

underscores the important role WGS plays in identifying new mechanisms of antimicrobial resistance.

**Disclosures.** All authors: No reported disclosures.

**607. Scope and Predictive Genetic/Phenotypic Signatures of “Bicarbonate [NaHCO<sub>3</sub>]-Responsivity” and β-Lactam Sensitization among Methicillin-Resistant *Staphylococcus aureus* (MRSA)**

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**Background.** Selected MRSA strains become susceptible to β-lactams (e.g., oxacillin [OX]; cefazolin [CFZ]) in vitro when tested in a standard medium (cation-adjusted Mueller–Hinton Broth; CA-MHB) supplemented with NaHCO<sub>3</sub> (“NaHCO<sub>3</sub>-responsivity”). In vivo activity of β-lactams was demonstrated for MRSA strains with this phenotype in a rabbit endocarditis model (Ersoy et al Antimicrob Agents Chemother 2019). The current study was designed to: (i) determine the prevalence of the NaHCO<sub>3</sub>-responsive phenotype in a large collection of clinical MRSA isolates; and (ii) identify genetic and phenotypic predictors of this phenotype. **Methods.** 58 recent MRSA bloodstream isolates representing contemporary clonal complex (CC) genotypes were screened for the NaHCO<sub>3</sub>-responsive phenotype by broth microdilution MICs in CA-MHB, with or without NaHCO<sub>3</sub> supplementation (25–44 mM).

**Methods.** 58 recent MRSA bloodstream isolates representing contemporary clonal complex (CC) genotypes were screened for the NaHCO<sub>3</sub>-responsive phenotype by broth microdilution MICs in CA-MHB, with or without NaHCO<sub>3</sub> supplementation (25–44 mM).

**Results.** 43/58 (74.1%) and 21/58 (36.2%) were rendered susceptible to CFZ and OX, respectively, in the presence of NaHCO<sub>3</sub>; 20 of the 21 OX-susceptible strains were also susceptible to CFZ in the presence of NaHCO<sub>3</sub>. High baseline β-lactam MICs (i.e., MICs in CA-MHB alone ≥64 μg/mL) was not predictive of NaHCO<sub>3</sub> responsivity. The CC8 genotype was correlated with NaHCO<sub>3</sub> responsivity for OX, but not CFZ (*P* < 0.05).

**Conclusion.** The NaHCO<sub>3</sub>-responsive phenotype is relatively common for both OX and especially CFZ among clinical MRSA isolates. Identification of specific genetic factors linked to this phenotype remains ongoing. Confirmation in relevant animal models that this phenotype is predictive of β-lactam efficacy *in vivo* could provide a solid foundation for a paradigm shift in antimicrobial susceptibility testing of MRSA.

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**608. Emerging Methicillin Resistance Mechanism in *mec* Gene-Negative *Staphylococci* not Detected by Reference Methods**

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**Background.** β-lactam resistance in *Staphylococci* is mediated by *mec* genes usually diagnosed by disc diffusion Cefoxitin test (DDFOX) and PCR testing. Here, we report methicillin-resistant *Staphylococcus lugdunensis* and *Staphylococcus aureus* strains lacking *mec* gene misdiagnosed by reference methods. Since the strains are not β-lactamase hyperproducers we investigated the molecular basis of the methicillin resistance.

**Methods.** We tested 2 *S. lugdunensis* isolates (SL1, SL2) collected from distinct blood cultures of the same patient and 2 *S. aureus* isolates (SA1, SA2): (i) by DDFOX, (ii) for Oxacillin MIC by agar dilution (AD), (iii) by VITEK<sup>2</sup> (bioMérieux) for Oxacillin MIC (V2 OXA) and Cefoxitin Screen Test (V2 OXSF), (iv) for *mecA*, *B*, *C* genes by PCR and (v) by whole-genome sequencing (WGS).

**Results.** The 4 isolates were methicillin susceptible by DD FOX and *mec* negative. However, all the isolates displayed variable results for V2 OXA MIC (0.5 to ≥4 mg/L) and for V2 OXSF (POSITIVE, NEGATIVE). For SL1 and SL2 isolates, the V2 OXSF growth curve atypical pattern has led to investigating the OXSF wells. The plates inoculated with the broth extracted from the OXSF well showed 2 colony morphotypes (small “P” and regular “G”) for both isolates. The small colonies (SL1P, SL2P) were Oxacillin resistant (V2 OXA MIC ≥ 4; AD MIC = 4) and V2 OXSF POSITIVE whereas the regular colonies (SL1G, SL2G) were Oxacillin susceptible (V2 OXA MIC = 2; AD MIC = 0.5) and V2 OXSF NEGATIVE. The 4 morphotypes were cefoxitin susceptible by DDFOX and *mec* negative. Interestingly, WGS revealed a GdpP truncation in the N-terminal domain only found in *S. lugdunensis* small colonies (SL1P, SL2P) phenotypically resistant to Oxacillin. GdpP is a cyclic diadenosine monophosphate phosphodiesterase enzyme which function is the hydrolysis of a signaling nucleotide.

**Conclusion.** We described *mec* negative *S. lugdunensis* and *S. aureus* strains expressing heterogeneous methicillin resistance detected by the VITEK2 OXSF test.

*S. lugdunensis* subpopulation of small colonies resistant to oxacillin is associated with a truncation of GdpP protein previously described in *S. aureus*. Interestingly GdpP loss of function in *Staphylococci* is associated with a reduced growth and may arise as a result of the selective pressure of exposure to β Lactams.

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**609. Differing Genotypic Contexts Between *E. coli* and *A. baumannii* Modulate the Role of bla<sub>ADC-7</sub> in the Development of Antibiotic Collateral Sensitivity**

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**Background.** Antibiotic resistance is a global health crisis. While persistent drug discovery of novel antibiotics has previously been relied upon to thwart resistance, evolution inevitably perseveres. While genes conferring antibiotic resistance have previously been characterized, it is unclear how varying genetic contexts can change the antibiotic resistance phenotype a given gene confers.

**Methods.** The DH10B strain of *E. coli* was transformed with a bla<sub>ADC-7</sub> plasmid. In 12 evolutionary replicates, the modified *E. coli* strain and a clinical strain of *A. baumannii* containing the same resistance gene were passaged daily for 10 days on cefepime gradient agar plates with gradually increasing concentrations of cefepime. MICs of cefepime and a diverse set of 15 other drugs were determined for the parental strains and after the final passage passage. MIC of cefepime after intermediary passages were determined for select replicates. Lastly the bla<sub>ADC-7</sub> gene after the final passage was sequenced.

**Results.** At the end of 10 passages, collateral sensitivity in *A. baumannii* was observed to tigecycline and fosfomycin in 5 and 6 replicates respectively, out of 12 total. 4 out of 12 *E. coli* replicates displayed collateral sensitivity to minocycline (Figure 1). In the third *E. coli* replicate, Sanger sequencing revealed a novel S286R mutation in bla<sub>ADC-7</sub> appearing in passage seven which preceded a several log fold increase in the MIC of cefepime (Figures 2 and 3). No additional mutations were found in the other evolutionary replicates.

**Conclusion.** Patterns of resistance varied among antibiotics of the same class, (e.g., tetracyclines, fourth-generation cephalosporins) in both *E. coli* and *A. baumannii*; however, *A. baumannii* expressed less widespread collateral resistance than *E. coli*. A previously undiscovered S286R mutation in bla<sub>ADC-7</sub> coincided with a pronounced increased in resistance to cefepime. Further studies are required to determine whether this mutation gives rise to a structural change in the protein product. Given that no other mutations were found, resistance to cefepime and subsequent collateral resistance to other antibiotics may have developed due to epigenetic changes or mutations outside the bla<sub>ADC-7</sub> genes. Indeed, future experiments with whole-genome sequencing may reveal such changes.

Figure 1: Log change in MIC of 16 drugs after 10 passages of Cefepime in 12 evolutionary replicates

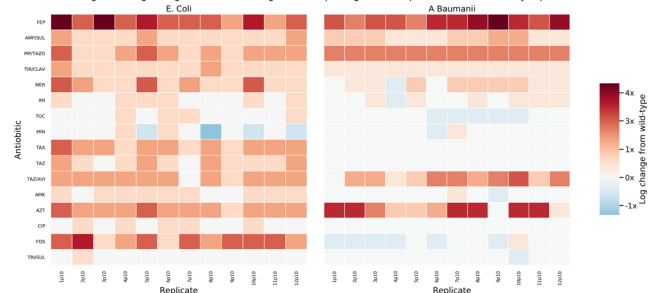


Figure 2: MIC of cefepime for *E. coli* replicate 3

