inner colonies within the zone of inhibition around the fosfomycin disk confound interpretation of susceptibility testing. The goal of this study was to estimate the frequency of these non-susceptible inner colony E. coli mutants and to identify their resistance mechanisms.

Methods. Disk diffusion testing of fosfomycin was performed on 650 E. coli clinical isolates at UMPC between 2011 and 2015 (496 were ESBL-producing). For E. coli strains producing inner colonies within a non-susceptible zone of inhibition (≤12-15 mm in diameter), disk diffusion testing was repeated to confirm that stable resistance had developed. Both the parental strains and their corresponding most proximal inner colony mutants were subjected to MIC testing, whole-genome sequencing, qRT-PCR, and carbohydrate utilization studies.

Results. Of the 650 E. coli clinical isolates, 69 (9.9%) produced non-susceptible inner colonies. Whole-genome sequencing revealed deletion of uhpT in 4 of the E. coli strain inner colonies, while the remaining two strains contained non-susceptible mutations in uhpA and uhpC, respectively. Both genes are required for expression of uhpT. Carbohydrate utilization showed that all six inner colony mutants had decreased growth on minimal medium supplemented with glucose-6-phosphate compared with their parent strains. Expression of uhpT was absent in the mutant strains with deletions of uhpT and lower in mutants with mutations of uhpC and uhpA compared with their parents by qRT-PCR.

Conclusion. Among E. coli clinical isolates studied, occurrence of non-susceptible parent mutants by qRT-PCR, and carbohydrate utilization studies. All authors: No reported disclosures.

32.2. The Gastrointestinal Tract Is A Major Source of Echinocandin Drug Resistance in a Candida glabrata Colonization Mouse Model

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Background. Gastrointestinal (GI) Candida commensals may be a major source of invasive candidiasis and a hidden reservoir of antifungal resistance. Candida glabrata resistance rates are increased greater than those of other species. Herein, we present a C. glabrata GI colonization model to explore how antifungal drugs affect resistance acquisition and systemic breakthrough infections.

Methods. Immunocompetent mice were treated with antibiotics to clear native GI bacterial flora and then inoculated via gavage with C. glabrata. Fecal samples were collected throughout the study to assess fungal GI colonization. Daily administration of caspofungin (CSF; 5 or 20 mg/kg ip), cinchoninate inhibitor nikkomycin Z (Nz; 100 mg/kg oral), or saline was initiated on day 3 post inoculation. CSF-resistant colony frequencies were determined through selection of fecal samples on CSF-supplemented media, and FKS mutations were identified using the newly developed molecular beacon diagnostic assays. Dexamethasone was administered to induce immunosuppression. Upon completion of the experiment, blood, and organs were harvested and yeast burden levels determined.

Results. Daily therapeutic dosing (5 mg/kg) of CSF resulted in no reduction in fecal burdens, little resistance (0-10%), and organ breakthrough rates similar to control groups. Treatment with high dose (20 mg/kg) CSF caused a 2.5-log decrease in average burden, yet high levels (10/10 mice) of resistance (fsk2/2 mutants) were observed following 9 days of treatment. Although breakthrough rates decreased in this group, yeast recovered from organs contained fsk mutations. The largest reduction (3-log) in GI burdens was obtained within 3-5 days of high dose CSF plus Nz (100 mg/kg oral) treatment. However, echinocandin resistance was again observed from all mice (10/10) following 5-7 days of treatment. Treatment with the therapeutic dose plus Nz left GI burdens unchanged, but did significantly reduce organ breakthrough rates (20%; P < 0.05).

Conclusion. We have developed a C. glabrata GI colonization and dissemination model. Systemic breakthrough depends on both gut C. glabrata population composition and serum/tissue drug level.

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Pfizer: Grant Investigator, Research support. Astellas: Consultant and Grant Investigator, Research support and Speaker honorarium. F2G: Consultant, Consulting fee

32.3. Genomic Pathways Associated with Daptomycin (DAP) Resistance in DAP-Susceptible Enterococcus faecium Harboring Substitutions in LiaFSR
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Background. DAP is used off-label for treatment of severe enterococcal infections. DAP resistance has been associated with changes in LiaFSR, a three-component regulatory system that controls the cell envelope stress response to antibiotics. In particular, substitutions in LiaS (T120A) and LiaR (W73C) seem to predispose to development of DAP-R during therapy, without increasing the DAP MIC above the clinical breakpoint. Using a PK/PD model of simulated endocardial vegetation, we evaluated the genomic pathways for DAP-R under different DAP dose schemes.

Methods. A DAP-susceptible E. faecium (HOU503; MIC 3 mg/ml) harboring the LiaS-R substitution was subjected to simulated doses of 6, 8, and 10 mg/kg/d in the model for 14 days using a starting inoculum of 106 CFU/ml. Sixteen DAP-R isolates were recovered from the SEV model: five isolates from 6 mg/kg (D6 isolates from days 2 to 4); five isolates from 8 mg/kg (D8 isolates from days 2 to 8); and six isolates from 10 mg/kg (D10 isolates from days 1 to 14) which were subjected to whole genome sequencing. Reads from each sequencing were aligned against the HOU503 genome for SNP analyses. Variant calling was done with GATK, SamTools, and the low-frequency variant detector from CLC Genomics Workbench 8.5. Variants detected by the three callers were selected and annotated with SnpEff, then compared among the different groups of isolates accordingly to the DAP doses that were exposed.

Results. We detected a total of 16 proteins exhibiting substitutions consistently in all the DAP-R sequenced isolates; including mobile genetic elements (9), hypothetical proteins (2), a bacteriocin, a prophage, a gyrA subunit, a FtsK homolog, a GntR family transcriptional regulator, an FNR-like regulator, and a HIC-like protein, all of which showed high sequence identity with a functional regulator that represses expression of a T6SS in E. coli. This regulator was associated with the HOU503 isolate recovered from patients following 6 mg/kg D6. No significant changes were observed in the LiaS-R substitution.

Conclusion. Using a humanized SEV PK/PD model and SNP-based analyses, we were able to uncover possible novel genetic pathways associated with the development of DAP-R via the LiaFSR system in enterococci.

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