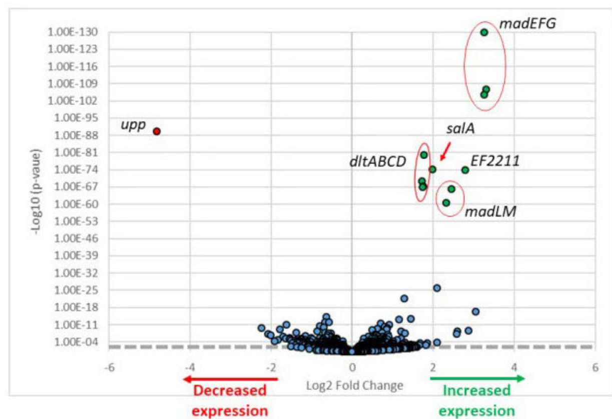


**Figure 1. RNA Sequence analysis of the MadRS Regulon.** Total RNA was extracted from *E. faecalis* OG1RF$madS_{A202E}$, which results in activation of the MadRS system, and wild type OG1RF at mid-exponential phase. Log2-fold change in OG1RF$madS_{A202E}$ as compared to OG1RF is shown on the x-axis, with p-values (significance) shown on the y-axis.

Figure 2

***Conclusion.*** The MadRS system plays an important role in mediating resistance to AP both *in vitro* and *in vivo*. The novel ABC-transporter MadEFG is likely to play a major role in preventing enterococcal killing by the human cathelicidin LL-37.

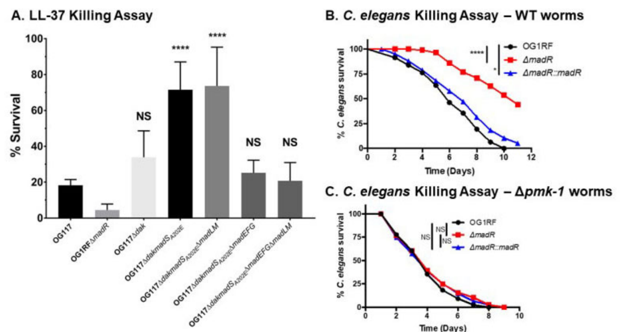


**Figure 2. MadRS orchestrates resistance to APs of the innate immune system *in vitro* and *in vivo*. A)** LL-37 killing assay. Strains were grown to mid-exponential phase in brain heart infusion (BHI) broth, and diluted to a final inoculum of $10^3$ cells/mL. LL-37 (50 µg/mL) was added, and cells were incubated for 2 hours at 37° C followed by colony counts. Statistical comparison for each group was made to OG117 using one-way ANOVA, with Tukey correction for multiple comparisons. **B)** Survival of *C. elegans* incubated with OG1RF, OG1RFΔ*madR*, and OG1RFΔ*madR*/pAT392::*madR* (complementation in trans on a plasmid). **C)** Survival of *C. elegans* lacking *pmk-1*, with impaired ability to produce innate immune peptides. *, p<0.05, ****p<0.001, NS – not significant.

***Disclosures.*** **William R. Miller, MD**, **Entasis Therapeutics** (Scientific Research Study Investigator)**Merck** (Grant/Research Support)**Shionogi** (Advisor or Review Panel member) **Cesar A. Arias, M.D., MSc, Ph.D., FIDSA**, **Entasis Therapeutics** (Scientific Research Study Investigator)**MeMed** (Scientific Research Study Investigator)**Merck** (Grant/Research Support)

### 28. Regulation of the Staphylococcal β-lactamase Plays a Major Role in the cefazolin Inoculum Effect

Sara I. Gomez-Villegas, MD[1]; An Q. Dinh, BS[2]; Jinnethe Reyes, MSc, PhD[3]; Diana Panesso-Botero, PhD[4]; Maria I. Reyes, n/a[4]; Maria I. Reyes, n/a[4]; Rafael Rios, MSc[5]; Oscar Ortega-Recalde, MD, MSc, PhD[5]; Oscar Ortega-Recalde, MD, MSc, PhD[5]; Lorena Diaz, Ph.D.[5]; Sandra Rincon, PhD[6]; Barbara E. Murray, M.D.[2]; Barbara E. Murray, M.D.[2]; Kavindra V. Singh, Ph.D.[2]; Kavindra V. Singh, Ph.D.[2]; Cesar A. Arias, M.D., MSc, Ph.D., FIDSA[7]; [1]University of Texas Health Sciences Center at houston, Houston, Texas; [2]Center for Antimicrobial Resistance and Microbial Genomics, UTHealth, Houston, TX,, Houston, Texas; [3]Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, Bogota, Colombia, Bogota, Distrito Capital de Bogota, Colombia [4]UTHSC at Houston Mc Govern Medical School, Houston, Texas; [5]Universidad El Bosque, Bogota, Distrito Capital de Bogota, Colombia; [6]Universidad el Bosque, Bogota, Distrito Capital de Bogota, Colombia; [7]CARMiG, UTHealth and Center for Infectious Diseases, UTHealth School of Public Health, HOU, TX; Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics, Universidad El Bosque, BOG, COL, Houston, Texas

**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

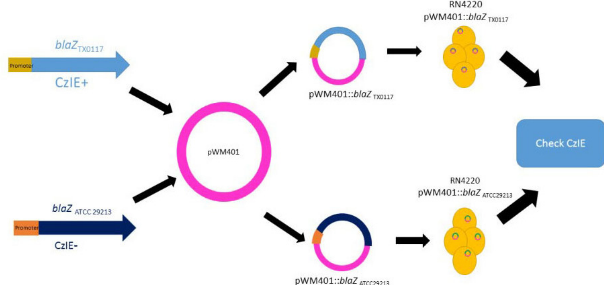


Figure 3. In trans expression of the bla Operons from a CzIE+ strain (TX0117) and a CzIE- strain (ATCC29213) in RN4220

***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.

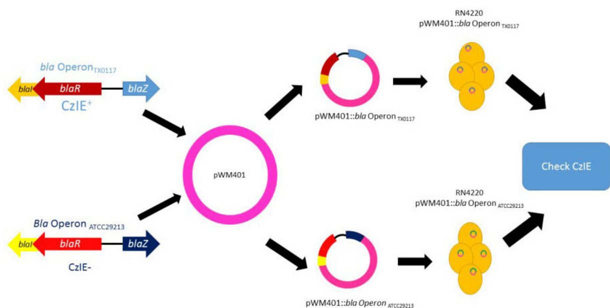


Table 1. MIC values of transformans after In trans expression of blaZ genes and bla Operons from a S. aureus CzIE+ strain(TX0117) and a CzIE- strain (ATCC29213) in RN4220

| 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